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Title:
Memory and The Ageing Brain

Dopamine, sleep and the hippocampus

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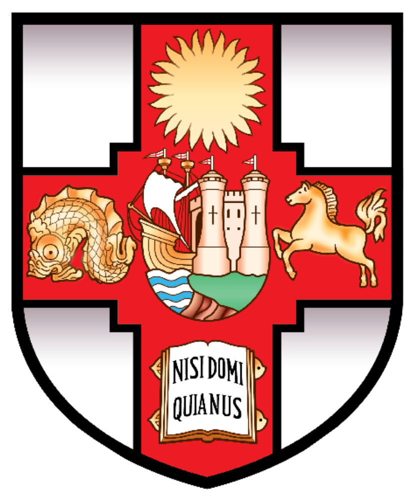
Memory and The Ageing Brain:

Dopamine, sleep and the hippocampus



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Abstract

Human brains selectively store knowledge about the world to optimise future behaviour. We automatically rehearse and contextualise, or discard information to create a robust collection of facts and events. The medial temporal lobe is central to a network of memory regions within the brain that select important memories for long term storage. Much of this memory selection is purported to occur automatically during sleep. Recent emerging data have suggested that dopamine might influence memory longevity. However, it has not been clear at which time point in the memory process dopamine is active, particularly whether dopamine biases memory at the time information is encountered or, later, during consolidation of memory during sleep.

In two independent double-blind randomised placebo-controlled studies of healthy older adults, I administered dopamine to temporally target memory evolution at different time-points in relation to learning using a verbal recognition task. Nocturnal dopamine enhanced efficiency of routine forgetting while sparing saliently tagged information. Importantly, dopamine administration did not affect encoding or retrieval, strongly suggesting that dopamine acts after encoding during memory storage processes. Analysis of polysomnography suggested that the behavioural tagging effect of dopamine was associated with increased spindle amplitude during slow wave sleep. Overnight dopamine also increased total slow wave sleep duration by 11%. No relationships were seen between memory and medial temporal lobe structures on structural MRI. However, volumes of hippocampal subfields CA2 and dentate gyrus and entorhinal cortex were all associated with slow wave sleep duration. Intriguingly, CA2 volume negatively correlated with slow wave sleep duration, but positively correlated with spindle density.

In summary, nocturnal dopamine optimises the memory selection processes by modulating slow wave spindles.

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Last but certainly not least: thank you mum and dad.

Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: .... DATE:......................

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Abbreviations:

AFS	Awakening from sleep
BF	Bayes Factor
BFW	Behaviour following waking
BOLD	Blood oxygen level dependent signal
CA1-4	Cornu Ammonis regions 1 to 4
COMT	Catechol-O-Methyltransferase
CSF	Cerebrospinal fluid
D'	D-prime, a measure of accuracy
D1	D1-like dopamine receptors
D2	D2-like dopamine receptors
DAT	Dopamine transporter
DBS	Deep brain stimulation
DG	Dentate gyrus
e.g.	exempli gratia (latin – For example)
EC	Entorhinal cortex
EC (I – VI)	Different cell layers of the entorhinal cortex
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electro-oculogram
FDR	False discovery rate
GTS	Getting to sleep
H_0	The null hypothesis
H_1	The alternative hypothesis
i.e.	id est (latin – in other words)
ICD	an implantable cardioverter-defibrillator
IUD	an intrauterine device
JASP	Jeffrey's Amazing Statistical Package software
L-DOPA	levodopa
LTD	Long-term depression (opposite of long-term potentiation)
LTP	Long-term potentiation
MAO	Monamine oxidase
MoCA	Montreal Cognitive Assessment
NREM	Non rapid eye movement sleep
p	p-value
PANAS	Positive and negative affective scale
pg	Page
PSG	Polysomnography
QOS	Quality of sleep
REM	Rapid eye movement sleep
SD	Standard deviation
SMHSQ	St Mary's hospital sleep questionnaire
SUB	Subiculum
SwR	Sharp wave ripple
t	t-test statistic
VTA	Ventral tegmental area
Δ / δ	Cohen's delta (standardised measure of effect size)

Chapter I: Background

Your accent, your deepest fears, hopes and expectations, your worldviews and ethical values – and the specific effortless sequence that makes your perfect cup of tea – all rely on combining complex pieces of information about the past to make a coherent representation of the present. Our ability to interpret current and future events, and to navigate our environment is filtered through and guided by our experiences. Memory is the process of maintaining and manipulating information over time. It is not a passive process but instead, we update our knowledge-base continuously as we learn new information.

Within the brain, changes in neuronal activity underlie memory. An engram, or a neurobiological representation of a memory, is born at learning (Tonegawa, Liu, Ramirez, & Redondo, 2015). The exact myriad of events that leads to the birth, storage and up-keep of engrams is not known, but it has become increasingly clear that it involves plastic changes in the brain function (Hebb,

1949; Squire, 1992). Several physiological processes, neurotransmitters and anatomical structures seem to be involved in these changes.

The involvement of the medial temporal lobe in memory has been postulated since the 1950s (Scoville & Milner, 1957), and these observations have been consistently confirmed since . In short, hippocampus, which lies deep within the medial temporal lobe, seems to be involved in the initial formation and storage of autobiographical memories, and, at least in some cases, in later retrieval (Rugg & Vilberg, 2013). Initially engrams are encoded in the hippocampus, but long term storage is more reliant on cortical regions (Rasch & Born, 2013).

Different proteins and neurotransmitters are thought to support these hippocampal processes. Both sleep and dopamine are stipulated to play a role in memory by modulating hippocampal function (J. Lisman, Grace, & Duzel, 2011; Rasch & Born, 2013).

In this chapter, I will first introduce the basic neuroanatomy and physiology of the hippocampal formation, dopamine and sleep (pg. 1), to provide necessary background to discuss these topics in the context of long-term procedural memory (pg. 33) and ageing (pg. 70).

Anatomy and neurophysiology

Hippocampus

The hippocampus is likely the most studied brain structure. It lies deep within the medial temporal lobe (Figure 1) and forms a part of the limbic system. Behaviourally, it has been associated with memory and sleep among other things (Buzsaki, 1986; Scoville & Milner, 1957).

The hippocampus is not a uniform structure. Instead, it is composed of structurally and functionally separable but interconnected subfields: the dentate gyrus, cornu ammonis (CA)1, CA2, CA3, CA4 and the subiculum (Duvernoy, Cattin, & Risold, 2013). These subfields receive input from sensory processing areas through the entorhinal cortex (Figure 2A).

One of the main processing pathways in the hippocampus is the trisynaptic loop (Figure 2B), which is thought to be involved in memory storage (Hyman, Van Hoesen, Kromer, & Damasio, 1986; Lomo, 2003). Information processing within the hippocampus begins when input from the entorhinal cortex enters the dentate gyrus along the perforant path. The dentate gyrus is the prime site for hippocampal neurogenesis (Gould et al., 1999; Kuhn, Toda, & Gage, 2018). Mossy fibres from the dentate gyrus then project to the CA3, which in turn projects information to the CA1 along Schaffer collaterals. The CA1 then completes the loop by sending projections back to the entorhinal cortex. Another major output route from the CA1 projects to the subiculum, which also sends and receives projections to and from the entorhinal cortex, as well as from other limbic regions and the neocortex.

Three main types of hippocampal neuronal activity, all of which occur along this route, have relevance to this thesis. These are the long-term potentiation and depression (Holscher, 1999)

and sharp wave ripple complexes, especially during sleep (van de Ven, Trouche, McNamara, Allen, & Dupret, 2016).

Long-term potentiation and long-term depression

Long term potentiation (LTP) is a process in which frequent co-activation of two neurons that share a synapse strengthens said synapse. LTP was initially discovered half a century ago in the rabbit dentate gyrus following targeted high frequency stimulation of the perforant pathway (T. V. Bliss & Lomo, 1973). Following its discovery, monumental effort has been made to study the relation of this process to memory (T. V. P. Bliss & Collingridge, 1993).

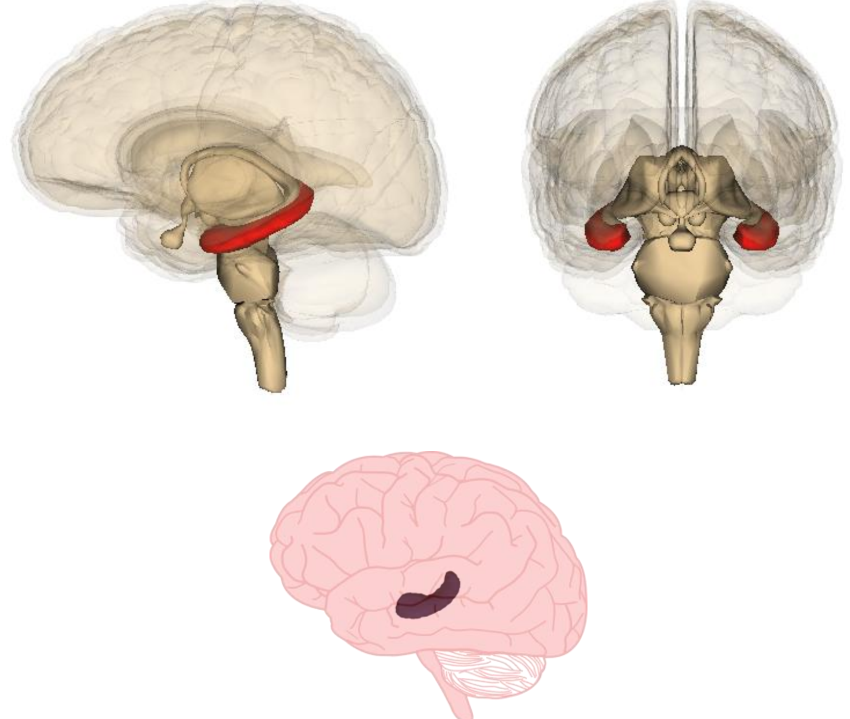
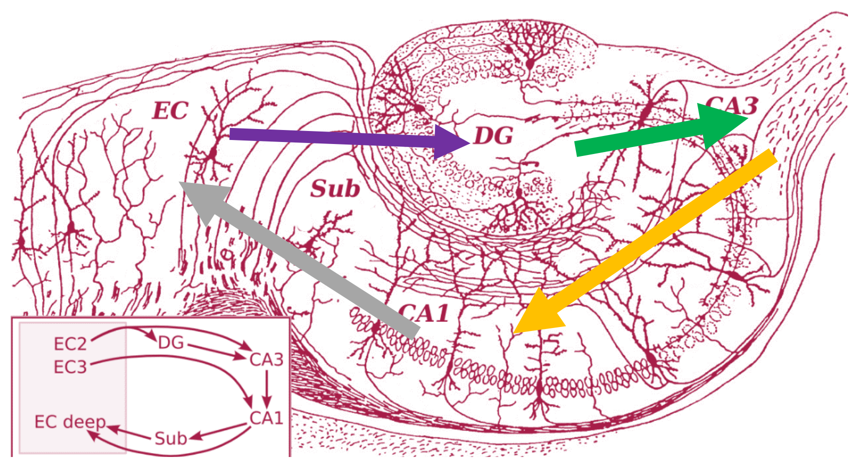


Figure 1: The hippocampus is in the medial temporal lobe

The hippocampus lies deep within the medial temporal lobe. The glass brain (top) shows the hippocampus in red. The bottom panel shows where the hippocampus lies in respect to the lateral surface. The bottom panel is not to scale.
Top panel: Life Science Databases (LSDB), Wikimedia Commons

A



B

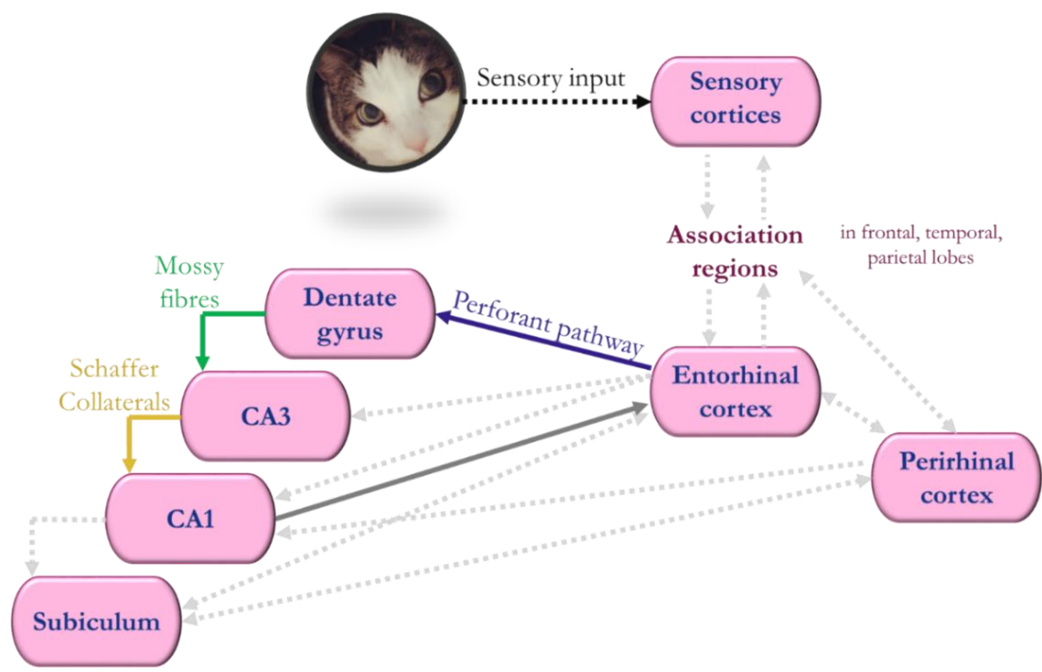


Figure 2: Hippocampal subfields and connections

Cross-section of the hippocampal body with a reference for the major pathways.
EC = entorhinal cortex, EC2, EC2, EC deep = different entorhinal cortex layers, DG = dentate gyrus, Sub = subiculum.
A simplified schematic of hippocampal subfield connectivity and major pathways. Solid lines together form the trisynaptic loop.

Panel A: Modified drawing by Santiago Ramón y Cajal (1911), Wikimedia Commons

Long term depression (LTD) on the other hand refers to the weakening of synaptic connections (Bear & Abraham, 1996). LTP and LTD are processes of Hebbian (Hebb, 1949) plasticity where the connectivity between two neurons is strengthened or weakened based on synaptic activity. This type of plasticity requires two events to occur: activation of synapse between cells and depolarisation that triggers action potentials in the postsynaptic neuron.

LTP and LTD are often considered the main ways in which the brain adapts to experience (T. V. P. Bliss & Collingridge, 1993). Perhaps weakening and strengthening of connections is what allows us to build coherent models of the world.

Morris et al (1986) were the first to demonstrate the behavioural relevance of LTP in a spatial learning task. Using a water maze, they found that a N-methyl-D-Aspartame (NMDA) receptor antagonist impaired learning as well as prevented hippocampal LTP (R. G. Morris, Anderson, Lynch, & Baudry, 1986). Later work by Manahan-Vaughan and others (1997) showed that the same concentration of NMDA antagonist that blocked learning and LTP was also blocking LTD in the hippocampus (Manahan-Vaughan, 1997). Together, these early findings suggest that both LTP and LTD are necessary for hippocampal spatial learning. Later evidence has strongly suggested that while LTP and LTD are both implicated in learning, they may support different processes (Kemp & Manahan-Vaughan, 2004, 2007). The basic mechanisms of hippocampal LTP and LTD are fairly well understood.

There are several ways in which LTP and LTD can occur. The NMDA-receptor-dependent LTP and LTD in the CA1 region of the hippocampus are perhaps the most well understood (Dudek & Bear, 1992; Manahan-Vaughan, 1997; Manahan-Vaughan, Kulla, & Frey, 2001). This type of plasticity requires changes in AMPA receptor availability that are triggered by NMDA receptor events. The AMPA receptors are glutamatergic and the number of these receptors affects the strength of the synapse.

The NMDA receptors are normally blocked by a magnesium ion. The positively charged magnesium is latched onto the strongly negatively charged NMDA receptor causing a blockade. This voltage-dependent magnesium ion block prevents postsynaptic glutamate binding (Mayer, Westbrook, & Guthrie, 1984). The opening of the NMDA receptor requires both presynaptic glutamate release as well as depolarisation of the postsynaptic neuron. Depending on the level of stimulation of the AMPA receptor, and the type of AMPA receptors stimulated, the magnesium ion influx can either trigger LTP or LTD. The former requires short bursts of activation causing strong depolarisation of the postsynaptic neuron, while the latter requires longer term weaker depolarisation or inactivation of the synapse (Bear & Abraham, 1996).

Long-term potentiation (LTP)

First, during LTP, glutamate that is released by the presynaptic neuron binds to AMPA receptors, causing an action potential. When the AMPA receptor is frequently activated by glutamate, this causes depolarisation of the postsynaptic neuron. Depolarisation refers to a rapid shift in the electric charge of the cell – in this case resulting in reduced negative charge in the postsynaptic neuron. As the magnesium ion block is voltage-dependent, the ion is released from the NMDA receptor into the postsynaptic neuron, and therefore the neuron becomes less negatively charged. During LTP this release is widespread across NMDA receptors on the cell surface.

The positive charge of the magnesium that influxes into the postsynaptic further increases depolarisation. As a result, more calcium influxes into the intracellular space. The calcium movement cascades events that lead to the release of the Ca2+/Calmodulin-Dependent Protein Kinase II (CaMKII) enzyme which triggers biochemical changes that lead to additional AMPA receptors being inserted onto the membrane. The additional AMPA receptors increase the likelihood of depolarising the postsynaptic neuron: **the synapse has now become stronger**.

Experimentally, these events have been associated with memory formation. Both genetic and pharmacological manipulations of both NMDA receptors and CamKII impact memory (J. Lisman, Schulman, & Cline, 2002). Furthermore, while LTP is most frequently studied within the CA3/CA1 synapse, it can also be observed along the CA1-subicular projections and elsewhere in the brain. Disrupting LTP in the CA1 (Tsien, Huerta, & Tonegawa, 1996) or along the CA1-subiculum pathway has been shown to impair memory maintenance (Commins, Gigg, Anderson, & O'Mara, 1998).

Therefore, in LTP, the repeated glutamate release from the pre-synaptic neuron makes the postsynaptic neuron more likely to respond to weaker signals from the presynaptic neuron by causing physiological changes in the synapse. The strengthening of this synapse supports memory formation.

Long-term depression (LTD)

LTD is less well understood than LTP but it is also posited to be important for memory. Again, there are several different types of LTD. The best understood process involves reductions in AMPA receptors due to insufficient magnesium release from AMPA receptors. While LTP typically occurs following a short high intensity stimulation of a postsynaptic neuron, LTD can also happen following a longer lasting low intensity stimulation or after an action potential.

In a simple model, the type of low intensity stimulation that triggers LTD depolarises the postsynaptic neuron but less intensively than that required for LTP. Therefore, only some magnesium ions are removed from the NMDA receptor: the postsynaptic neuron is therefore not sufficiently depolarised to cause widespread magnesium ion release – as seen in LTP. The modest magnesium release does not trigger the insertion of further AMPA receptors onto the cell membrane, i.e. LTP does not occur. Instead, it cascades events that trigger the *removal* of AMPA receptors: **the synapse has become weaker**.

One interesting hypothesis of LTD is that it may allow "space" for new memories to form. By strategically "forgetting" or re-setting synapses, LTD allows for new memories to be born or important ones to be strengthened (Manahan-Vaughan & Braunewell, 1999; Stanton, 1996; Tsumoto, 1993). This hypothesis is often called the reversal hypothesis: LTD is considered the reverse, or the mirror image of LTP. This process may also, at least in part, support systems consolidation where newly acquired information is "removed" from the hippocampus during sleep to be stored preferentially in the neocortex. LTD may be necessary to allow space for new memories to be born, as will be discussed later in this chapter.

However, **this explanation of LTD and LTP is simplistic.** These processes can take place in several different brain regions and they are not mere mirror images of one another (H. K. Lee, Barbarosie, Kameyama, Bear, & Huganir, 2000; Manahan-Vaughan, 1997). For example, LTP and LTD may be dependent on *distinct* NMDA receptor subunits (Liu et al., 2004). LTP and LTD are not merely involved in memory persistence either, but seem to support encoding as well (Kemp & Manahan-Vaughan, 2007). While the forgetting account is pervasive, it is worth noting that it is unlikely to be the only means by which LTD supports memory, and not everyone sits in its support. Indeed, some have coined the forgetting hypothesis of LTD as being "fatally flawed" (Kemp & Manahan-Vaughan, 2007).

Alternatively, LTP and LTD have been suggested to work in parallel to modulate the signal-to-noise ratio for forming associations that underlie memories (P. Dayan & Willshaw, 1991). Furthermore, while here I have presented a short account of how the NMDA receptor dependent processes work, there are other types of LTP and LTD. For example, LTD within the dentate gyrus is *independent* of NMDA receptors (Poschel & Manahan-Vaughan, 2007).

While it is increasingly clear that both LTP and LTD are involved in memory persistence and modulation, their exact roles and functions are still much debated.

Oscillations in the hippocampus

The brain has several mesoscopic activity patterns, known as oscillations, which are typically denoted by different letters of the Greek alphabet. While LTP and LTD refer to synaptic events, oscillation patterns are the outcome of several different action potentials firing synchronously. Cross-frequency coupling, or the temporal interaction of the different oscillation "bands", are thought to be associated with sensory, motor and cognitive processes (Canolty & Knight, 2010).

These oscillations can be observed recording electroencephalograms (EEGs) using sensors placed either directly on the brain's surface or inside tissue, or on the scalp. Recordings from within the brain are known as local field potentials (LFP). These recordings are taken from the extracellular space and therefore they represent small populations of cells. EEG recordings of hippocampal activity require access to the intracranial space and cannot be recorded from the scalp. In humans these recordings can be obtained during brain surgery, typically from patients with severe epilepsy. For this reason, majority of the direct hippocampal recordings are made in animals.

The hippocampus has three main types of oscillation patterns: theta, sharp waves and gamma. The former two are specific to the hippocampus while gamma can be recorded across several brain regions. All three are thought to support memory (Buzsaki, 1986; Nyhus & Curran, 2010).

The different types of hippocampal oscillations are also associated with different behaviours. For example, during wakefulness, when a rat is exploring, rearing or sniffing, the LFPs recorded from the CA1 are dominated by theta activation (Vanderwolf, 1969). However, when a rat is eating or grooming, the theta activity is swiftly replaced by intermittently occurring sharp waves (Buzsaki, Leung, & Vanderwolf, 1983).

Chapter I: Background

Anatomy and neurophysiology

Sharp waves are typically recorded from the CA1 stratum radiatum layer. They are short (40-100ms) large amplitude negative deflections. What is interesting about them is that they are typically – though not always – cross-frequency coupled with short bursts of CA1 pyramidal activity known as sharp wave ripples (SwRs) (Buzsaki, 1986; Buzsaki, Horvath, Urioste, Hetke, & Wise, 1992; O'Keefe, 1976).

SwRs can be seen in hippocampal EEGs during restfulness (when the animal is alert but still) and during sleep. They have been recorded across several species, including in humans (Buzsaki, 1986, 2015; Buzsaki et al., 1992; Buzsaki et al., 1983; Buzsaki, Logothetis, & Singer, 2013).

SwRs have several distinct features (Buzsaki, 2015) such as temporal coordination patterns between several neurons (Buzsaki et al., 1992). SwRs are also associated with transient changes in the excitability of hippocampus and adjacent structures (Buzsaki, 1986, 2015; Csicsvari, Hirase, Czurko, Mamiya, & Buzsaki, 1999). For the relevance of this thesis, the most important observation of SwRs are patterns of spike trains that occur during them.

Although SwRs have a synchronous appearance, the cells activated during a SwR fire in a specific temporal order. More precisely, the sequential firing patterns observed during SwRs mimics that seen during wakefulness, except that the firing is faster paced (Skaggs & McNaughton, 1996; M. A. Wilson & McNaughton, 1994). In other words, during SwRs neuronal firing patterns associated with wakeful activity are rapidly replayed. It is thought that these patterns of neuronal replay support memory transfer from the hippocampus to neocortical regions during sleep.

It is thought that, during encoding theta oscillations support learning (synaptic consolidation) while later, during sleep and rest, the SwRs support the transfer of information from the hippocampus to the neocortex. In subsequent sections of this thesis I will elaborate on this theory by presenting behavioural evidence in support.

Dopamine

Dopamine was first synthesised in 1910 but it was not recognised as a neurotransmitter until much later (Carlsson, 1993; Hornykiewicz, 1966; Marsden, 2006). Since then, it has been shown to play key roles in behaviours ranging from lactation and movement to reward and addiction. Dopamine is also implicated in multiple neurological and psychiatric diseases including depression, schizophrenia and Parkinson's disease (Knickmeyer et al., 2014; Marsden, 2006).

Parkinson's disease is hallmarked by movement-related symptoms (tremor, rigidity and bradykinesia are core features plus gait imbalance and dystonia) that are driven by loss of dopamine cells in the midbrain (Jankovic, 2008). To pharmacologically treat dopamine depletion, typically either a dopamine agonist or L-DOPA (levodopa) is given orally. L-DOPA, unlike dopamine, can cross the blood brain barrier, and when it does it is centrally converted into dopamine (Figure 3). This is important because the finding that Parkinson's disease is caused by loss of dopaminergic neurons in the brain and that it can be treated by L-DOPA opened up an avenue to study the effects of dopamine depletion and supplementation on several cognitive processes.

Once dopamine has been produced and released into the synapse (Figure 4), it will either excite or inhibit the post-synaptic neuron, depending on the site of action and the receptor type. There are five heterogenous dopamine neuron receptor types, coined D1-5, that may affect cognition and behaviour in disparate ways (Granado et al., 2008; Lazenka, Legakis, & Negus, 2016). These are divided into two families by their properties; D1-like (D1 and D5) and D2-like receptors (D2, D3 and D4) (Civelli, Bunzow, & Grandy, 1993). D1 and D2 receptors are the most prominent and they were the first to have been identified (Carlsson, 1993). Hereafter, D1 and D2 will be used to refer to the families of receptors. These classes differ in genetic, signalling and anatomical properties, as reviewed elsewhere (Beaulieu & Gainetdinov, 2014). In short, they have

two main modes of action: the D1 receptors are stimulatory and get activated by dopamine, and the D2 family are inhibitory.

L-DOPA affects activity of both receptor types indiscriminately, and in most human studies it is difficult to disentangle the effect of different receptors on cognition. Given its widespread pharmacological use, primarily in Parkinson's disease, placebo-controlled trials using L-DOPA or dopamine agonists provide an accessible approach in studying dopamine's effects on cognition.

After reuptake from the synapse, dopamine can be re-used or metabolised into hemovanillic acid or noradrenaline. This is important because noradrenaline affects many cognitive processes, including memory (Kobayashi & Yasoshima, 2001; Swanson-Park et al., 1999). Both dopamine agonists and L-DOPA increase noradrenaline (L. Dayan & Finberg, 2003). As manipulating dopamine levels will inevitably also alter levels of noradrenaline, interpreting experiment outcomes from dopamine's effects on behaviours is difficult.

To further complicate things, dopamine at different quantities appears to affect behaviour in disparate ways. Evidence from Parkinson's disease led to the observation that dopamine replacement therapies could both enhance and impair cognition (Gotham, Brown, & Marsden, 1988). As L-DOPA acts indiscriminately across the brain, dopamine-laden regions relatively unaffected by Parkinson's disease can become overdosed, impairing their function (Cools, Barker, Sahakian, & Robbins, 2001). It therefore seems that there is an optimal dose of dopamine (Chowdhury, Guitart-Masip, Bunzeck, Dolan, & Duzel, 2012; Cools & D'Esposito, 2011; Gjedde, Kumakura, Cumming, Linnet, & Moller, 2010), and dopamine's relationship with cognition can be described by an inverted U-shaped curve (Figure 5).

Dopamine is also substantiated in several brain regions (Figure 6). It is most concentrated in the ventral tegmental area (VTA) and substantia nigra, where the loss of these dopaminergic neurons

underlies the hallmark motor symptoms of Parkinson's disease. However, dopaminergic neurons are also prominent in the hippocampus.

Figure 3: Dopamine synthesis

The production of dopamine begins with the aminoacid tyrosine, which can be exogenously sourced (e.g. through diet or supplements) or produced by endogenously. Tyrosine first resides in the extracellular space but in order to synthesise into dopamine the tyrosine transporter protein introduces it into the cytoplasm. Tyrosine transporters are specific to the dopaminergic synapse. In the cytoplasm, tyrosine is converted into L-DOPA. The DOPA decarboxylase enzyme then breaks L-DOPA down to dopamine. In the dopaminergic synapse, this is the final product. In other synapses, or following re-uptake, dopamine may be further broken down into hemovanillic acid or adrenaline

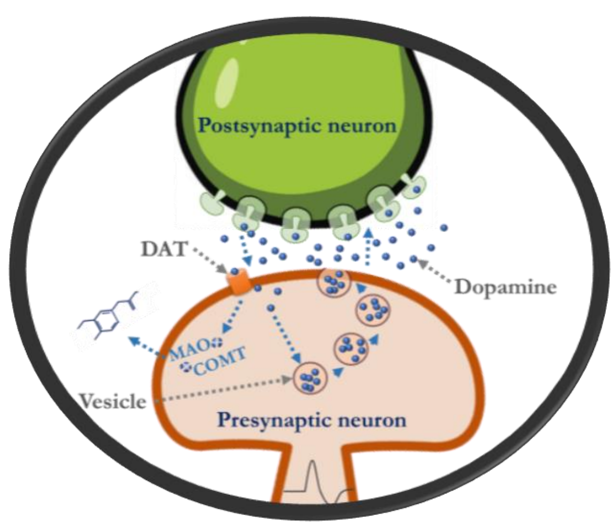


Figure 4: Dopamine and the synapse

An action potential stimulates voltage-gated calcium channels on the presynaptic plasm membrane to activate. An influx of calcium into the presynaptic space is triggered, causing for vesicles to exocytose and release the dopamine into the synaptic cleft. Then, dopamine can either return to the presynaptic cell or bind to the postsynaptic cell. The dopamine binding in the postsynaptic cell can inhibit or exhibit the cell. Dopamine can be transported back into the presynaptic cell for recycling by the dopamine transporter protein (DAT), or it can be metabolised by several enzymes. Prominent examples are monamine oxidase (MAO) and Catechol-O-Methyltransferase (COMT), which break it down into hemovanillic acid.

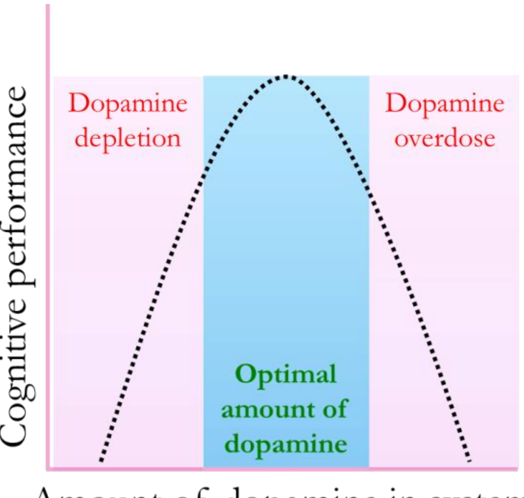


Figure 5: Dopamine and cognition: An inverted U-shape

Dopamine's relationship with cognition can be describes by an inverted U-shaped function where too low or too high a dopamine load can cause impairment.

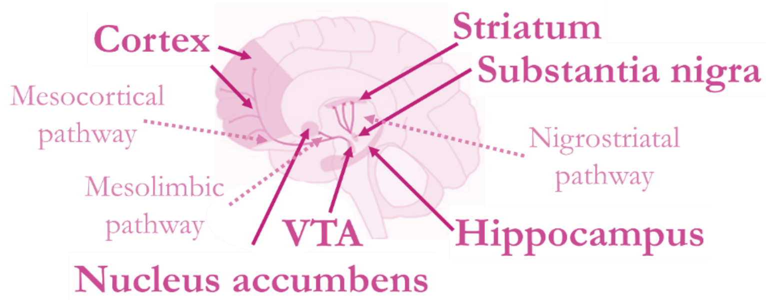


Figure 6: Dopamine distribution and pathways

Dopamine projections connect the midbrain to other regions via several pathways. The largest pathways are the nigrostriatal pathway (substantia nigra to the striatum), the mesolimbic pathway (VTA to limbic structures) and the mesocortical pathway (VTA to cortex). The nigrostriatal pathway is the most prevalent pathway, containing around 80% of all dopaminergic neurons in the brain. It travels from the substantia nigra to caudate putamen and modulates communication between the motor cortex and the cortex via the thalamus. The loss of dopaminergic neurons in the substantia nigra is the landmark pathology of Parkinson's disease. Both mesolimbic and mesocortical pathways originate from VTA. The former connects to the accumbens, ventral striatum and amygdala, and the latter to the prefrontal, the cingulate and the entorhinal cortices. VTA also projects directly into the CA1 of the hippocampus.

Dopamine and the hippocampus

Dopamine is abundant in the hippocampus (Table 1) (Gasbarri, Packard, Campana, & Pacitti, 1994; Gasbarri, Sulli, & Packard, 1997). Dopamine is required for memory related plasticity in the hippocampus (Frey, Schroeder, & Matthies, 1990; Y. Y. Huang & Kandel, 1995; Otmakhova & Lisman, 1996, 1998), and interfering with hippocampal dopamine has been shown to affect both LTP and memory (Axmacher et al., 2010; O'Carroll, Martin, Sandin, Frenguelli, & Morris, 2006). Dopaminergic neurons from the ventral tegmental area (VTA) in the mid-brain project to the hippocampus and back through the VTA-hippocampal loop. From the other side of the loop, it has been shown that rats have enhanced learning in response to dopaminergic simulation of the VTA (Bao, Chan, & Merzenich, 2001).

The VTA-loop is activated by novel or salient information, or cues that predict saliency (J. E. Lisman & Grace, 2005; Wittmann, Bunzeck, Dolan, & Duzel, 2007). The dentate gyrus and the CA3 attempt to predict likely future events based on previously occurred events, and they feed this information into the CA1. Simultaneously, CA1 receives direct cortical inputs about sensory information. In other words, the dentate gyrus and the CA3 help CA1 combine stored information (memories from CA3 and dentate gyrus) with information from the environment. These inputs are compared within the CA1, which signals to the subiculum in response to salient events (Hasselmo & Wyble, 1997; J. E. Lisman, 1999; J. E. Lisman & Grace, 2005). The signal then travels through the subiculum, activating the VTA-hippocampal loop (see Figure 7).

The activation of the VTA-hippocampal loop therefore increases dopamine activation, creating an optimal environment for LTP and therefore supporting the early stages of memory. Storing memories for the longer term, however, benefits from additional processes including sleep.

Subfield	DAT	D1-like	D2-like
Humans			
CA1/2	[neuron]	[neuron][neuron][neuron]	[neuron][neuron]
CA3	[neuron]	[neuron]	[neuron]
Dentate Gyrus	[neuron][neuron]	[neuron]	[neuron]
Monkeys	DAT	D1-like	D2-like
CA1/2	-	[neuron][neuron]	[neuron][neuron][neuron]
CA3	-	[neuron][neuron]	[neuron]
Dentate Gyrus	[neuron][neuron][neuron]	|	[neuron]
Rats	DAT	D1-like	D2-like
CA1/2	[neuron]	[neuron][neuron]	[neuron]
CA3	[neuron]	[neuron][neuron][neuron]	[neuron]
Dentate Gyrus	[neuron][neuron]	[neuron][neuron][neuron]	[neuron][neuron]

Table 1: Dopamine distribution in the hippocampus

Relative density from minimal (|) to significant ([neuron]) to more abundant ([neuron][neuron][neuron]) labelling. Note that labelling technique differences may, at least partially, explain inter-species differences.
DAT = Dopamine transporter.
Based on (Shohamy & Adcock, 2010)

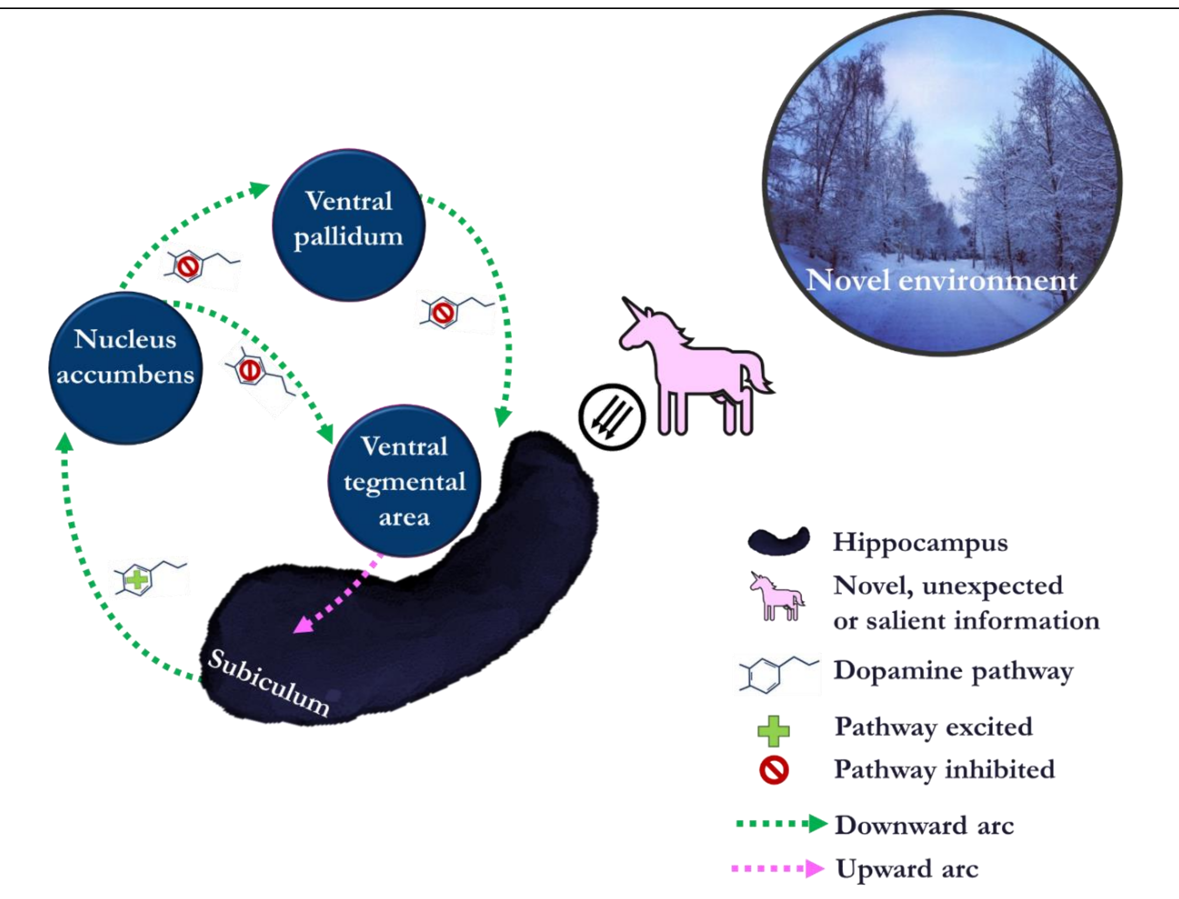


Figure 7: The dopaminergic VTA-hippocampal loop

The VTA-hippocampal loop is activated when the subiculum receives signal from the entorhinal cortex and the CA1 in response to a novel environment. The ventral subiculum will then excite the nucleus accumbens which in turn inhibits the pallidum and the VTA. This inhibition causes an "inhibition of inhibition", making dopaminergic neurons in the VTA more permeable, and more likely to fire in response to salient events (pink unicorn). The VTA then signals to the hippocampus, completing the loop. This theory is presented in full in Lisman & Grace (2005).
VTA = ventral tegmentum.

Long-term potentiation and dopamine

The aforementioned Hebbian (Hebb, 1949) type of plasticity underlying LTP and LTD may not be sufficient to explain the modulation of synaptic plasticity that underpins memory formation. In accordance to this simplistic account, events that co-occur several times should be always remembered. However, it is intuitive that several events or stimuli can co-occur but not be retained in memory unless if they are deemed important. As I will outline later in this chapter, behavioural relevance is pivotal for memory persistence. Therefore, several neurotransmitters and cortical higher-order processes are likely to modulate memory.

One of these is the neurotransmitter dopamine. This model of memory-associated LTP that includes dopamine release was coined the neoHebbian framework by Lisman, Grace and Duzel (2011). Below, I will outline a short account of the mechanism by which dopamine may stimulate protein synthesis.

In the hippocampus, stimulating dopaminergic neurons, and therefore increasing dopamine release in the synapse, increases synaptic transmission by triggering glutamate receptor 1 (GluR1) expression on the cell surface (W. B. Smith, Starck, Roberts, & Schuman, 2005). GluR1 is a type of AMPA receptor. Further evidence has also suggested the role of brain-derived neurotrophic factor (BDNF) in triggering AMPA receptor synthesis in the hippocampus (Lu, Christian, & Lu, 2008). BDNF is a protein that regulates several neurodevelopmental events including dendritic connectivity, maturation and growth of neurons.

BDNF is also involved in activity-dependent synaptic regulation by signalling movement of proteins to promote synaptic maturation and plasticity. Together, BDNF and D1 receptor mediated pathways activate mitogen-activated protein kinase, or MAPKs (Yoshii & Constantine-Paton, 2010). MAPKs are signalling molecules that regulate several cell functions including gene expression. Activation of the MAPK can induce protein synthesis on the postsynaptic cell

membrane. Indeed, both memory and hippocampal late phase LTP can be blocked by interference of MAPK action in mice (Kelleher, Govindarajan, Jung, Kang, & Tonegawa, 2004). Dopamine can also regulate MAPK activity. Stimulating D1 cascades events that lead to increased MAPK activation (Valjent et al., 2005). Therefore, dopamine can influence late-phase LTP by increasing protein expression via activation of protein kinases.

The release of dopamine into the synapse is dependent on higher-order processes including reward, motivation and novelty (J. E. Lisman & Grace, 2005). Therefore, dopamine is likely to be the missing link or one of the missing links that aid memory *selection*, possibly via activation of the VTA-hippocampal loop. Later in this chapter I will discuss how both dopamine and these behaviours are associated with memory persistence in humans and animals.

Sleep

During a full night of sleep, the brain fluctuates between different sleep stages (Figure 8): Wakefulness, Stage 1, Stage 2, slow wave sleep (Stage 3), and rapid eye movement (REM) sleep. Stages 1-3 are also referred to as non-REM (NREM). These stages are characterised by unique neural signatures (Table 2). Stage 2, slow wave sleep and REM are most frequently associated it memory. Slow wave sleep is characterised by high amplitude delta oscillations (slow waves) and high frequency spindles, which can be seen on scalp EEG traces. Slow waves originate from the cortex while spindles are produced by the thalamus. Slow wave spindles predominantly occur during delta depolarisations ('upstates'). During the 'downstates' of the spindles, the hippocampus and surrounding regions produce sharp-wave ripples (SwR). SwRs are high frequency (100 –250 Hz) oscillations resulting from synchronous, rapid, neuronal firing. During some SwRs, patterns of neuronal firing seen during learning are re-activated (Figure 10). These 'replay' events are closely linked with later memory for the associated learning (Molle, Yeshenko,

Marshall, Sara, & Born, 2006). Slow wave oscillations, spindles, SwRs and replay are therefore temporally linked, and they have all been associated with memory (Battaglia, Sutherland, & McNaughton, 2004; Clemens et al., 2007; Siapas & Wilson, 1998).

Spindles and SwRs are also seen during REM and Stage 2, and SwRs are also seen during wakeful rest. In the following sections I will show that these sleep-events are relevant for memory persistence, particularly during slow wave sleep.

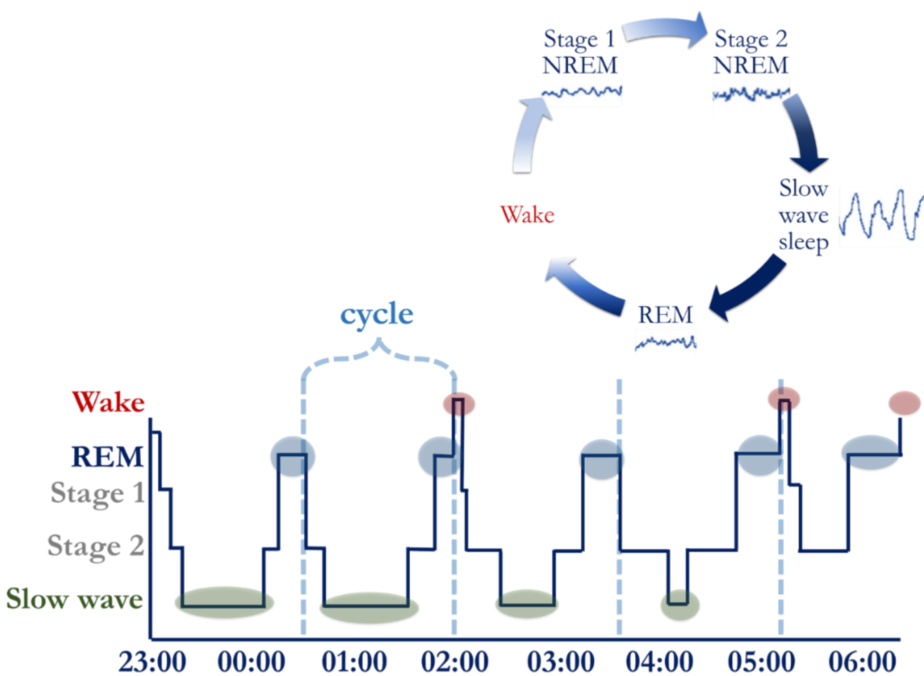


Figure 8: Sleep cycles and stages

Sleep stages vary in ultradian cycles lasting around 1-2 hours. These cycles are characterised by disparate neural oscillatory signatures. A hypothetical example of a sleep hypnogram demonstrating how proportionately more time is spent in slow wave sleep (green) during the first half of the night, while REM is more dominant later (blue). The transitions between cycles begins when a sustained period of REM ends. Note that this is an optimal schematic of sleep. It is possible for someone to go directly from Wakefulness to Stage 2 or even REM or slow wave sleep – especially if wakefulness is a short awakening at night.

Sleep stage	Frequency (Hz)	Amplitude	PSG signal	Behaviour
Drowsy	9-11	Low	Alpha in occipital channels, blinks.	Eyes closed, relaxed but awake
Stage 1	4-8	Low	Theta. Slow eye movements on EOG and elevated EMG	Very light sleep, easy to wake up
Stage 2	11-16	Slight elevation	Theta, K complexes and spindles. Absent EOG, reduced EMG	Light sleep
Slow wave sleep	1-4	High, at least 75µV	Delta dominates. Spindles continue. Absent EOG, reduced EMG	Deep sleep, Difficult to wake up
REM	9-11	Low	Mostly theta. As awake but with sawtooth waves (2-6Hz). Low EMG but rapid movements in EOG	Atonia but eye movements are present.

Table 2: Human sleep stages
Different sleep stages are characterised by different patterns of neural events. The frequency and amplitude band characteristics for each sleep stage are subject to individual variation.

PSG = Polysomnogram
EMG = electromyogram
EOG = electro-oculogram
REM = Rapid Eye Movement

Note that previously there was also Stage 4. This is now no longer scored and instead Stage 3 / slow wave sleep includes both former stages 3 and 4.

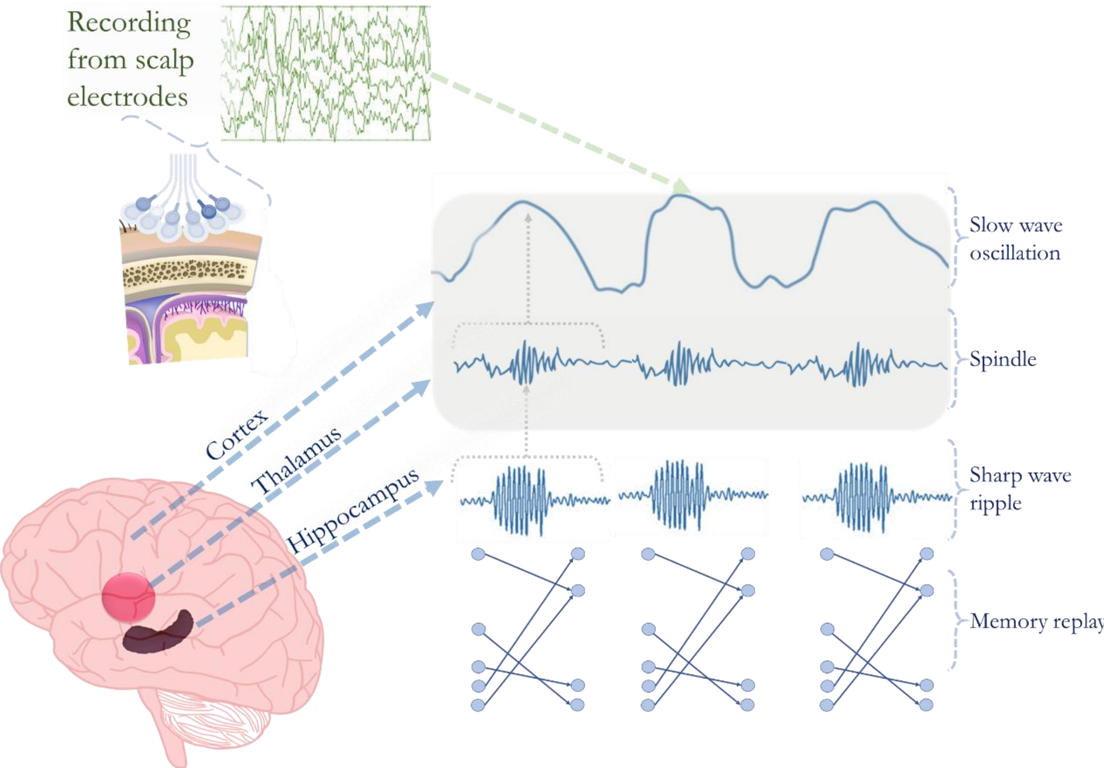


Figure 9 The relationship between slow wave oscillations, spindles and ripples
During slow wave sleep, the neocortex generates slow wave oscillations. Spindles take place during the up-states of these oscillations (arrow connecting spindle to slow wave), while ripples are associated with the down-states of spindles (arrow connecting ripple to spindle). **Note that the size of the ripples is magnified here for illustration**. During ripples, the memory engram is replayed. Slow wave oscillations and spindles can be seen on scalp EEG (green shade) but sharp wave ripples and replay events require intracranial access.
The sleep events in this figure are not to scale.

Dopamine and circadian rhythms

The relationship between sleep and dopamine offers a rich area of study, because dopamine is intimately involved in the regulation of sleep-wake cycles. Animal studies show that firing of dopaminergic cells is different during wakefulness compared to sleep, with more bursting activity and enhanced dopamine release in the nucleus accumbens and forebrain during wakefulness (Eban-Rothschild, Rothschild, Giardino, Jones, & de Lecea, 2016; Monti & Monti, 2007). Similarly, enhancing overall levels of dopamine causes changes in sleep and wakeful states. Central administration of a D1 or D2 agonist reduces REM and slow wave sleep and increases behaviours associated with wakefulness (Isaac & Berridge, 2003), while conversely, in a macaque model of Parkinson's disease, central D1 agonist increases REM duration and daytime wakefulness (Hyacinthe, Barraud, Tison, Bezard, & Ghorayeb, 2014).

The traditional view held that dopamine promotes wakefulness and does not play a role in sleep processes (Jones, Bobillier, Pin, & Jouvet, 1973; M. M. Lima, Reksidler, & Vital, 2008). Instead, dopamine is now widely accepted to play a role across circadian cycles –particularly in regulating REM sleep. The electrophysiological trademarks of REM closely resemble those of wakefulness: during REM, as during wakefulness, theta activity originating from the brain stem and projecting to cortical regions is abundant. Some of these projections are dopaminergic (M. M. Lima, Reksidler, et al., 2008; Saper, Scammell, & Lu, 2005) and dopamine levels in rats are elevated both during wakefulness and REM compared to slow wave sleep (Lena et al., 2005).

Another line of evidence which shows that increased dopamine levels promote wakefulness comes from targeted REM deprivation. In these paradigms, volunteers (or animals) sleep otherwise normally but are woken up at each REM onset. A REM rebound effect – where the proportion of total sleep time spent in REM is increased – follows sleep deprivation, selective REM deprivation or stressful events (Suchecki, Tiba, & Machado, 2012; Vogel, 1975). In rats,

REM deprivation causes an increase in striatal dopamine and impairs cognitive performance (Proenca et al., 2014). In drosophila, sleep deprivation causes short-term memory deficits but suppression of D1 activity can rescue the cognitive deficits (Seugnet, Galvin, Suzuki, Gottschalk, & Shaw, 2009). Blocking D2 receptor activity after REM deprivation can also reduce subsequent REM (M. M. Lima, Andersen, et al., 2008). These studies suggest that increased dopamine following REM deprivation may impair cognitive performance during subsequent wakefulness and induce REM during subsequent sleep.

To further complicate matters, different dopamine receptors have disparate effects on wakefulness. Downregulating or impairing either of the two most expressed receptor types, D1 or D2, causes somnolence in mice (Cromwell, Berridge, Drago, & Levine, 1998; Kelly et al., 1998; Vallone et al., 2002; Zweifel et al., 2009), while D3 downregulation increases alertness (Accili et al., 1996; Xu et al., 1997). Findings relating to D4 or D5 receptors have been inconclusive or yielded no differences (Monti & Monti, 2007).

Dosage may also introduce a biphasic effect of dopamine on sleep. For example, smaller doses of central D2 agonists apomorphine or bromocriptine were associated with increased REM and slow wave sleep in rats, while larger doses had the opposite effect. The D2 agonist pergolide also had a biphasic effect on slow wave sleep and wakefulness, but it inhibited REM at all given doses (Monti, Hawkins, Jantos, Dangelo, & Fernandez, 1988; Monti, Jantos, & Fernandez, 1989).

However, a word of caution is required when interpreting rodent studies: rodent sleep is ultradian rather than circadian in nature, so differences between human and rodent sleep/awake cycle regulation are likely.

It is difficult to piece together a unitary role of dopamine in sleep from the animal literature. Dopamine's effects on sleep seem to depend on the receptor type, dose and organism. However,

it is possible that these findings are conflated by small sample sizes and biased reporting of scientific studies.

Dopamine and sleep in humans

Parkinson's disease, which is characterised by accelerated dopamine neuron loss in the midbrain (Braak et al., 2003), is associated with multiple sleep-related dysfunctionalities (Swick, 2012; Videnovic & Golombek, 2013). Increased daytime somnolence and sleep attacks are side effects of dopamine replacement therapies (Homann et al., 2002). These attacks are characterised by sudden sleep onset, affecting around 6.6% of patients, with those on higher doses being at elevated risk (Montastruc et al., 2001; Tan et al., 2002). While the exact cause of sleep attacks is unknown, they are a side effect of all dopamine replacement drugs in Parkinson's disease patients and therefore likely to reflect some dopamine dysfunction. This is somewhat paradoxical given dopamine promotes wakefulness.

Sleep impairments in Parkinson's disease also originate from the same mesocorticolimbic regions that promote wakefulness (Rye, 2004) and they are increased with chronic dopamine replacement therapy (Nausieda, Weiner, Kaplan, Weber, & Klawans, 1982). Up to 90% of Parkinson's patients have co-morbid sleep disturbances, with the most common complaints being insomnia, REM behaviour disorder, sleep apnoea, restless leg syndrome and excessive day-time somnolence (Gagnon et al., 2002; Kales, Ansel, Markham, Scharf, & Tan, 1971). Around 15-47% of Parkinson's patients have REM behavioural disorders (Gagnon et al., 2002). These are characterised by REM episodes during which atonia – or the lack of muscle tone characteristic of stage REM (Table 2) – is absent. Instead, patients can "act out" their dreams during sleep.

An interesting observation in Parkinson's disease patients with REM behavioural disorders is that the Parkinsonian motor control symptoms, such as impaired smoothness and speed of

movement or speech articulation, are absent during these episodes (De Cock et al., 2007). While to our awareness this has not been directly studied, a possible explanation is that during REM increased dopamine release in substantia nigra ameliorates motor control symptoms mimicking the effects of dopamine replacement therapy.

There have been few efforts to study how L-DOPA and dopamine agonists acutely alter sleep. An early observation was that unmedicated Parkinson's patients had later sleep onset and frequent arousals during nocturnal sleep (Kales et al., 1971). These problems were mainly associated with slow wave sleep (Stage 3) with REM being relatively unaffected. Onset of dopamine replacement therapy by L-DOPA was associated with changes in REM – with some patients having longer and some shorter REM durations. These effects returned to baseline with chronic treatment after 2 weeks. In 4 spouse-controls L-DOPA caused an acute, small, increase in REM but no other changes. Due to the discrepant findings and a small sample, it is difficult to draw conclusion from this study.

In another study, nocturnal L-DOPA did not enhance sleep in patients with Parkinson's disease following a period of time OFF dopamine replacement (Wailke, Herzog, Witt, Deuschl, & Volkmann, 2011). It is possible that there was no difference OFF L-DOPA due to insensitivity caused by chronic dopamine replacement treatment. In contrast, others have reported increased daytime somnolence with both L-DOPA and pramipexole (a D2-agonist)(Contin et al., 2003; Pal, Bhattacharya, Agapito, & Chaudhuri, 2001). While L-DOPAs effects in modulating sleep in healthy humans is less clear, in rats, L-DOPA treatment has a clear benefit in restoring circadian rhythm impairments (Boulamery, Simon, Vidal, & Bruguerolle, 2010).

Not many studies have attempted to disentangle the effect of dopamine-medication on sleep in healthy individuals, but those that exist suggest that increased dopamine activity impairs sleep architecture. In young healthy men, a single dose of the D2 agonist pramipexole delivered

nocturnally reduced time in slow wave sleep and in REM and increased time in Stages 1-2, without affecting total sleep time (Feld, Besedovsky, Kaida, Munte, & Born, 2014). In other studies with healthy young adults ropinirole, also a D2 agonist, reduced sleep onset time (Ferreira et al., 2002) and pramipexole increased daytime somnolence at 3.5h and 5.5h delays from administration (Micallef et al., 2009).

Similar findings have been found with acute pramipexole in restless legs patients, although in this cohort chronic use improved sleep (Saletu, Anderer, Saletu-Zyhlarz, Hauer, & Saletu, 2002). In this patient group, clonazepam, which alters dopamine activity, improves sleep efficiency and quality (Saletu et al., 2001), but it is more likely that clonazepam modulates sleep through GABA rather than by increasing dopamine activity.

In humans, sleep deprivation is likely to reduce dopamine release. In line with this, sleep deprivation in Parkinson's disease has been shown to ameliorate motor symptoms caused by reduced dopamine in the midbrain – possibly by making patients hyperresponsive to dopamine-increasing medications (Bertolucci, Andrade, Lima, & Carlini, 1987; Reist, Sokolski, Chen, Coskinas, & Demet, 1995). In healthy adults, sleep deprivation decreased D2 binding in the ventral striatum as measured by [^{11}C]-raclopride binding potential in a positron emission study (Volkow et al., 2012; Volkow et al., 2008). The magnitude of the dopamine suppression was also associated with increased sleepiness. Note that this is at odds with the findings that REM deprivation increase striatal dopamine in rodents (Proenca et al., 2014).

While most of the available evidence suggests that dopamine only regulates wakefulness and REM, it may be implicated in slow wave sleep as well. First, slow wave sleep duration is reduced in Parkinson's disease (Kales et al., 1971) (Wailke et al., 2011). Second, this decrease can be partially but not fully rescued with dopamine replacement therapy (Diederich, Paolini, & Vaillant, 2009). Third, in healthy humans, glucose metabolism and therefore brain activity in midbrain and

forebrain regions expressing dopamine and in the hippocampus is increased during non-REM sleep (Nofzinger et al., 2002). Fourth, Feld et al (2014) found that a dopamine agonist reduced slow wave sleep. Finally, as I will outline in Chapter V (pg. 158), dopamine may play a role in memory-related plasticity during slow wave sleep.

The effect of dopamine-enhancing agents can be paradoxical, with low doses possibly acting as sedatives and high doses having the opposing effect and promoting wakefulness (Monti, Hawkins, Jantos, D'Angelo, & Fernandez, 1988). The relationship between dopamine in and sleep is further complicated by the disparate effects of different receptor types. It is possible that different patients have different patterns of dopamine loss across the brain due to disease processes, age, and unknown factors. Acute and chronic effects of dopamine replacement therapies are likely to yield different effects as well. For this reason, studies in healthy populations are pivotal.

Together these findings suggest that dopamine's relationship with sleep is complex, and it is difficult to predict what, if any, acute effects dopamine-enhancing medications may have in healthy people. As outlined above, there is overwhelming evidence that dopamine is implicated in the maintenance of wakefulness. The available literature also suggests that dopamine, particularly at D2 receptors, is required for REM sleep. Yet, much of the evidence comes from animal and patient studies and relatively little is known about the effects of L-DOPA or D2 agonists/antagonists in healthy individuals or in prodromal disease states. Also, there is little evidence of dopamine affecting slow wave sleep, a stage which is also impaired in Parkinson's disease (Wailke et al., 2011).

Exploring dopamine, hippocampus and sleep in humans

Due to the complex nature of the interactions between hippocampus, dopamine and sleep, to fully begin to understand dopamine's role on human cognition requires integrating evidence from several methodological modalities.

Neuroimaging, such as magnetic resonance, lesion studies and EEG can be used to study the function and structure of the hippocampus in humans. Direct recordings can also be obtained from patients undergoing intracranial surgery, and while sharp wave ripples and replay are not visible in scalp EEG, sleep spindles can be used as a proxy to study them.

Dopamine in humans

Common approaches to study dopamine are:

1. **Genetics**: Differences in cognitive performance by gene-load of dopamine-activity-altering genes, e.g. , e.g. (de Frias et al., 2004).
2. **Patient studies:** Cognitive changes in patients that have either increased (e.g. Schizophrenia) or decreased (e.g. Parkinson's disease) levels of dopamine, e.g. (Dubois & Pillon, 1997).
3. **Drug interventions:** Assessing changes associated with dopamine-level altering medications in healthy controls and patient populations, e.g. (J. P. Grogan et al., 2018; Shohamy, Myers, Geghman, Sage, & Gluck, 2006) and this thesis.
4. **Neuroimaging**: Positron emission tomography allows to detect individual variability in dopamine binding. Functional magnetic resonance and electroencephalograms can also be used to study cognition-related variability in neural responses in relation to genetics, pharmacological manipulations or disease states, e.g. (Salami et al., 2019).

In the following section, I will discuss these topics in the context of procedural human long-term memory.

Long-term memory

Long-term memory refers to memory that persists from minutes to lifetimes. According to the multi-store model of memory (Atkinson & Shiffrin, 1968), before information enters long-term storage, it is first processed by the sensory system, which has little storage capacity and from where it passes through rapidly. If this information is attended to, it then progresses into short-term memory. The duration of these memories is only a few seconds, and the short-term memory system can only hold 7 ±2 items at any given time. A proportion of these memories are retained for a longer duration as they enter long term memory.

In contrast, the levels of processing model (Craik & Tulving, 1975) proposes that instead of having distinct storage systems, memories are processed on a continuous scale ranging from shallow to deep processing. A combination of the two models is likely to hold true. For information to flow into long-term memory, it needs to be held in short-term memory first, but it is unlikely that there is a hard boundary between the two processes (Figure 10).

A healthy memory system cannot retain every piece of information encountered in the environment. Even if the storage capacity were limitless, the behavioural utility of non-selective memory is questionable. For example, it is not necessary to remember details about noises of traffic when you navigate to a new location, but the route itself is salient. The memory system instead prioritises salient memories over irrelevant information. In this chapter, I will discuss different memory stages and processes considering their involvement in selecting memories – not all information is equally encoded, consolidated, retrieved and forgotten

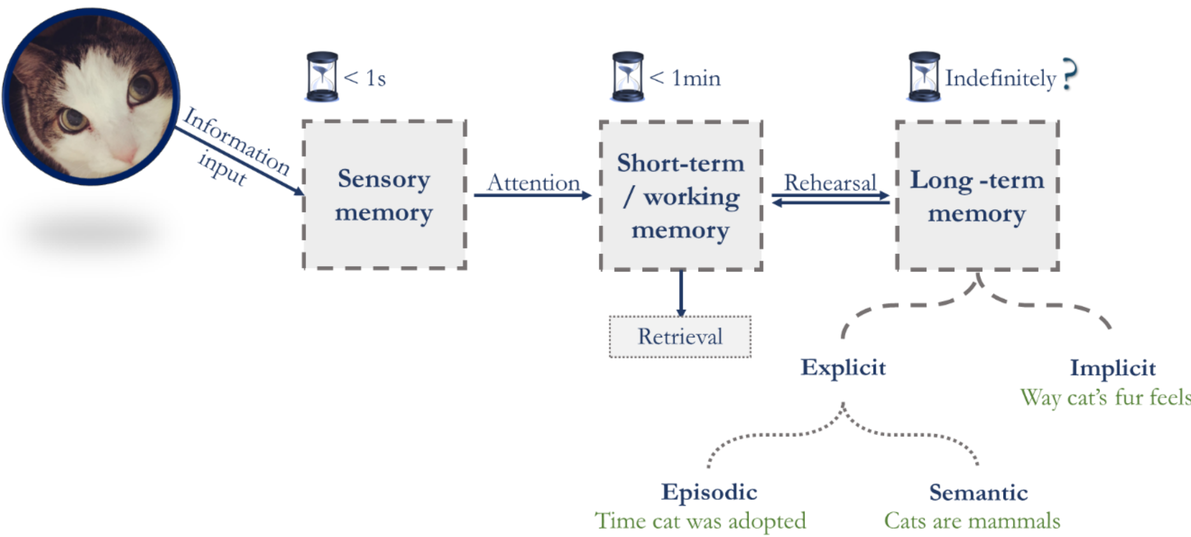


Figure 10: Types of memory

Before entering long term memory, information is first processed by sensory memory, which lasts only some fractions of a second. After that, information flows into short-term storage, also called working memory. The storage capacity here is only up to a minute after which information is being transferred into long term storage.
Long term memories can roughly be divided into explicit (or declarative) and implicit (or procedural) memories. This division reflects the level of conscious awareness one can have of the information; explicit memories refer to information you can name and describe, while implicit memories consist of information that is more procedural. Explicit memories can further be divided into episodic and semantic, where the former refers to information that is personal and unique to the individual, and the latter to facts and concepts. (Atkinson & Shiffrin, 1968; Craik & Tulving, 1975)

Making memories

Encoding, which is heavily reliant on the hippocampus, is the initial step in explicit long term memory formation (Scoville & Milner, 1957). The idea that memory encoding involves transcribing the external experience into an internal representation of the world was first suggested over a century ago by Richard Semon. He coined this internal representation as the 'mnemic trace' or the engram (Semon, 1909). He suggested that an engram is born at encoding and reactivated when memories are accessed later. While several aspects of his theory – such as engrams being inherited – did not survive the test of time (Peitikainen, 2007), successfully encoded memories do create biophysical or biochemical changes in response to the external environment.

During encoding, explicit memories are transcribed into an engram within the hippocampus (Figure 11). This representation takes the form of synchronously activated cell assemblies, and as these cells fire together the connections between them become stronger during subsequent memory processes, see page 39 (Manahan-Vaughan & Braunewell, 1999; Tonegawa, Liu, et al., 2015; Tonegawa, Pignatelli, Roy, & Ryan, 2015). The hippocampus is likely not required for procedural encoding nor is it the only site implicated in explicit memory encoding. Instead, widespread cortical networks are also involved at encoding (Kensinger, Clarke, & Corkin, 2003; Squire, Genzel, Wixted, & Morris, 2015).

There is little uncertainty about the involvement of the hippocampal formation in explicit encoding. In short, in humans, the extent of recruitment of the hippocampus during encoding predicts later memory performance. This subsequent memory effect, or difference due to memory, can be studies using paradigms where participants' brain activity is measured during learning. A common finding is that hippocampal activation predicts subsequent memory. This effect can be seen in intracranial (Elger et al., 1997; Long, Burke, & Kahana, 2014) and scalp

EEG (Kamp, Bader, & Mecklinger, 2017), and in functional magnetic resonance imaging (Hayes, Hayes, Williams, Liu, & Verfaellie, 2017; Kim, 2019). Note that in scalp EEG signal source localisation is difficult and one cannot be certain where the effects in those studies originate from.

The subsequent memory effect not only demonstrates the intimate link between neural activity at encoding and subsequent memory, but it also indicates that not all information is equally encoded. Instead, encoding is selective, and strength may vary with information characteristics such as saliency.

Saliency-tagging

Salient or rewarded information is typically prioritised for memory. I will refer to this saliency-driven difference in memory strength as the tagging effect. At encoding, contextual information or previous experiences influence tagging of some information over others. During subsequent memory stages, this tag guides the selection of memories to be stored. A possible anatomical underpinning of the tag is likely within the hippocampus. Newly synthesised proteins induced by salience – or tags – optimize the environment for synaptic changes that underlie memory persistence (Ballarini, Moncada, Martinez, Alen, & Viola, 2009; Frey & Morris, 1997a, 1997b; Redondo & Morris, 2011). Thus, the synaptic changes that underlie memory persistence (page 39) effectively capture this saliency tag.

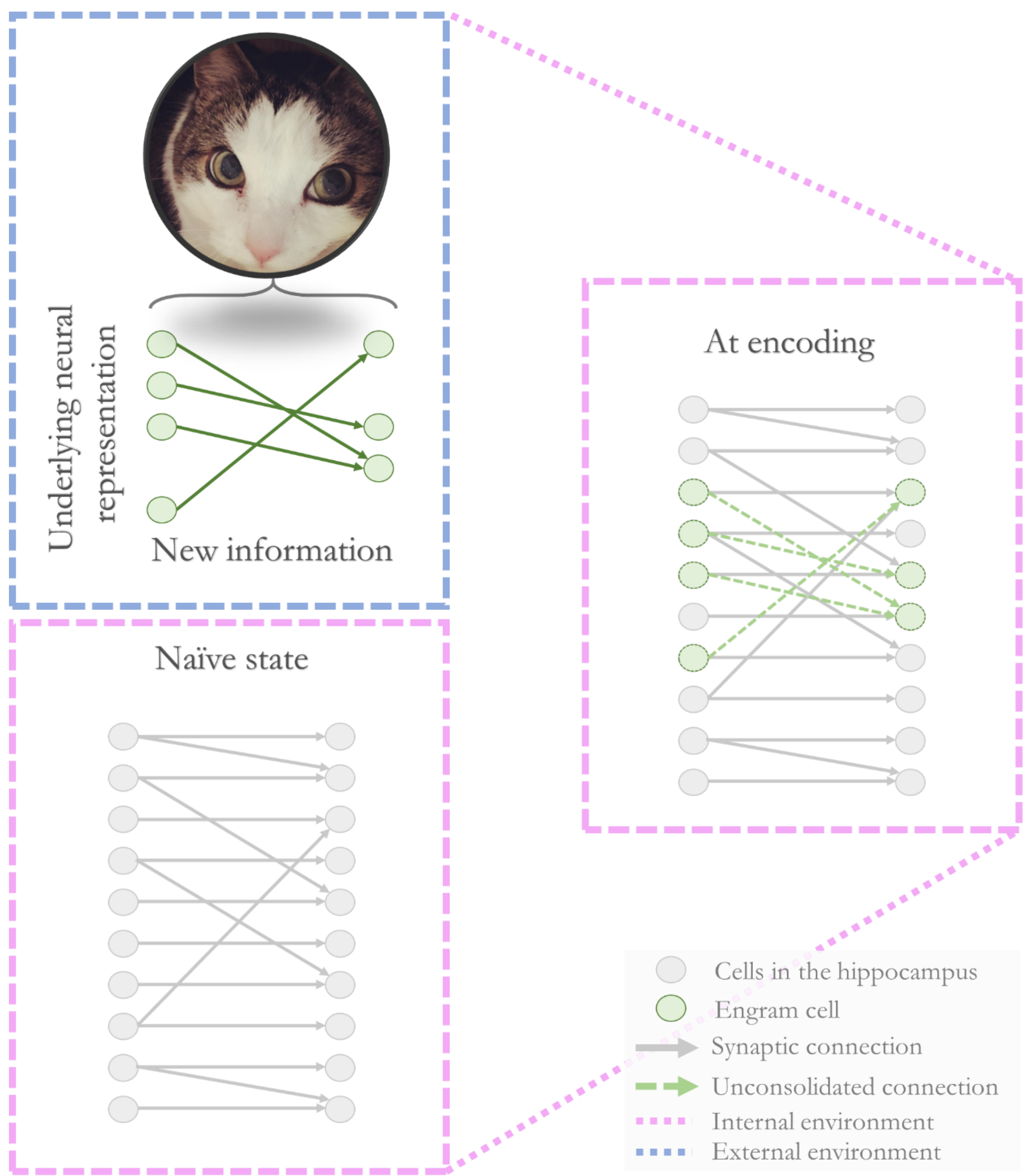


Figure 11: Engrams and encoding

Information (cat) in the external environment (top left) influences the state of "naïve" hippocampal cells (bottom left). Encoding creates a unique neural representation corresponding to the newly acquired information (right).

The subsequent memory effect provides a platform for studying tagging. Human intracranial recordings have shown that theta activity in the hippocampus and cortex during encoding

predicts subsequent memory performance (Long et al., 2014). Cortical theta refers to low frequency scalp EEG oscillations that are often associated with memory encoding. Theta rhythms can also be recorded from the hippocampus where they may be linked to learning and memory (Hasselmo, 2005). When theta activity is induced in the rat prefrontal cortex during encoding, the animal has enhanced memory for behaviourally salient information (fear conditioning), but not for non-salient information (Jarovi, Volle, Yu, Guan, & Takehara-Nishiuchi, 2018).

These tagging processes may involve dopamine and the dentate gyrus. Dopamine is one of the most abundant neurotransmitters in the human nervous system, and its role in reward and saliency has been well established in animal and human studies (Bromberg-Martin, Matsumoto, & Hikosaka, 2010; Schultz, 1998). Photostimulation of dopamine in the dentate gyrus induces a reduction in synaptic connectivity and reduces both theta oscillations and subsequent learning. A behavioural reward yielded similar effects for upcoming learning trials (Du et al., 2016). Learning was enhanced before a reward and impaired after. These findings suggest that dopamine in the dentate gyrus may improve encoding of salient information by shielding it from interference. Tagging therefore continues to bias memory selection during subsequent memory stages.

Saliency can also tag engrams retroactively. When participants learn neutral information which is subsequently associated with rewards, the previously neutral memories benefit from saliency-tags (Braun, Wimmer, & Shohamy, 2018; Patil, Murty, Dunsmoor, Phelps, & Davachi, 2017). After learning salient information, functional connectivity during rest between the ventral tegmentum and the hippocampus is increased, and the magnitude of this increase predicts persistence of salient but not non-salient information (Gruber, Ritchey, Wang, Doss, & Ranganath, 2016).

In this study (Gruber et al., 2016), functional connectivity was measured from the two regions using resting state fMRI. In this kind of a paradigm, two regions are considered to be

functionally connected when the blood oxygen level dependent signal, an indirect measure of brain activation, from these regions follows a similar timeline. In other words, when brain activity in two regions is temporally correlated, they are considered to be functionally connected. The ventral tegmentum and the hippocampus are also structurally, or synaptically, connected. I.e. there are structural pathways supporting communication between the two regions (J. E. Lisman & Grace, 2005).

Given functional connectivity between the dopaminergic ventral tegmentum and the hippocampus is associated with memory persistence for salient information, it is likely that this functional connection supports memory through dopamine.

Therefore, the tagging effect is likely a result of neuronal dynamics during and after encoding biasing salient encoded items to be preferentially consolidated in the long term. Given that the neuroanatomical basis of this learning seems to rest on the ventral tegmentum and the hippocampus, it is likely modulated by dopamine.

Maintaining memories

Once explicit memories are encoded, consolidation is required to make them last. The dual processing theory of memory suggests that at least two complementary routes to memory persistency are at an interplay: systems and synaptic consolidations (Mcclelland, Mcnaughton, & Oreilly, 1995). Both processes are, at least partially, supported by the hippocampus and are primarily studied along the CA1/3 Schaffer collaterals. During initial stages of memory storage, the hippocampus supports rapid learning by encoding information into neural representations with immediate effect (Figure 12). Early synaptic consolidation processes begin rapidly, within seconds from learning (Bonstrup et al., 2019). Meanwhile, systems consolidation refers to a process whereby information is moved from within the hippocampus to the neocortex for long-

term storage. The hippocampus works as a switchboard during this process, signalling repeats of the activation present at encoding.

Memory and long-term potentiation

Over half a century ago, neurons and synapses were first suggested to be modifiable by experience. The Hebb rule (Hebb, 1949), or the "neurons that play together stay together" rule, states that the strength of a synapse is dependent on the interaction between the presynaptic and postsynaptic neurons. In short, if cell X excites cell Y, their connection will be strengthened and maintained.

The study of long-term potentiation (LTP) sprung support to Hebb's theory. In the CA1, LTP follows Hebbian principles (Wigstrom & Gustafsson, 1986): LTP requires both presynaptic input (cell X response) and postsynaptic depolarisation (activation of cell Y in response to cell X activity). There is little doubt about the importance of Hebbian LTP for memory persistence (T. V. P. Bliss & Collingridge, 1993; Lomo, 2003). However, from anecdotal experience information in the environment can co-occur repeatedly but it is not until this information bears some importance/salience that we learn associations. This observation can be explained by the 'neoHebbian' framework first proposed by Lisman et al (2005, 2011).

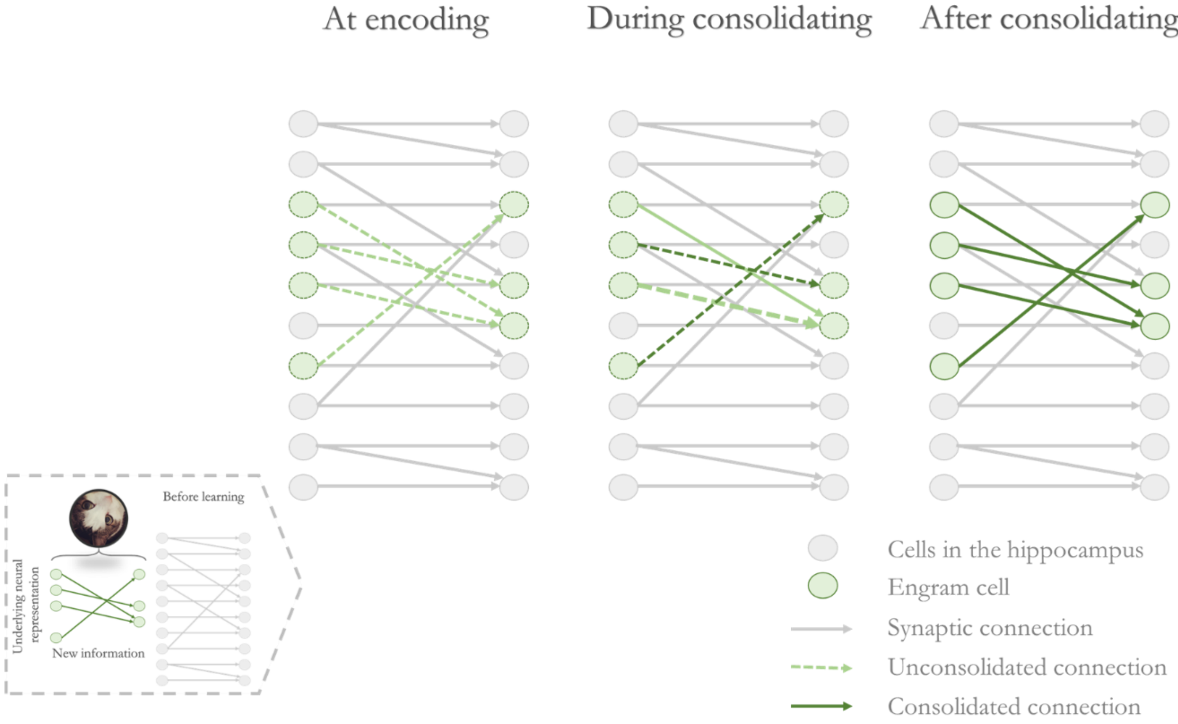


Figure 12: Early consolidation

During early consolidation processes, the strength of memory-associated synapses becomes gradually strengthened over time. Later, during systems consolidation (not shown here), these connections within the hippocampus weaken as information is stored in a more widely distributed network.

NeoHebbian framework

What might signal salience at the synapse? According to the neoHebbian view, for LTP to *persist,* in addition to Hebbian conditions, a release of dopamine is required (J. Lisman et al., 2011; J. E. Lisman & Grace, 2005). Importantly, early LTP does not seem to be dependent on dopamine, while late LTP is – suggesting dopamine plays a role in memory persistence. Dopamine's role in late LTP has been demonstrated several times (Edelmann & Lessmann, 2013; Granado et al., 2008; Hamilton et al., 2010; Y. Y. Huang & Kandel, 1995; Lemon & Manahan-Vaughan, 2006; Matthies, Schroder, Hollt, & Krug, 1997; Otmakhova & Lisman, 1998; Papaleonidopoulos, Kouvaros, & Papatheodoropoulos, 2018) and these have been outlined earlier in this chapter.

These cell-level dopamine-driven changes in synaptic strength are directly associated with behavioural changes in memory persistence. Therefore, dopamine is associated with salience and it can induce late LTP in the hippocampus, but it is not required for early LTP.

Abundance of evidence to support the link between dopaminergic LTP and memory comes from animal studies. For example, an injection of a D1 agonist preceding encoding impairs memory when performance is tested 30 minutes later but memory is improved, compared to control rats, when tested after a 12-hour delay (Floresco & Phillips, 2001). As increased dopaminergic activity would have still been present at the 30-minute test, these data do not reveal whether dopamine impairs encoding or retrieval. However, they do suggest that D1 activity is important for consolidating memories long term, over a period that coincides with sleep.

These findings are in line with other research in animals (Rossato, Bevilaqua, Izquierdo, Medina, & Cammarota, 2009) that have found that dopamine controls persistence of long-term memories. An injection of a D1 antagonist into dorsal hippocampus impairs long-term memory when injected 12 but not 9 hours after learning. In another group of animals, a D1 agonist to the hippocampus enhanced memory 12 but not 9 hours after learning. These effects were associated with changes in protein synthesis in the ventral tegmental area, suggesting that the VTA-hippocampal loop is associated with memory persistence. Together these findings suggest that the timing of dopamine in relation to memory is crucial: it may impair encoding and enhance consolidation.

Support from humans

At least one study has found supporting evidence of the neoHebbian model in humans. Recordings from Deep Brain electrodes inserted for deep brain stimulation from the substantia nigra in 23 Parkinson's disease patients showed that 25% of neurons were novelty sensitive,

indicating their role in memory (Kaminski et al., 2018). These, mostly dopaminergic, neurons activated ~500ms after stimulus onset during correct trials in a recognition memory test. The activity changed at each trial and it was predictive of recognition accuracy. Moreover, spiking patterns were temporally associated with prefrontal theta oscillations and the functional connectivity between substantia nigra and theta also predicted recognition memory accuracy.

In a previous study neurons in the hippocampus have been shown to activate in response to novelty in this same task (Rutishauser, Mamelak, & Schuman, 2006). The results from these studies lend first-in-human direct support for the neoHebbian model and the VTA-hippocampal dopaminergic loop's involvement in memory (J. Lisman et al., 2011).

From synapse to system

Memory traces do not rely on the hippocampus indefinitely. Instead, the recruitment of wide-spread cortical networks is also required for memories to persist. This memory migration into the cortex is referred to as systems consolidation. During this process, information becomes reorganised and more widely distributed in the cortex while hippocampal involvement is weakened (Frankland & Bontempi, 2005). Initial accounts posited that memories are first stored in the fast-learning hippocampus from where they *transfer* to the neocortex, which is a slow learner. Memories then become wholly independent of the hippocampus. However, memories do not literally migrate outwith the hippocampus to the neocortex. Instead the relative weighting of contribution of different regions in the memory network are changed over time. Both the hippocampus and the neocortex are likely to be involved in episodic memory persistence from the onset of encoding to late consolidation and possibly retrieval (Genzel et al., 2017; Squire et al., 2015).

Yet, the weightings between hippocampal and neocortical memory representations do change over time. When participants learn a memory task and they are tested later in an fMRI scanner,

the duration between learning and test is predictive of recruited brain networks (Takashima et al., 2009). If tested 15 minutes later, the hippocampus is more heavily recruited during test, and the connectivity between the hippocampus and cortical regions is increased compared to a 24-hour test. In comparison, at a 24-hour test, functional connectivity within cortical regions is increased. While these findings are congruent with the current understanding of memory maintenance, this study did not account for qualitative differences in encoding strength. The memory task at 15 minutes would have been easier than it was at 24 hours, which could, in part, explain the results. Encoding strength is tightly interlinked with consolidation speed.

Indeed, the position that neocortical learning is universally slow-paced has been challenged. Initially in simulations that showed that information congruent with previous experience can be 'fast mapped' to the cortex rapidly (McClelland, 2013), and soon after experimentally in humans (Coutanche & Thompson-Schill, 2014). Further evidence comes from a virtual navigation task. During initial exploration of the virtual environment, efforts to navigate relied more heavily on hippocampal-cortical connectivity, consistent with early phases of consolidation. With prolonged exploration of the virtual environment, less hippocampal activity was required, and processing became reliant on the connectivity between cortical networks instead (Brodt et al., 2016). This is analogous to systems consolidation but could be seen in a single fMRI session in this study. In other words, these findings support the notion that rapid systems consolidation is possible during repeated exposure.

Similar findings have been seen in a verbal memory task where participants studied a word list seven times with each repeat followed by an immediate recall (Himmer, Schönauer, Heib, Schabus, & Gais, 2019). Rapid change-over between memory systems was observed again. The effect persisted after a full night of sleep. Interestingly, if volunteers spent a similar amount of time awake instead, the hippocampus had "forgotten" about the supposed systems consolidation that took place during learning. There are two main implications from these studies. First,

repeated testing seems to provide a fast route for systems consolidation, and second, systems consolidation may require sleep to stabilise.

In sum, initial consolidation begins rapidly after learning and is supported by synaptic plasticity. Behavioural tagging during learning may bias the synaptic environment in way that enhances the likelihood of this plasticity. Systems consolidation is typically slower and takes place later, but it can be accelerated by previous information or repetition. Intentional awake repetitions of learnt information, such as repeat study or exploration, provide a fast route for consolidation, possibly by reactivating neuronal assemblies present at learning. This may also be supported by previous knowledge: presumably repeated information may be deemed as more salient and therefore become tagged for prioritised storage.

Dopamine, encoding and consolidating

Dopamine's roles during different memory stages are unclear. While some have found dopamine to be selectively involved in consolidation (Bethus, Tse, & Morris, 2010), other evidence has linked it with active forgetting, working memory and encoding (Berry, Cervantes-Sandoval, Chakraborty, & Davis, 2015; Cools & D'Esposito, 2011; Du et al., 2016). As memory persistence is modulated by novel events both before and after learning, dopamine is likely involved not just in the consolidation but also in the encoding stage.

Dopamine and memory in Parkinson's disease

Dopamine's effects on cognition are often studied in Parkinson's disease patients using paradigms were patients abstain from dopamine replacement therapies (OFF treatment) for short periods of time. Coulthard et al (2012) investigated probabilistic learning in 22 Parkinson's disease patients ON and OFF their usual treatment (Coulthard et al., 2012). During learning

participants made left / right judgements in response to four cartoon characters – e.g. four different hamsters. Each hamster was associated either with disparate probabilities of positive and negative feedback depending on the left / right judgement: for two hamsters a left press resulted in an 80% probability of positive ("Well done you caught it!") and a 20% probability of negative ("Bad luck" displayed on screen) feedback, and vice versa for the other two hamsters. Participants completed 30 study trials per hamster, with feedback at each trial.

Medication status did not affect initial learning – i.e. whether participants were ON or OFF dopaminergic medication they performed equally well toward the final learning trials. After a 20-minute delay, being ON medication increased performance by ~15%. Therefore, dopamine did not play a role in the initial learning process but following repeated repetitions of trials, subsequent performance was enhanced. This suggests that dopamine during learning may accelerate fast systems consolidation during learning over repeated blocks.

A limitation of this study was that participants were either ON or OFF medication throughout the testing sessions, and therefore isolating between the effects of dopamine on encoding, consolidating and retrieving is not possible.

In another study in Parkinson's disease, dopamine had disparate effects on memory depending on the timing of administration in relation to the memory stage (J.P. Grogan, Bogacz, Tsivos, Whone, & Coulthard, 2015). When patients encode a list of 12 words OFF compared to ON medication, memory performance is enhanced 24 hours later. If patients are ON medication during the subsequent night and memory test, their performance is further enhanced. At an earlier 30-minute test with the same items, participants' memory was impaired if they were ON medication. The most optimal performance in this task was reached when patients were OFF medication during learning but ON during the subsequent night. These findings suggest that in

Parkinson's disease, dopamine during encoding impairs memory, while nocturnal dopamine enhances it.

Note that this finding is opposite to that seen in drosophila (and some rodents), where dopamine enhances encoding and accelerates forgetting (Berry, Cervantes-Sandoval, Nicholas, & Davis, 2012; Berry, Phan, & Davis, 2018; Castillo Diaz, Hernandez, Capella, & Medina, 2019). Berry et al (2012, 2018) conducted a series of studies in which they modulated dopamine neuron activity acutely and reversibly, visualised dopamine cell activity and observed behavioural deficits in dopamine mutant drosophila. Blocking dopamine post-learning enhanced memory persistence and stimulating dopaminergic neurons boosted forgetting, while dopamine stimulation during encoding enhanced memory (Berry et al., 2012). The dopamine activation that enhances encoding simultaneously blocked consolidation of old memories (Berry et al., 2018).

An alternative explanation that bridges the gap between humans (J.P. Grogan et al., 2015) and flies is that at dopamine has a dual effect on memory that is driven by salience: dopamine accelerates forgetting for non-salient items and spares salient information. According to this explanation, dopamine between learning and the subsequent 30-minute test enhanced routine forgetting, effectively "impairing" performance. The repeated test of the same items at 30-minutes tagged them as salient, after which subsequent dopamine enhanced memory for this information. Rather than enhancing consolidation globally, dopamine selectively shielded salient information from memory decay and accelerated nocturnal forgetting.

Dopamine and memory in randomised controlled trials

To fully understand dopamine's roles in memory and cognition, pharmacological trials in healthy humans are also needed. While dopamine's effects can be studied in Parkinson's disease, the hallmark pathology of substantia nigra dopamine loss may also affect cognition (Dubois & Pillon, 1997). An advantage in studying dopamine as an intervention in healthy individuals, rather

than by withdrawing regular medication of people with Parkinson's disease, is that it allows using double-blinded designs. Findings from placebo-controlled trials in healthy humans have shed further light to dopamine's role in memory.

Dopamine accelerates the rate of language learning over multiple days, possibly by enhancing repeated learning. Healthy young adults were given 100mg of L-DOPA 1.5h before learning a language task. This was repeated over five separate learning sessions (Knecht et al., 2004). At each visit, participants completed 400 learning trials, during which they learnt to associated pseudowords with images. Each word-image pair was presented four times over the session (Breitenstein & Knecht, 2002). The L-DOPA mediated enhancement on word-learning started to emerge on the second day. L-DOPA accelerated the initial learning over the first 2-3 days, and it slowed down forgetting over time compared to placebo. The L-DOPA mediated enhancement in memory persisted at delayed tests a week and a month later, after the treatment period and training had finished (Knecht et al., 2004). Very similar effects of L-DOPA on word-learning have been found by others, where beneficial effects only begun after repeated testing (Shellshear et al., 2015). Dopamine therefore enhances language learning over study periods spread over multiple days.

It is difficult to differentiate when dopamine was influencing memory from these trials. Participants in both studies were dosed prior to learning and exogenous dopamine was active throughout testing (Knecht et al., 2004; Shellshear et al., 2015). It is tempting to say that dopamine is only acting at encoding but encoding processes may be different when information is re-encoded, particularly if that re-encoding event is associated with a memory test as it was here. Information learnt over multiple days, as opposed to on the same day, is also more resistant to forgetting (Ezzyat, Inhoff, & Davachi, 2018). These findings further support dopamine's involvement in prioritising memory persistence for salient information, possibly by enhancing the tagging effect.

Other studies have targeted different memory processes more carefully. In one such study, while L-DOPA was active during both learning and encoding, fMRI was used to study the underlying neural signature of L-DOPA at encoding (Chowdhury et al., 2012). Old adults were dosed with 150mg L-DOPA (or placebo) 90 minutes preceding a reward-scene learning task in the scanner. During encoding, participants learnt 120 still scenes that were either rewarded (£1.00) or not (£0.00). Recognition was probed 2h and 6h later outside the scanner using the Remember-Know paradigm (which is discussed later). Recollection for neutral scenes was enhanced for a group of participants receiving a "medium dose" (based on body weight), while no effect of was seen in low or high dose groups. This effect was seen both by splitting the participants into groups based on body weight and by fitting a quadratic curve.

Furthermore, the L-DOPA-mediated enhancement in memory was independent of hippocampal activity during learning. While the subsequent memory effect – where hippocampal activity at encoding predicts later memory – is not always seen in elderly (e.g. (Morcom et al., 2010), in this study it was present during placebo but not during the L-DOPA visit (Chowdhury et al., 2012). The authors concluded that L-DOPA wiped out effects of preferential encoding of rewarded information by boosting consolidation globally. In conjunction with the behavioural findings, this study suggests that dopamine does not modulate encoding but rather enhances consolidation.

Similar findings have been found in healthy young individuals. Nocturnal D2 agonist pramipexole given after learning, to target consolidation, wipes out the tagging effect between high and low reward items by enhancing memory for low reward (Feld et al., 2014). It is notable that pramipexole was given after learning, to target consolidation. Together with these two studies suggest that dopamine at encoding does not modulate memory in healthy individuals regardless of age. Interestingly, high reward information did not benefit from subsequent dopamine in either study. Dopamine may instead tag low reward items retrospectively to increase

consolidation, while high reward items do not benefit as they are already at "ceiling" when it comes to the reward-mediated memory boost. I suggest that as participants knew they would be tested on all items, even non-rewarded information carried a saliency-tag.

Sleep and memory

Sleep has long been recognised as playing a role in consolidating memories (Stickgold, 2005; van de Ven, Trouche, McNamara, Allen, & Dupret, 2016). The initial observation on sleep's role in memory was made by Ebbinghaus, who pioneered memory research over a century ago. He noted that forgetting curves were not linear but instead they were slowed down during sleep (Ebbinghaus, 1885; Jenkins & Dallenbach, 1924). Seemingly, sleep protects memories from decay. However, memories during sleep are not stable. Instead, while we sleep, they are selectively stored or forgotten, or adapted to integrate into our previous network of knowledge through the process of systems consolidation.

Here, I will demonstrate that sleep supports memory consolidation and maintenance. Sleep also supports several other cognitive and physiological restorative functions – from stabilising mood and satiety to clearing brain amyloid and regulating insulin (Li, Kechter, Olmstead, Irwin, & Black, 2018; Reutrakul & Van Cauter, 2018; Shokri-Kojori et al., 2018). Given that during sleep memories – at least in past – migrate from the hippocampus to cortical regions, sleep and consolidation might also restore encoding capacity in the hippocampus. Indeed, hippocampal activation is reduced when retrieving memories that have been consolidated overnight (Wang & Morris, 2010).

Memory replay

What then may support the gradual information transfer between the hippocampus and the cortex? The sharp wave ripples touched upon earlier, (see Figure 9 on page 20), seem to play a key role in bolstering systems consolidation, at least for some types of memories. In rodents, a well-replicated finding is that sequences of hippocampal place cell firing active at learning are spontaneously replicated at a higher frequency during sharp-wave ripples (Skaggs & McNaughton, 1996; M. A. Wilson & McNaughton, 1994). While they take place both during wakefulness and sleep, sleep seems to provide an optimal environment to support hippocampal replay (Carr, Jadhav, & Frank, 2011; Foster & Wilson, 2006; Molle, Yeshenko, Marshall, Sara, & Born, 2006; Ramadan, Eschenko, & Sara, 2009).

Not only do the ripples reactivate patterns of neuronal activity, they also support consolidation. Abundant evidence for this comes from experiments on animals. For example, ripples induce LTP in the hippocampus (Sadowski, Jones, & Mellor, 2016), and artificially suppressing ripples impairs memory (Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009). In primates, ripples during wakefulness and sleep are temporally associated with suppressed activity in thalamic and cortical regions (Logothetis et al., 2012; M. Y. Yang, Logothetis, & Eschenko, 2019). This cortico-thalamic silence may provide an optimal window for systems consolidation by reducing interference.

Sleep spindles, which are time-locked to ripples, also support the kind of communication between medial temporal lobe and the cortex that is needed for systems consolidation. Connectivity between cortical regions and the hippocampus is enhanced during slow wave spindles (Siapas & Wilson, 1998; Sirota, Csicsvari, Buhl, & Buzsaki, 2003). In line with this observation, the density of spontaneous ripples during slow wave sleep is associated with enhanced memory for the repeated events (Ramadan et al., 2009). Intracranial recordings from

patients with epilepsy also show that ripples are associated with neocortical slow oscillations (Axmacher, Elger, & Fell, 2008). Spindles and slow wave activity after learning has also been shown, in scalp EEG, to *mediate* the process of integrating newly learnt information into an existing knowledge framework (Tamminen, Lambon Ralph, & Lewis, 2013). Together these studies robustly show that hippocampal activity during sleep promotes memory consolidation.

Note that this sleep-dependent replay parallels the "fast route" online systems consolidation driven by repeated learning trials (Brodt et al., 2016; Himmer et al., 2019). Replay involves spontaneous unintentional repeats of learning-related neuronal firing, possibly analogous to repeated exposure to stimuli.

Finally, while there is less evidence of replay in humans, some affirmation comes from patients with parasomnias. A behavioural re-enactment of a learnt motor task has been video recorded in one sleep-walking patient during slow wave sleep (Oudiette et al., 2011). In the daytime the patient underwent vigorous training of a motor-reaction time task where obvious, large and uncommon hand and arm gestures were required. At night sequences of the learnt task were spontaneously repeated. However, the replay was not linked to spindles.

Nevertheless, these evidence together build a robust case that replay during sleep and awake plays a role in memory persistence. While studies in humans are scarce, there have been several attempts to induce replay in humans.

Targeted memory re-activation

Efforts to study memory replay in healthy humans have also been made. An analogue to memory replay can be induced artificially using targeted reactivation paradigms. In these studies, participants typically learn paired associates in the day-time – such as a smell or a sound paired with other information, such as a word (e.g. "*meow* - Finland", "*squeak* - Greece"). During sleep,

the associated sound is played (e.g. "*meow*"), and participants are more likely to retain information related to the sound ("Finland") than other information ("Greece"), even though participants are not consciously aware of the sound (Rudoy, Voss, Westerberg, & Paller, 2009; Shimizu et al., 2018).

Targeted memory reactivation may be particularly beneficial during slow wave sleep. When a distinct odour was present during both learning and slow wave sleep, performance in an association task was enhanced (Rasch, Buechel, Gais, & Born, 2007). The presentation of the odour during slow wave sleep was also associated with increased hippocampal activity during an fMRI scan. No beneficial memory effect was found if the odour was presented during REM sleep, after learning during wakefulness, or during slow wave sleep but not at learning. Others have also found that targeted memory activation during slow wave sleep increases spindles and subsequent memory (Cairney, Guttesen, El Marj, & Staresina, 2018). These findings give indirect support to memory replay affecting hippocampally-mediated memory. They also suggest that, as seen in rodents and primates, slow wave sleep provides an optimal timing for memory consolidation.

Evidence that targeted memory reactivation is causally related to replay comes from rats. When memories are reactivated during slow wave sleep by presenting a sound, the overall incidence of replay events does not increase. Instead, the sound-associated sequences are preferentially replayed (Bendor & Wilson, 2012). This suggests that the replay does not enhance memory overall but rather biases it. This raises the question: what factors play a role in selecting which memories will be re-activated during sleep?

Salient memories

Sleep-dependent consolidation is not equal: some information is retained while others are lost (Figure 13). Encoding-related events may bias memory selection during subsequent sleep. Salient

- rewarded, novel and unusual – information may particularly benefit from sleep. In line with this, hippocampal ripples, which are time-locked to spindles, have recently been shown to selectively contribute to the consolidation of rewarded items (Michon, Sun, Kim, Ciliberti, & Kloosterman, 2019).

When learning salient information is followed by sleep, it is prioritised for storage even when tested 3 months after learning (Igloi, Gaggioni, Sterpenich, & Schwartz, 2015). Importantly in this study, this effect only applied to salient information. While the tagging effect was present months later when the volunteer had taken a nap after learning, in the absence of post-learning sleep, rewarded and non-rewarded memories decayed equally. It is not just rewarded information that is prioritised during sleep.

Personal values and can also guide consolidation. When participants were learning foreign-language words, the degree to which sleep facilitated learning was associated with how much they valued the language they were learning (van Rijn, Lucignoli, Izura, & Blagrove, 2016)

Sleep therefore preferentially consolidates behaviourally salient information. However, when participants do not know that their memory is going to be tested later, they perform equally well regardless of whether they have slept or not (Wilhelm et al., 2011). When participants know they will be tested, memory is enhanced. This enhancement is associated with an increased spindle count during slow wave sleep – but no such relationship was seen when information was not behaviourally salient. Similarly, sleep-mediated increases in memory persistence are biased toward prospective memories (Diekelmann, Wilhelm, Wagner, & Born, 2013) and other memories that are relevant for future behaviours (Fischer & Born, 2009; Rauchs et al., 2011).

Other evidence that sleep benefits memory selectively for salient information comes from studies showing that sleep enhances memory for overall concepts, or the gist, of the memory but not for specific information. For example, when participants learn objects with shared category

features and specific individual features, sleep improves memory for the former but not the latter (Schapiro et al., 2017). It may be much more beneficial for the memory system to extract the gist while disregarding minute details.

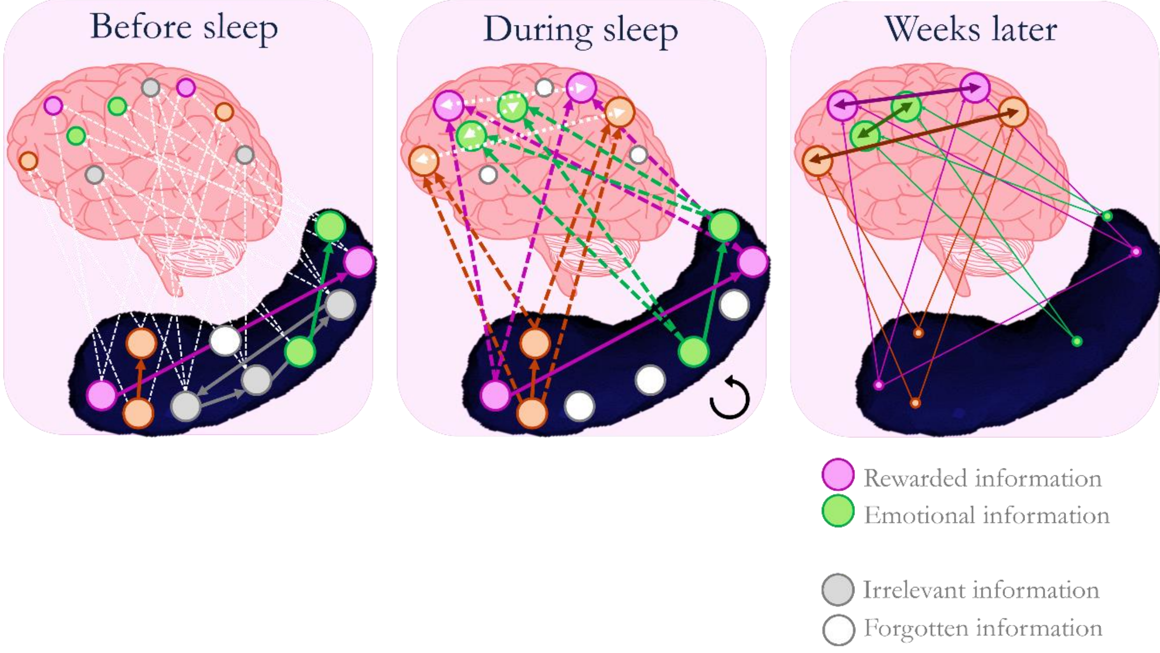


Figure 13: Long term consolidation and forgetting, and sleep

Before memories have stabilised (left) they rely heavily on hippocampal nodes and connections, and they are weakly connected to cortical regions. During repeated reactivations, which often take place during sleep (middle), the cortex becomes more involved in memory storage and begins to form novel associations between nodes. Memories that are salient are also prioritised for spontaneous reactivations, while irrelevant information is selectively lost. Weeks later (right) memories rely more heavily on cortical nodes and connectivity. Some hippocampal connections between nodes may even become lost as the "cortical gist" of the memories is prioritised over fine grain detail stored in the hippocampus.

Sleep stages

Throughout sleep, the brain fluctuates across different sleep stages. These stages are likely be disparately associated with cognition. Many animal studies report slow wave spindles to be temporally associated with memory replay. Studies in rats have mostly associated consolidation with sleep events, such as spindles, during slow wave sleep. Rat slow wave sleep is analogous to human non-REM sleep, and therefore it encompasses sleep events that takes place across human stages 1, 2, and slow wave sleep. In humans spindles are abundant in stage 2 and several studies show that spindles during both stage 2 and slow wave mediate memory performance (Andrade et al., 2011; S. M. Fogel & Smith, 2006; Genzel et al., 2017; Squire et al., 2015).

While here I have mostly discussed slow wave sleep, the dual processing hypothesis stipulates that different sleep stages serve to consolidate different types of information. In short, REM has been suggested to serve implicit memories, while slow wave sleep has been suggested to serve consolidation of explicit memories (Gais & Born, 2004; Rasch & Born, 2013; Rauchs, Desgranges, Foret, & Eustache, 2005). Yet, the picture is unlikely to be this clear-cut as there are some exceptions with emotionally loaded information being preferentially consolidated during REM (Harrington, Johnson, Croom, Pennington, & Durrant, 2018; Wiesner et al., 2015).

Forgetting

A healthy memory system cannot consolidate all information that is encoded. Even if the storage capacity was limitless, the behavioural utility of non-selective memory is questionable. As we have seen, the memory system prioritises salient memories over irrelevant information, and strategic forgetting of what is not later required likely allows important items to be better retained. In other words, blocking consolidating or encoding of competing information may

support consolidation of salient information. For memory to be successful, a degree of routine, strategic forgetting may be necessary.

Initially, it was thought that forgetting was caused by interference from competing stimuli alone, and that sleep enhanced memory by blocking this interference (Jenkins & Dallenbach, 1924). Since this view has been extended by including passive, and later active, memory decays as alternative routes for forgetting (Hardt, Nader, & Nadel, 2013). Sleep not only plays a role in consolidating memories but it also facilitates forgetting (Berry et al., 2015; Davis & Zhong, 2017; Feld & Born, 2017).

It is not entirely clear what facilitates routine forgetting in a healthy brain but increased cognitive load may increase the demands for strategic forgetting. In a word-learning task memory was enhanced by sleep when volunteers learnt 160 word-pairs but not when they learnt 320 (Feld, Weis, & Born, 2016). In the 320-word condition participants performed equally well whether they had slept or not. This suggests that during sleep instead of consolidating some of the word-pairs, the memory system actively suppressed consolidating or triggered active forgetting of these items, mimicking processes like those caused by interference during wakefulness. Under pressure from a large amount of memories to consolidate, sleep may facilitate active forgetting in favour of other salient information.

Several studies in drosophila have identified molecular and cellular structures that support routine forgetting, including pioneering studies linking it to forgetting (Berry et al., 2012). Drosophila are a commonly used model to study the effect of brain chemicals on sleep and disease. As in humans and other mammals, in drosophila dopamine regulates sleep-wake cycles and arousal (Andretic, van Swinderen, & Greenspan, 2005; Kume, Kume, Park, Hirsh, & Jackson, 2005) .

Dopamine's effects on learning fluctuate with circadian rhythms. In drosophila, increased dopamine during wakefulness increases routine forgetting and decreased dopamine during rest inhibits it (Berry et al., 2015). In drosophila, dopamine is required for both encoding and active forgetting (Berry et al., 2012; Berry et al., 2018), and while these are mediated by the same set of neurons, they are associated with separate receptors. Therefore, one dopamine neuron can have disparate effects on memory and cognition.

Research in rodents has localised these effects into the VTA. Castillo Dias et al (2019) conducted a series of experiments with D1 antagonists and agonists injected into the VTA, hippocampus or nucleus accumbens at varying delays *after* learning. D1 projections from the VTA to the nucleus accumbens were found to enhance memory persistence, while projections from the VTA to the hippocampus accelerated active forgetting (Castillo Diaz et al., 2019). Therefore, D1 receptors in the VTA, projecting to the hippocampus might provide a neurophysiological basis for forgetting. Blocking dopamine activity in either inhibits active forgetting, while exciting D1 receptors accelerates it, at least for salient information. In light with the findings in drosophila, this suggests that the dopaminergic connections to and from the hippocampus can active forgetting.

In sum, memory is not just an outcome of successful consolidation but instead forgetting is an active process that can be triggered by behavioural or pharmacological interventions. The effects of dopamine on forgetting are difficult to interpret in the context of the findings where these same regions enhance memory persistence. The above results may be specific to cocaine-related memory. Acute cocaine can increase memory persistence in mice (Introinicollison & Mcgaugh, 1989; Janak, Keppel, & Martinez, 1992).

It is also possible that disparate sub-populations of dopaminergic neurons have disparate effects on memories depending on the type of memory, time since learning and so on. Indeed, Berry et al (2018) demonstrated that the same neurons at different receptors both enhances encoding and

forgetting. Some have suggested that dopamine influences memory persistence via two separate routes – one involving the locus coeruleus and the other the hippocampal-VTA-loop (Duszkiewicz, McNamara, Takeuchi, & Genzel, 2019). It is not presently clear why dopamine seems to induce forgetting in some studies and consolidation in others.

A possible explanation is that of publication bias and the file drawer effect. In short, publication bias refers to the phenomenon where publishing positive studies is incentivised in a way that leads to studies with negative results being underreported or ending in the file drawer. Selective publishing of results can lead to several low-power studies with discrepant findings.

The file-drawer effect can be estimated from published results. When studies are poorly powered, as they are in neurosciences (Button et al., 2013), published findings become less reliable. First, the likelihood of false positive findings increases as smaller samples increase the likelihood of chance findings. Second, true effects become less likely to be detected which means that when they are found, observed effect sizes are likely to be larger than the size of the true effect – as the true effect may be too small to be observed in a small sample. Furthermore, the distribution of p-values is heavy-tailed when the alternative hypothesis is true (i.e. when there is an effect very low p-values are more likely) but equally distributed when the null hypothesis is true (i.e. when there is no effect every p-value is equally likely).

Using reported summary statistics from published research studies one can attempt to quantify the magnitude to which publication bias is affecting the reliability of findings from a given field. I am not aware of any meta-analyses that have systemically tried to quantify the magnitude to which publication bias is a problem in the dopamine/memory literature. However, using data from over 3800 published papers, Szucs and Ioannidis (2017) estimated that up to 50% of published findings in cognitive neurosciences may be false (Szucs & Ioannidis, 2017).

Several of the aforementioned studies have low power and small effects. This increases the likelihood of false positives and therefore studies being selectively published. This is likely to at least partially explain the discrepancies in findings.

Retrieval

Recollection and familiarity

The final memory stage in the traditional stage model is retrieval. During retrieval, memories are reconstructed in a process that mimics the neuronal firing patterns associated with learning. This process is also supported by hippocampal sharp wave ripples (Joo & Frank, 2018). Retrieval can either take the form of free recall or recognition.

In experiments, recall is prompted either by cueing learnt items or by free recall. Both free recall and recognition can be used to test either episodic or semantic memory. In episodic recognition tasks participant are asked to judge if an item was presented previously (OLD) or not (NEW). This task is considered episodic because participants are asked about items presented in a *specific* situation and context (Migo, Mayes, & Montaldi, 2012). The reasoning is, that participants are recollecting the specific situation in which they learnt the tested item. Yet, it is intuitive that participants can make OLD – NEW judgements in the absence of recollection for the learning context. Participants can therefore make two types of correct OLD judgements – ones that are based on recollection of the learning context and ones based on familiarity with the presented item.

Thus, recognition memory can be divided into recollection and familiarity. Recollection refers to episodic memories about events – where contextual information relating to the recollected information is recalled. Familiarity instead relates to knowing that information was encountered

before, in the absence of recollection. These can be considered analogous to episodic (recollection) and semantic (knowing) memories (Brown & Aggleton, 2001; Tulving, 1985). A classic example to illustrate this distinction is recognising a person on the street as *familiar* with no *recollection* of who they are.

One approach for dissociating recollection and familiarity experimentally is to use the Remember-Know procedure (Tulving, 1985). In this task, participants are prompted follow-up questions after OLD – NEW judgements. Upon recognising information as OLD participants are asked to determine if they REMEMBER (recollect) or KNOW (familiar) the item, or – in case of a forced-choice task – if they made a GUESS (Figure 14).

The end outcome of both recollection and familiarity is recognition. While behaviourally these are separable processes, there is great controversy associated with the neuroanatomical basis of recollection and familiarity. The dual processing view holds that recollection and familiarity are achieved by separable neurophysiological processes. In accordance to this view, the perirhinal and lateral entorhinal cortices support familiarity while the hippocampus supports recollection (Bowles, Duke, Rosenbaum, McRae, & Kohler, 2016; Diana, Yonelinas, & Ranganath, 2007; Rugg & Yonelinas, 2003; Yonelinas, Kroll, Dobbins, Lazzara, & Knight, 1998).

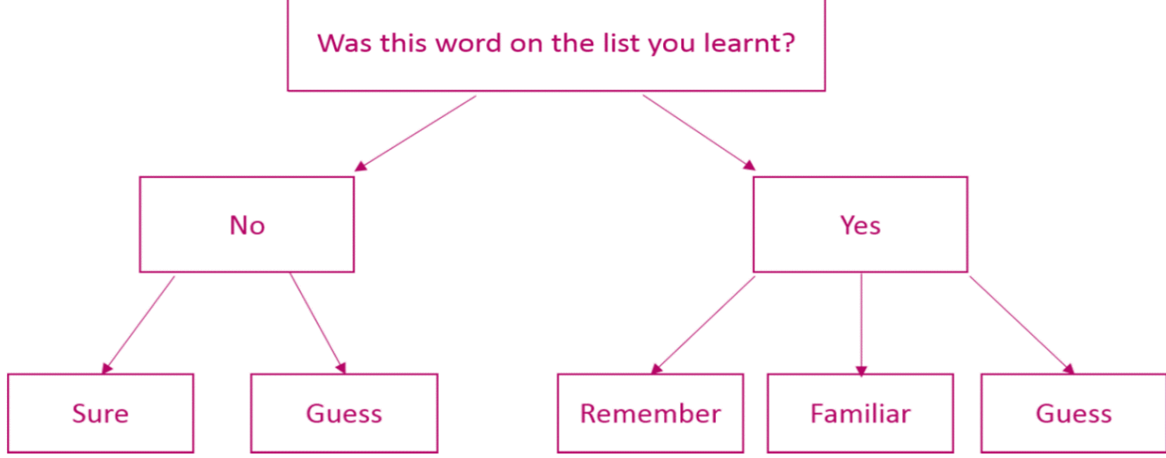


Figure 14: Example of a Remember Know task

In contrast, others regard the difference between familiarity and recollection a question of memory strength: both processes are mediated by the same medial temporal lobe regions that are involved in processing recognition memory strength (Brezis, Bronfman, Yovel, & Goshen-Gottstein, 2017; Wixted & Squire, 2010). However, evidence from a recent fMRI study suggests that the hippocampus is not sensitive to recollection strength but to the *quantity* of recollection (Mayes et al., 2019).

This study (Mayes et al., 2019) used a modified version of the Remember-Know task where, during recognition and cued recall, participants were asked to judge the memory strength for recognised words (on a scale from 1 to 3), whether they were recollected, or if they were distractor words. Participants completed both recognition and cued recall trials while in the fMRI scanner. During each recognition trial, participants saw a word (e.g. HAMSTER), and they had to make one of the following responses: 1, 2, 3, Recollected, New; where 1, 2, and 3 corresponded to memory strength. During the cued recall trials, participants saw a cue for a word (e.g. HAM _ _ _ _) to which they responded to as in the recognition trials. After they had made a response, they were shown two words (e.g. HAMSTER, HAMBURG), and they were asked to determine which one they had thought of during cued recall.

Crucially, this task allows isolating memory strength, accuracy and quantity. Quantity was measured as the number of recollected items in the cued recall condition. For accurate cued recall, spontaneous recollection of the word itself was required, so any recollections would have reflected a change in the *amount* of recollection, rather than a switch between familiarity and recollection. The hippocampus was sensitive to the amount of recollections, but not to recollection strength or accuracy (Mayes et al., 2019). This suggests that the difference between recollection and familiarity cannot be explained by strength alone.

A disadvantage of the traditional Remember-Know procedure is the heterogeneity in its adaptation. The fine difference between recollection and familiarity is difficult to explain to participants in the context of experimental tasks – for a layperson the difference between remembering, knowing and recognising is often neither clear nor intuitive. This may lead to participants making Remember-Know judgements that are analogous to judging memory strength. Care needs to be taken when instructions are delivered to participants. One approach to limiting bias based on participants' understanding of the task, is to ask them to justify their answers. Whenever a REMEMBER response is made, the investigator can ask participants to explain what they recollect.

While this approach partially limits participants' likelihood of erroneously identifying recollected items as familiar, it does not address the issue of memory strength: the average recollected memory is likely to be stronger than the average familiar item, and it does not solve the problem of erroneously identifying recollected items as familiar. Indeed, recent evidence suggests that hippocampal activation seen during familiar trials in fMRI studies may be explained by participants inaccurately judging familiarity (Mayes et al., 2019).

Familiarity is further complicated by previous knowledge of the words presented. Typically, real words are used in these tasks. In a task designed this way, each item is familiar from some previous context or contexts. Others (C. N. Smith et al., 2014) have argued that familiarity judgements in verbal Remember-Know tasks measure associative memory between the word and the context of the learning situation, rather than *true* familiarity. This view suggests that familiarity judgements rely on the hippocampus. The hippocampus plays a key role in integrating pieces of information with one another, and thus supporting associative memory (Bird, 2017). These limitations and discrepancies in findings and implementations of these tasks make it difficult to interpret what the underlying physiology related to these tasks is.

It is therefore not entirely clear whether familiarity and recollections are truly independent. However, the medial temporal lobe structures do seem to play some role in supporting both types of judgements. In rats, attempts to isolate familiarity and recollection have been made by applying response deadlines during recognition memory. In accordance to this reasoning, under time constraints recollection is eliminated and rats will rely on familiarity – while when no time constraints are applied, they will use recollection and familiarity equally. Results from this task behaviourally mimic response patterns seen in humans (Sauvage, Beer, & Eichenbaum, 2010). In rats there is a clear involvement of the lateral entorhinal and perirhinal cortices during familiarity judgements, while hippocampal subfields CA1 and CA3 are not recruited under time constraints (Atucha, Karew, Kitsukawa, & Sauvage, 2017). These findings suggest that the dentate gyrus and the perirhinal cortex support familiarity, while the CA1-CA3 support recollection.

However, there are obvious caveats to this approach. Separating familiarity from recollection is a difficult task even in humans and participants typically need training and detailed explanations to understand the difference. While faster responses in rats may certainly resemble recollection in some ways, these results should be taken with a pinch of salt. Furthermore, there is on-going debate about the involvement of hippocampus in recognition memory (Bird, 2017).

Retrieval modulates memory

Retrieving information is not a passive process of accessing something that has been stored. Instead, memory traces are recreated rather than accessed, and the act of retrieval is accompanied by a myriad of events that can cause plasticity in both retrieved and contextually related information.

Perhaps the most widely recognised effect of retrieval is that it improves memory for the retrieved information. Active retrieval enhances subsequent memory opposed to re-study (C. Yang, Potts, & Shanks, 2018). This has been coined the testing effect – testing one's memory

enhances persistence of the tested memory trace (Wing et al, 2013). The hippocampus, temporal cortical regions and the prefrontal cortex are recruited to a higher extent during re-testing, compared to passive re-study. This activation pattern is remarkably similar to that seen during initial encoding for items that are later correctly retrieved, i.e. the subsequent memory effect covered earlier in this thesis. However, in this study they did not control for task difficult.

Intuitively, active memory testing is more difficult than passive restudy, which may bias their findings (Wing, Marsh, & Cabeza, 2013). Yet, their results are consistent with the wider theoretical framework, and they suggest that retrieval can enhance encoding processes – possibly by retroactively tagging previously learnt information as salient. Alternatively, previous knowledge of the tested information may guide re-encoding during memory test. It is not clear whether these are separate processes.

In line with encoding-related activity being triggered by testing, retrieval has been suggested to trigger awake replay in rats (Carr et al., 2011; Foster & Wilson, 2006). For example, in situations where a rat is faced with a cued-recall, and needs to make decisions between multiple different routes, fast-paced replay of the hippocampal place cell activity corresponding to each possible route takes place. This might reflect decision making processes about future paths (Johnson & Redish, 2007; van der Meer, Johnson, Schmitzer-Torbert, & Redish, 2010). Note that dopaminergic neurons are likely to play a key role in orchestrating this type of action selection (Schultz, 1998; Westbrook & Frank, 2018).The strength of this replay is associated with memory success (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010).

Indeed, retrieval has been suggested to act as a fast route to offline consolidation (Antony, Ferreira, Norman, & Wimber, 2017). Repeated testing increases enhances memory by creating more “elaborate” memory traces (Rosburg, Johansson, Weigl, & Mecklinger, 2015). This

suggests that wakeful re-activation of memories by re-test can trigger processes that resemble systems consolidation.

Wakeful reactivation on its own is unlikely sufficient to support healthy memory function. Perhaps wakeful reactivation and rehearsal provide a quick and easy route for consolidation that still requires subsequent sleep for information to "solidify". As we are unable to rehearse all new information it is unlikely that wakeful reactivation alone could replace sleep-dependent consolidation.

To my awareness there is no literature to show whether wakeful reactivation can also trigger hippocampal clearance of "nuisance" information (Figure 13). After periods of sleep-deprivation, learning new information is impaired (M. P. Walker, 2008). It seems that during sleep processes that support selectively retaining some information over other allows for the memory systems to "reset" to optimise subsequent memory performance. However, it does seem to provide a parallel route to learning that might mimic processes that promote memory selectivity during sleep.

Carefully selecting which memories to keep likely serves a behavioural benefit. When we are required to retrieve information, such as a new co-worker's name, it is likely because this information has some value. It may be that during retrieval-test the practised items are tagged as salient. Simultaneously, as contextual information is not tested, it may be preferentially forgotten in order to allocate cognitive resources where they are needed. Wimber et al (2015) coin this type of forgetting the 'dark side' of remembering (Wimber, Alink, Charest, Kriegeskorte, & Anderson, 2015). However, strategic forgetting may carry a behavioural benefit that is likely to generalise outwith the laboratory environment.

Retrieval induced forgetting

Paradoxically, retrieving previously learnt information can induce forgetting of contextually related information. Retrieval-practice is a commonly used three-stage paradigm (Anderson, Bjork, & Bjork, 1994) used to study retrieval-induced forgetting. First phase (1) is the initial encoding stage where participants learn to associate pairs of items, often categories paired with some of its constituent items (e.g. ANIMAL – cat, ANIMAL – hamster, FOOD – chocolate, FOOD – peanut, COUNTRY – Finland, COUNTRY - Australia). Then (2), memory for a proportion of the items is practiced by retrieval, using cued recall (e.g. ANIMAL – ha_ _ _ _ _, FOOD – ch_ _ _ _ _ _ _). Following this retrieval-practice phase, test items fall into three subgroups:

a.	practised items	hamster	chocolate
b.	unpractised related items	cat	peanut
c.	unpractised unrelated items	Finland	Australia

The latter category acts as a baseline. A common and well-replicated finding is that compared to the baseline practised items (hamster, chocolate) are better retrieved at the final test stage (3) while unpractised items are more likely to be forgotten in comparison:

a.	practised items	→	IMPROVED
b.	unpractised related items	→	IMPAIRED
c.	unpractised unrelated items	→	UNAFFECTED

Interestingly, the practice-induced tagging effect is increased by both an active suppression of the unpractised related items (cat, peanut) and an enhancement of the practiced items (hamster, chocolate) – in relation to the unpractised and unrelated items (Figure 15).

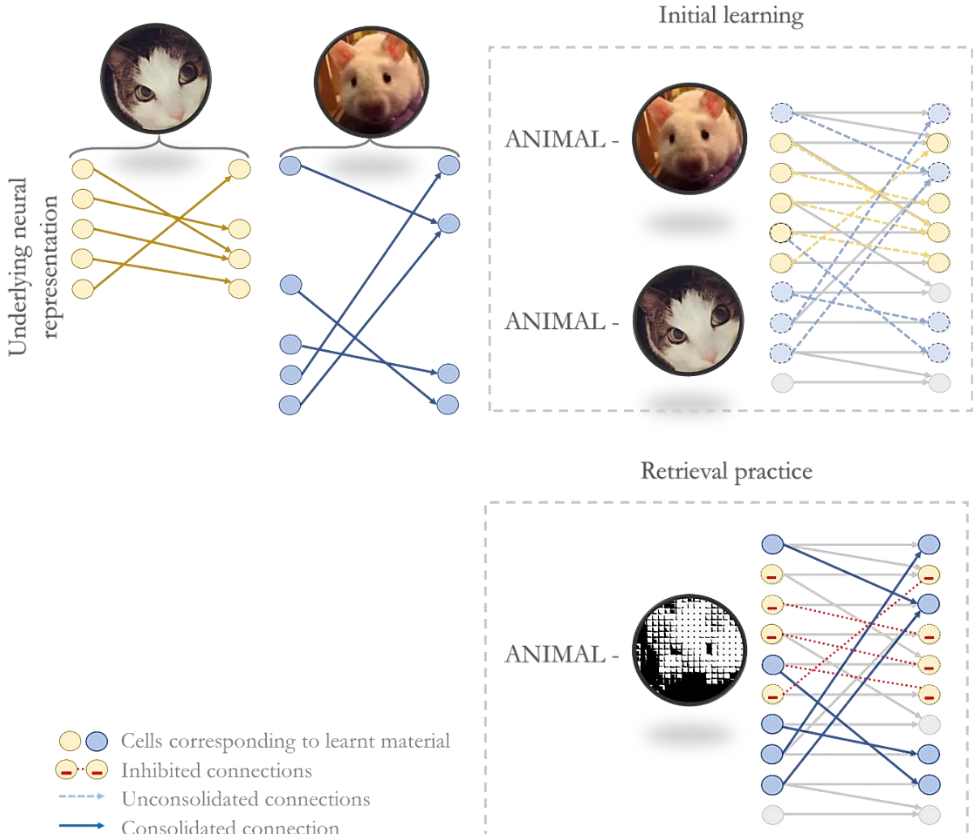


Figure 15: Retrieval and memory

During learning related information is learnt equally well. Later, when items are selectively retrieved practised items (hamster) are tagged. To solve the cognitive demands associated with the saliency-tag, "competing", or contextually related information is actively suppressed.

What then might mediate this active forgetting process? Using distinct categories of visual stimuli (faces and objects), Wimber et al (2015) isolated unique fMRI signals corresponding to different stimuli during a retrieval induced forgetting task. In this study, participants first viewed the visual stimuli whilst in an fMRI scanner in order to create a template of the brain activation that corresponds to each viewed image. Participants then learnt to associate two competing associates (images) with the same word. For example, they would be shown the word SAND with a picture of Marilyn Monroe (first associate) in one trial and a picture of a hat (second associate) on a second trial.

Subsequently, while in the MRI scanner, the participants were encouraged to selectively retrieve the first associate on cue: they were shown the cue (SAND) and asked to retrieve the first

associate (Marilyn Monroe). The rationale is, that upon presenting the cue, memory traces for both associates would automatically become active. However, the active cuing of one over the other associate would encourage participants to actively suppress memory of the second associate.

Over several cued reactivations of the first associate, the neural activation during cue (SAND) representation activated the same patterns of BOLD responses as were active during the initial viewing part of the study. In other words, the association between the cue and the first associate became stronger. Simultaneously, and in the absence of cueing retrieval for the second associate, the neural activity during retrieval started to become more *dissimilar* to patterns of activation associated with the second associate.

At retrieval practice the activity associated with the practiced item was therefore upregulated while the neocortical activity associated with the unpractised related items was suppressed. This is remarkable given the unpractised items were not presented at these trials, and yet the neural

representation corresponding to them was altered. No such alteration was seen in control items that were present during learning but not manipulated using cued retrieval.

Furthermore, the level of suppression of these items was further associated with was also behaviourally relevant and predicted subsequent memory for the items. Together, this provides strong evidence that retrieval can induce active forgetting of contextually or semantically related information.

Others have shown that the retrieval induced forgetting has neural processing benefits – i.e. it reduces the burden on cognitive control mechanisms at play during selective retrieval (Kuhl, Dudukovic, Kahn, & Wagner, 2007). These effects are likely driven by processes during retrieval that directly inhibit competing information (for review, see (Storm & Levy, 2012)).

Coming of age

Old age is associated with several physiological and behavioural changes in the brain and in cognition. A short outline of the changes relevant to this thesis is given below.

Hippocampus

Hippocampal connectivity is affected by old age (I. A. Wilson, Gallagher, Eichenbaum, & Tanila, 2006). Within the hippocampus, projections between the dentate gyrus and the CA1 deteriorate with old age. Similar changes in connectivity are seen along the perforant pathway that projects from the entorhinal cortex to the dentate gyrus, and between dentate gyrus and CA3, and along the Schaffer collaterals (CA3/1 synapse). Along the perforant pathway, the threshold for long term potentiation (LTP) is increased together with a decrease in LTP magnitude (Barnes, Rao, & Houston, 2000). The dopaminergic firing from the VTA to the hippocampus and entorhinal cortex is also decreased with old age (Penner & Mizumori, 2012).

Hippocampal volume has also been shown to decrease with old age. A systemic review of 28 studies and 3422 participants assessing the volume changes across different age groups showed that hippocampal atrophy accelerated with age (Fraser, Shaw, & Cherbuin, 2015). In adults under the age of 55, the annual rate of volume reduction was small, between 0.1% and 0.7%, while in those over 70 years of age, the average annual atrophy rate was between 0.9% and 1.4%. There was no effect of laterality or gender. Age-related atrophy in the hippocampus also has functional relevance and it is associated with memory decline (Salami, Eriksson, & Nyberg, 2012).

Of the hippocampal subregions, the CA1 and the dentate gyrus are the most affected by old age. Several cell types within the CA1 are affected by age (Hayakawa, Kato, & Araki, 2007). While

neurogenesis in the dentate gyrus continues throughout the lifespan, it is considerably slowed down with age (Gould et al., 1999; Heine, Maslam, Joels, & Lucassen, 2004; Jinno, 2016; Kuhn et al., 2018). This decline in neurogenesis may be partially reversible: several studies have shown that environmental enrichment, exercise and diet can reduce the effect of ageing, while increased stress and increase it (for a review see Kuhn, Toda & Gage, 2018). The age mediated LTP reduction in the Schaffer collaterals can also be rescued pharmacologically (Billard & Freret, 2018).

Microscopic scale changes in hippocampal structure and function are difficult to study in living humans but magnetic resonance imaging can be used to assess larger morphological and functional changes in ageing. Functional connectivity measured between CA1/subiculum boundary and the entorhinal cortex is decreased in old compared to young adults (Dalton, McCormick, & Maguire, 2019), and decreases in hippocampal connectivity along the perforant path in old age are associated with cognitive impairment (Yassa et al., 2010). These findings convincingly support what has been seen in animals.

In the human hippocampus, another method, that has not yet gained large-scale popularity, is to study hippocampal structure integrity using magnetic resonance imaging. T2 relaxometry can be used to assess microstructural integrity of brain parenchyma beyond macrostructural MR volumetry. The T2 relaxation time – or transverse relaxation time – is a quantitative MRI parameter that is a measure of loss of transverse magnetisation through relaxation.

Image contrast in structural MRI is an outcome of voxel-wise differences in relaxation times and proton density, which have different characteristics in each tissue types. The T2 MRI signal is a measure of water movements and mobility. After a radio frequency pulse, protons align with one another in a transverse plane in the rotating frame reference. It is said that the protons are in phase, and the transverse magnetisation is high. Transverse magnetisation begins to decay due to

inherent T2 relaxation of protons resulting in loss of phase coherence. T2 relaxation time is the time take for 33% of transverse magnetisation been lost.

Relaxation times refer to the duration protons take to dephase after the radio frequency pulse has been sent. This is influenced by the properties of the tissue they are measured from. Relaxation is typically slower in water (e.g. cerebrospinal fluid) than in dense tissues (e.g. bone), but other properties such as concentrations of myelin, macromolecules, proteins, and paramagnetic atoms (e.g. iron in blood) strongly enhance relaxation processes shortening thereby relaxation times (Meiboom & Gill, 1958; Papadaki et al., 2019)

Microstructural alterations together with altered water dynamics result in abnormal MRI relaxation times. Consequently, T2 relaxation time can reveal abnormal tissue properties even within the same structure and tissue type even prior to visible macrostructural changes. These changes in brain are affected by widely processes, such as maturation (Matsumae et al., 2001; Paus et al., 2001), pathology, cognition and healthy ageing (Callaghan et al., 2014; Knight, Wearn, Coulthard, & Kauppinen, 2019).

In addition to the absolute T2 relaxation time, the width of the distribution of the T2 relaxation times within a structure can be useful in detecting age-related changes associated with tissue integrity. Importantly, and as reviewed elsewhere (Tang et al., 2018), the T2 relaxation times are sensitive to early stages of disease related early pathology in dementia. These changes also precede volumetric changes (Callaghan et al., 2014; Knight et al., 2019). Early neurobiological changes associated with age and pathology that are missed out by looking at the volumes alone can thus be detected with T2 relaxometry.

Although the focus of this thesis is on human cognition, it is important to note that animal models of neurocognitive ageing and the hippocampus (reviewed in Leal and Yassa, 2015) are a

complementary research approach, albeit with major caveats to interpretation of animal cognition and sleep including lifespan and differences (Leal & Yassa, 2015).

Dopamine

While loss of dopaminergic neurons is typically associated with Parkinson's disease, a less severe reduction in density of such cells is associated with normal ageing (Kish, Shannak, Rajput, Deck, & Hornykiewicz, 1992). Several studies have shown age-related losses of dopamine. One such study estimated that striatal dopamine binding reduces by 6.6% per decade of age in healthy individuals (Werner et al., 2018).

Age-related dopaminergic alterations are also associated with changes in memory processing. For example, in a reward learning task, old compared to young participants were slower at making responses and this slowing was associated with reductions in theta band activity during encoding. The response time reduction was associated with a loss in structural integrity of the substantia nigra (Steiger & Bunzeck, 2017), suggesting that age-related dopamine dysfunction impairs reward learning.

Reductions in dopamine in the ageing brain are also associated with deficits in other dopamine-mediated behaviours such as decision making and learning from rewards. For example, older compared to younger elderly humans show reduced nucleus accumbens activity in response reward prediction errors, as measured by functional MRI. Performance differences in a reward learning were associated with age-related declines in the integrity of white matter pathways connecting the substantia nigra and the striatum (Chowdhury et al., 2013). In some elderly – but not all – administering L-DOPA restored the age-related deficits in reward learning together with the fMRI BOLD responses to match young adults.

Therefore, deficits in dopamine are seen in ageing are prominent and they affect cognition. Crucially, responses to dopamine treatments to target cognition also seem to affect young and old participants in different ways (Morcom et al., 2010). It is possible that due to the relatively widespread loss of dopamine network integrity in healthy ageing, studying dopamine's effects on cognition is this population is particularly fruitful.

Circadian rhythms

Ageing is associated with multiple sleep-related problems, most commonly with poor sleep quality and delayed sleep onset latency. Deep sleep, or slow wave sleep, is greatly reduced in ageing.

The causes of age-related sleep disturbances are not fully known. One plausible physiological explanation are age-related effects on brain regions regulating circadian rhythms. In the hypothalamus, the suprachiasmatic nucleus is a key area for the maintaining sleep homeostasis. Both neuron count (Roberts, Killiany, & Rosene, 2012) and neuronal firing (Nygard, Hill, Wikstrom, & Kristensson, 2005) in this region reduce with old age. These physiological changes with advanced age lead to functional decline in the suprachiasmatic nucleus and regulation of the 24h body clock (H. C. Chang & Guarente, 2013; Satoh, Imai, & Guarente, 2017).

Whatever the cause, the health implications of poor sleep in the elderly are concerning. Several processes that maintain homeostasis are activated during sleep, including clearance of metabolic waste and glucose regulation (Tasali, Leproul, Ehrmann, & Van Cauter, 2008; Xie et al., 2013). Disruptions in sleep's restorative functions can have adverse effects on health. Both sleeping too little or too much is associated with increased all-cause mortality (da Silva et al., 2016; Dew et al., 2003), with impaired sleep-wake rhythmicity of the circadian clock and high wakefulness after sleep onset (WaSO) score being the best sleep-related predictors of mortality in elderly (Wallace et al., 2018).

Round-up

In this section, I have shown that salient information increases dopamine activity and release in the hippocampus, ventral tegmentum and locus coeruleus. These releases seem to influence synaptic plasticity, consolidation, memory reactivation, and persistence in the hippocampus. Sleep also seems to play a pivotal role in these processes. While much is known about the interactions between sleep, memory, dopamine and the structure and function of the hippocampus, several questions remain. For example, does exogenous dopamine influence memory and sleep in humans? If so, which memory processes does it modulate (Figure 16)? Is this influence dependent on salience, sleep, or hippocampal structure? These are just some of the questions I will attempt to address in the subsequent chapters.

I will report findings across two double-blind placebo-controlled trials. Given the individual differences and age-related changes in dopaminergic systems, here I used crossover designs to allow within-subject comparisons between single doses of L-DOPA and placebo. I also tested older people exclusively for two reasons – first, due to the drop-out of dopaminergic neurons that comes with old age which has been shown to affect the impact of taking dopaminergic medications. Second, because of the similarities with the target group with early dementia or mild

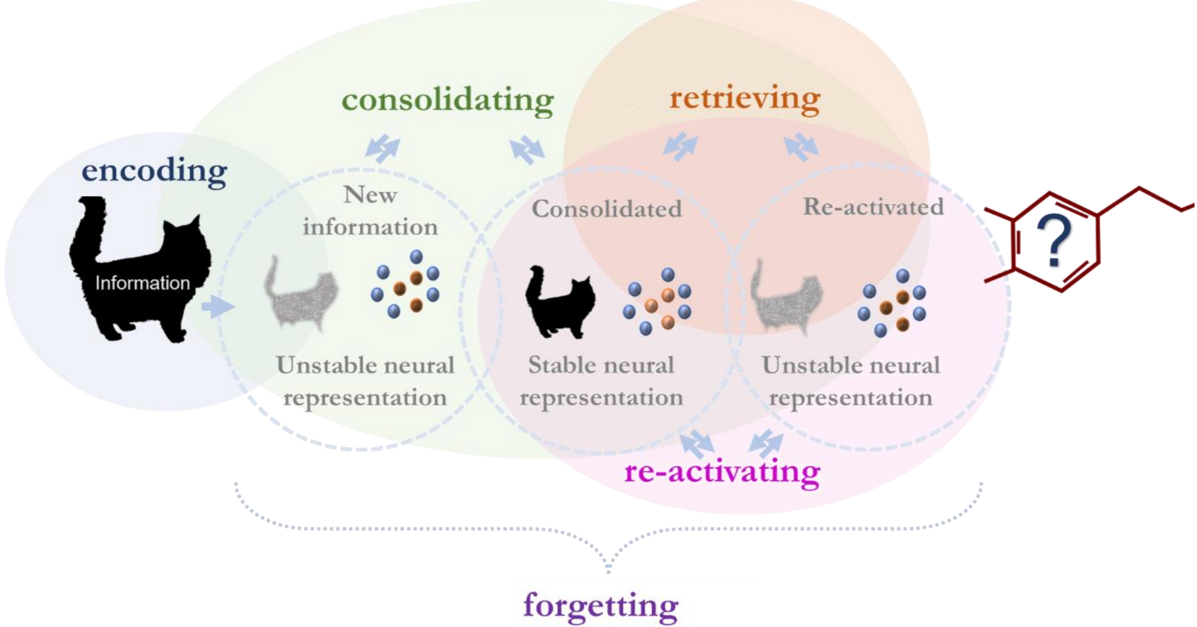


Figure 16: Memory stages and dopamine

Several memory processes together contribute to successful memory formation. Each of these processes also provide an opportunity for forgetting. It is not entirely clear which processes are influenced by dopamine.

cognitive problems, in whom we may want to try cognitive enhancement to ameliorate mild memory symptoms in the future.

Several techniques that are outlined in more detail in the next chapter are used here:

1. Behavioural tests
    a. The Remember-Know task which is the main outcome across Chapters III to VI
    b. The trail making task to control for dopamine's effects on executive control
2. Questionnaires
    a. Sleep questionnaires to measure self-reported sleep
    b. Mood questionnaires as a control for dopamine's effect on mood
3. Polysomnography
4. Structural MRI of medial temporal lobe subregions
    a. Volumetry
    b. T2 relaxometry

Aims and objectives

Aim:

The aim of this thesis is to investigate if dopamine's role on modulating different memory processes by carefully timing the administration of L-DOPA to target different aspects of memory evolution: encoding, consolidation, and retrieval. A secondary aim is to investigate the relationship between sleep physiology, hippocampal anatomy and L-DOPA's effects on memory consolidation.

Objectives:

To assess the effect of L-DOPA compared to placebo on encoding and retrieval separately ((Chapter III).

To assess the effect of L-DOPA compared to placebo on verbal memory tagging and consolidation (Chapter IV).

To assess the effect of nocturnal L-DOPA compared to placebo on nocturnal sleep architecture (Chapter (V).

To identify which elements of sleep architecture are associated with memory in elderly (Chapter V).

To assess whether L-DOPA's effects on sleep are associated to its effects on memory consolidation. (Chapter V).

To *explore* the relationship between memory, sleep and hippocampal and entorhinal cortex volumes and T2 relaxation times (Chapter VI).

Chapter II: General methods

This thesis comprises two studies:

DARet (**D**op**a**mine & **ret**rieval): A randomised double-blind study of levodopa's retrieval effects on reinforcing learning and episodic memory.

Chapter III

DOPAMIND (Targeting **dopa**mine to treat impaired **m**emory consolidation **in** neurodegenerative **d**isease): A double-blind placebo-controlled trial.

Chapters IV, V and VI

Ethical and regulatory approvals

All study procedures were completed in accordance with the Declaration of Helsinki.

The DARet study was carried out in the Brain Center in Southmead Hospital, North Bristol NHS Trust, Bristol, UK, and ethical approval was received by the University of Bristol Faculty of Health Sciences Research Ethics Committee (REF:12161).

The DOPAMIND study was carried out at CRICBristol, University of Bristol, Bristol, UK, and monitored by University Hospitals Bristol on behalf of University of Bristol. Ethical approval was granted by the South West - Central Bristol NHS Research Ethics Committee (REF: 16/SW/0028), by the Medicines and Healthcare Regulatory Authority (REF: 178711), and by the Health Research Authority

Both studies were sponsored by the University of Bristol.

Participants

We recruited healthy elderly (65+ years) native or fluent English speakers with normal or corrected-to-normal vision. A power calculation based on previous work (J.P. Grogan et al., 2015) suggested a minimum sample size of 26 (μ (0) = 53.2, μ (1) = 62.4, σ = 18.6) in detecting exogenous dopamine administration's effects on verbal memory using the conventional threshold for power (α = 0.05, β = 0.80) in a similar verbal memory task to that used here. I

aimed to test 30 people fully in both studies. This sample size is in line with previous studies looking at behavioural effects of dopamine on memory (Feld et al., 2014; Shohamy et al., 2006)

Recruitment

Volunteers were recruited from the BRACE Bristol Healthy Volunteer database in the North Bristol NHS Trust, Bristol, England, and from Join Dementia Research which is a UK based National Institute for Health Research maintained volunteer database. Upon contact, a brief phone screening was completed to rule out common reasons for exclusion.

Study procedures took place at the Brain Center in Southmead hospital (DARet) and at the CRICBristol, University of Bristol, Bristol (DOPAMIND). Participants were reimbursed a small sum for their time, £40 for DARet (£20 per visit) and £90 for DOPAMIND (£30 per visit) in total, and travel expenses.

Several people assisted in recruiting for these two studies. Major contributions were made by Dr James McErlane, Dr James Selwood, Dr Lisa Knight, Alex Howat, Will Mears, Nerea Irigoras Izagirre, John Grogan, Rachel Williams and Beth Ford.

Inclusion criteria

The inclusion criteria for DARet and DOPAMIND were the same; volunteers were over 65 years of age, native or fluent English speakers, and had normal or corrected-to-normal vision allowing them to read text on a computer screen.

Exclusion criteria

The exclusion criteria were selected to exclude anyone with clinically significant neurological or psychiatric conditions, such as dementia or mild cognitive impairment, as well as to ensure participants' safety to take part in the two studies.

Therefore, anyone who could not safely take L-DOPA or motilium were excluded from the studies reported here. Participants who took part in DOPAMIND were also screened for MRI eligibility and clinically significant sleep disorders.

The full list of exclusions is given in Appendix N.

Design and procedure

Both studies are placebo-controlled double-blind crossover studies. Each volunteer completed both arms (placebo and L-DOPA), and one week was allowed as a washout period between visits.

DARet

The purpose of this study was to assess L-DOPAs effects on retrieval and encoding.

This study was designed together with Dr John P Grogan.

In this placebo-controlled double-blind crossover study, participants took part in two testing sets, each entailing two visits to the Brain Center in Southmead Hospital, Bristol, England on consecutive days and three follow-up phone calls (Figure 17). The testing sets were separated by

a drug washout period of at least seven days between doses. The study procedures are summarised in Figure 18.

Both testing sets followed the same structure, with the exception that on one of the sets co-beneldopa was given, and placebo on the other.

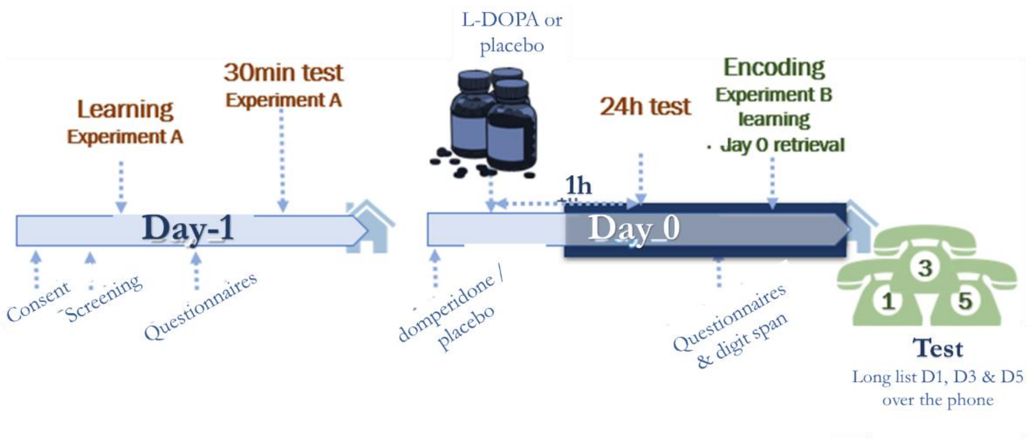


Figure 17: Study schematic for DARet

Volunteers were invited for two test sessions, each consisting of two consecutive days of testing. On the Day -1 the volunteers learnt an episodic verbal memory task, and their baseline performance was tested. On day 0 volunteers returned to site and they were dosed with L-DOPA or placebo before retrieval was tested. After this they learnt another episodic memory task before returning home. Their recall on this task was tested immediately and three times over the phone following a 1, 3, and 5 days' delays. The DARet study aimed to test the effect of L-DOPA on retrieval (orange, left) and encoding (pink, right).

Day -1

On the day preceding dosing, or Day -1 (D-1), volunteers were consented and screened to ensure they met the eligibility criteria. They then learnt two experimental tasks; a verbal episodic memory task and a reinforcement learning task. The latter is not reported here. The learning phase was followed by paper assessments, and 30 minutes later a baseline memory test.

Where necessary, volunteers' eligibility to take part was confirmed by a consultant neurologist (EJC) between D -1 and the day of dosing (D0).

Day 0

On Day 0 (D0), volunteers returned to the test site where they were first dosed with the domperidone and their blood pressure and heartrate was monitored. 30 minutes later, they received L-DOPA. At baseline and for 2 hours following Domperidone, volunteers' heart rate and blood pressure were monitored at 30-minute intervals as a safety procedure.

1h after drug administration, retrieval was tested for both tasks. Volunteers then completed the digit span test (J. P. Grogan et al., 2018), and they were offered a break before learning the second verbal memory task. Their learning for this test was measured immediately, and again over the phone 1, 3, and 5 days later.

On one of the testing sets the volunteer received a placebo and, on another L-DOPA, otherwise the testing sets were identical. A step-by-step for experimenters running the trial is appended (Appendix L).

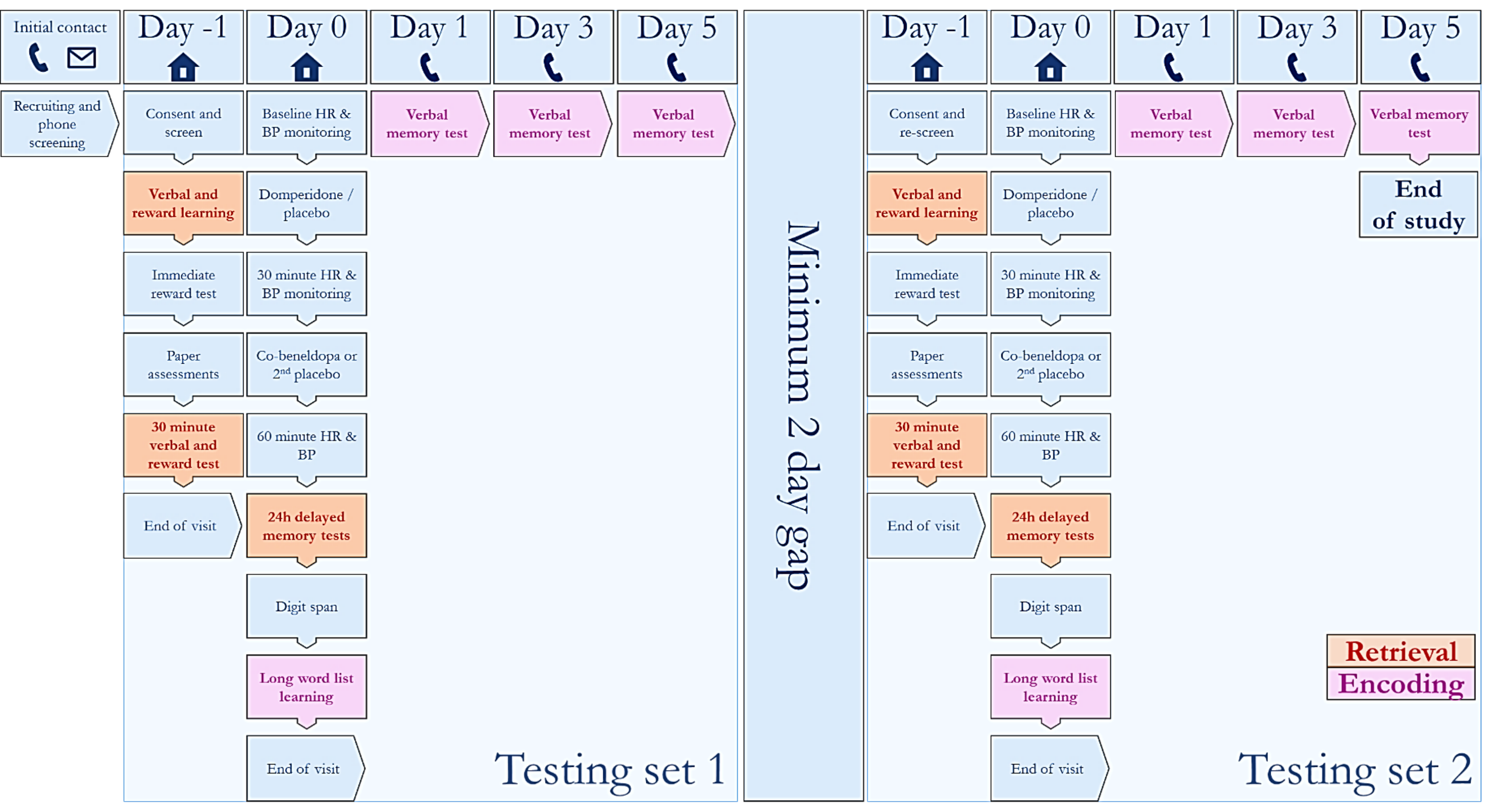


Figure 18: Summary of DARet study timeline

Each volunteer received both the co-beneldopa and the placebo but on different test visits. This study was designed to test L-DOPAs effect on both retrieval (orange) and encoding (pink).

DOPAMIND

A change to the DOPAMIND procedure was made after first 6 participants had been tested. Refer to Appendix A for details.

Screening

Following initial expression of interest, each volunteer was contacted over the phone by an experimenter who explained the study procedure. Interested volunteers were then screened for common exclusion criteria and, if they seemed eligible, booked for a full screening visit.

The screening visit started with a brief overview of the study and a tour of the sleep facilities before consenting. Following this, a brief medical history was recorded. If it was clear the volunteer was not eligible, the screening visit was terminated. Next, the participant completed short practice versions of the three experimental tasks (paired associates memory, verbal memory and motor sequence learning – note that only the verbal memory task is used in this thesis). A 12-lead ECG was taken and blood pressure, heart rate, body weight and height were recorded. Several paper assessments were also completed (Table 4, relevant paper assessments are also in Appendices F-J). Each screening visit took ~2.5 to 3h.

After the screening and before the first overnight visit a letter was sent to participants' GPs to inform them of participation. The participants also filled a sleep diary for 5-7 days prior to each overnight visit. Before they were booked for their first sleep visit, the consultant neurologist (EJC reviewed the screening information and confirmed eligibility.

Sleep visits

Each participant took part in two sleep visits. This means there were three visits in total

Refer to Figure 19 for a summary of the study timeline for each sleep visit. Apart from treatment allocation and payment, the sleep visits were identical and as follows:

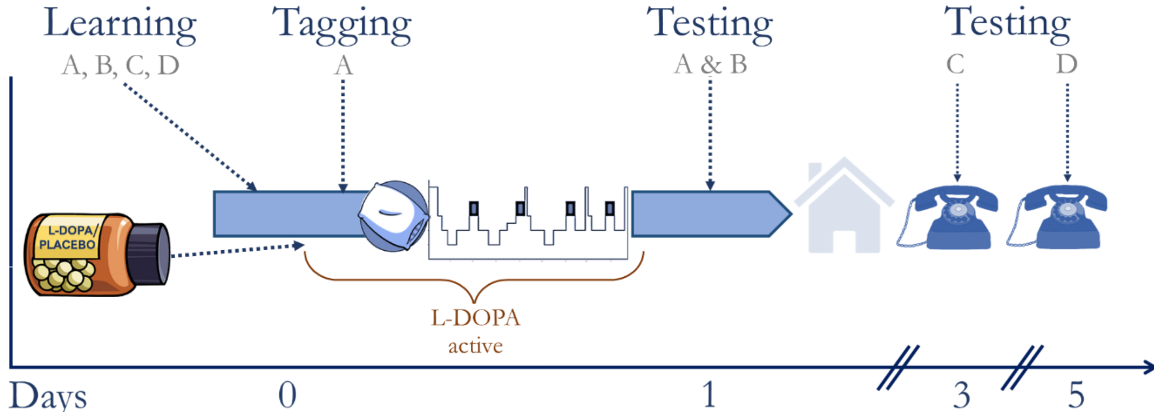


Figure 19: DOPAMIND study night timeline

Each study visit began with a confirmation of continued eligibility. This study was designed to test whether L-DOPA during sleep affects either of two processes.

Each volunteer completed two visits; on one of they received L-DOPA, on the other placebo. This figure is not to scale.

Evening

In the beginning of each visit, participants' continued eligibility to take part was ensured and baseline blood pressure, heart rate and electrocardiogram were recorded. Where there was uncertainty over eligibility, the consultant neurologist (Dr Elizabeth Coulthard) was consulted.

Volunteers was given dinner on-site, and the polysomnography set-up was started, prior to starting the experimental procedures. Each night's experimental testing schedule was worked backwards from the volunteers' natural bed time so that the testing began 3h and L-DOPA was given 2h before bed-time.

First, the participant completed the learning phases of the tasks, with domperidone being dosed in between. L-DOPA/placebo was given in a double-blind random order 30 minutes after learning the verbal task.

1h after dosing, the volunteers' memory on half the items for the paired associates and quarter of the items for the verbal memory task were prompted. This was followed by the Positive and Negative Affective Scale questionnaire as a control measure. Volunteers then had ~30 minutes to get settled before lights were switched off.

During the breaks between testing and monitoring, the PSG recording was prepared.

Dosing and monitoring

Prescribing, dosing and monitoring were performed by a study doctor. Details of participant physiological monitoring is given in appendix C.

Morning

Upon waking, the volunteers were given a couple of minutes before the experimenters removed the electrodes and participants were given a chance to shower and have breakfast. Morning memory was performed ~12h after learning where possible.

Visits started around 5-6PM in the evening and finished around 9-11AM the following day. At the end of both visits participants filled in a blinding verification form (Appendix K).

Phone calls

Following the visit, volunteers were contacted twice over the phone to test their memory on the remaining words, 3 and 5 days after learning.

For an example of each participants' timeline, see Figure 20.

Magnetic resonance

MRI scans were typically taken either at screening visit or in one of the mornings after all testing was completed. A small proportion of volunteers were scheduled for a separate visit for the MRI due to scanner availability clashes.

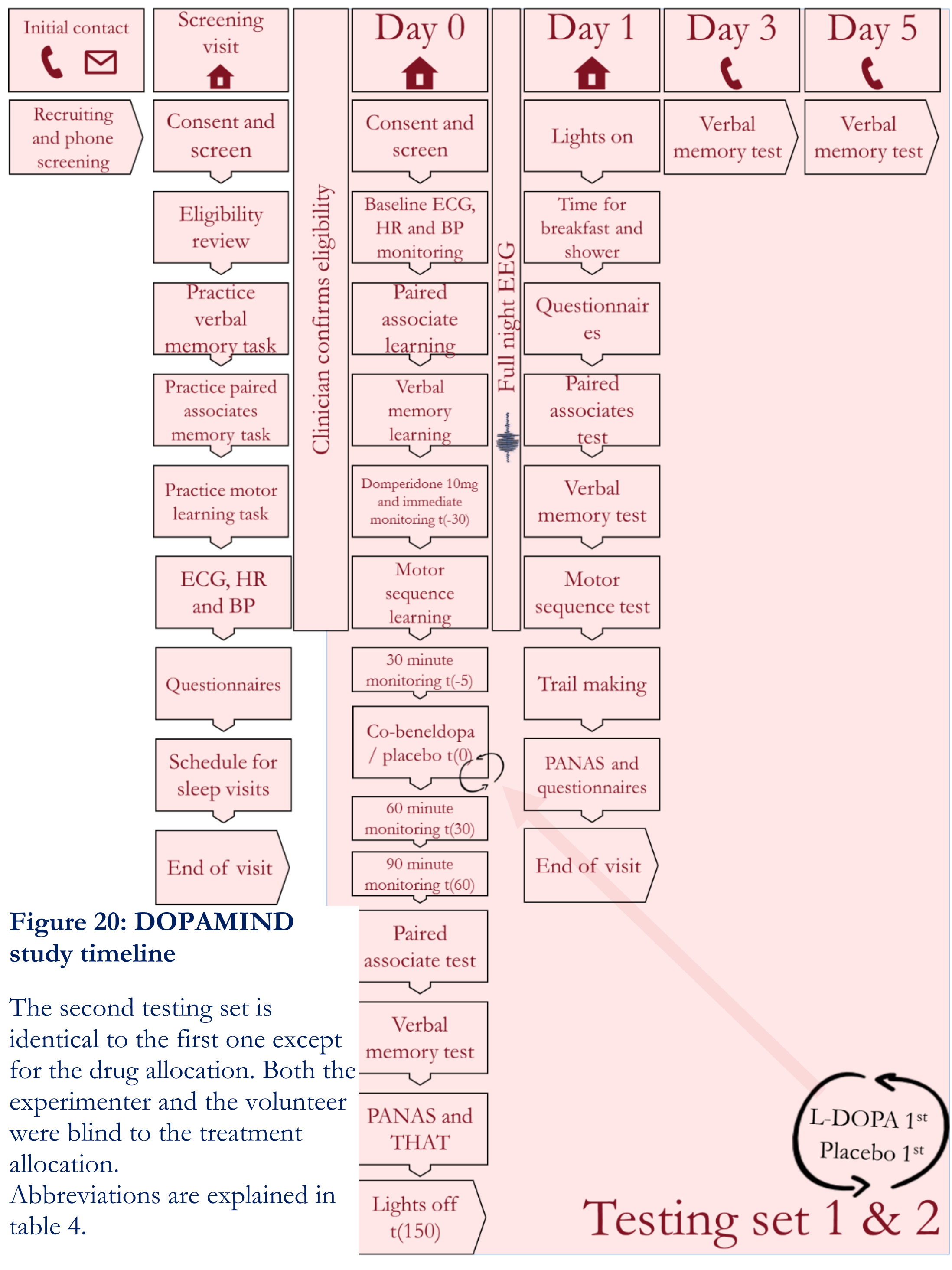


Figure 20: DOPAMIND study timeline

The second testing set is identical to the first one except for the drug allocation. Both the experimenter and the volunteer were blind to the treatment allocation.
Abbreviations are explained in table 4.

Materials

Both studies reported here were placebo-controlled within-subjects trials where participants were given L-DOPA and placebo at different test sessions in a random order. The randomisation and blinding procedures are given in Appendix O.

Treatment

In the DOPAMIND trial, participants received encapsulated co-beneldopa controlled release containing 200mg of L-DOPA. This form of L-DOPA is active in the system for up to ~12h.

In the DARet trial, participants received a 150mg L-DOPA dispersible. This type of L-DOPA reaches tMax in ~1.5h.

Further details about the treatment procedure is given in Appendix P.

Remember-Know task

The remember-know task (RKN (Tulving, 1987)) was used to assess verbal episodic memory. Slightly different paradigms were used in the different studies, and the delays between learning and retrieval varied across experiments. First, I will outline the broad principle of the tasks and then give an overview of the differences in administration between tasks. For summaries of differences between the two studies and how the drug administration relates to memory processes, see Table 3 and Figure 21.

Learning phase: Volunteers learnt a list of target words presented on a computer screen in a random order. For the DOPAMIND study, one list of words was learnt per condition (L-DOPA

and placebo), while for DARet, volunteers learnt two lists – one to prompt retrieval and another to prompt encoding.

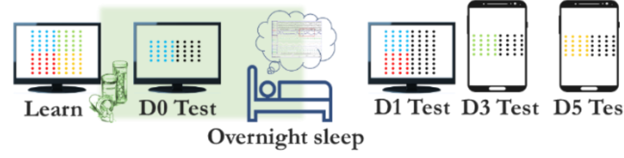
Test phase: Volunteers were shown a list of words with each word presented individually. Half of the items were targets (words present at learning) and half distractors (not present at learning). Participants were asked to judge whether a word was a target by judging it as 'OLD' (target) or 'NEW' (distractor). After each 'OLD' judgement volunteers were asked if they recollected the word ('REMEMBER'), recognised the word without recollection ('KNOW') or if they guessed ('GUESS'). They were instructed that a 'REMEMBER' response should be made only when they recollect the context of encoding, i.e. if they remembered what they thought of when they first saw the word. A 'KNOW' response should be made where there was no recollection. If a word was judged as 'NEW', volunteers chose if they were 'SURE' or made a 'GUESS'. The difference between 'REMEMBER' and 'KNOW' responses was explained to volunteers verbally with examples and then again on the computer screen before they started the task.

	DOPAMIND Learnt together		DARet	
			1st list	2nd list
	forgetting	**tagging**	**retrieval**	**encoding**
Total number of words	60	40	48	96
Number of breaks during learning	1	1	0	3
Number of times word present at learning	1	1	1	2
Instructions for learning	"Is this item alive"		"Read word aloud and try to memorise"	
Day -1 n target	N/A	N/A	24	N/A
Day 0 n targets	N/A	20	24	24
Day 1 n targets	20	40 (20 novel, 20 "tagged")	N/A	24
Day 3 n targets	20	N/A	N/A	24
Day 5 n targets	20	N/A	N/A	24

Table 3: Method: Remember-Know task differences

For the DOPAMIND study, volunteers learnt one list of 80 words. 60 of the words were used to assess memory persistence and forgetting curves, and 40 to assess saliency-tagging on L-DOPA. On Day 1 in the DOPAMIND study, the same targets as were tested as on Day 0, together with novel targets. Otherwise For the DARet study, volunteers learnt two separate lists, one with 48 words and one with 96. Recognition was tested with a proportion of targets (items present at learning) and a matched proportion of novel distractors. targets at test were always novel. Distractor words (words not present at learning) were never repeated twice.

DOPAMIND: Consolidating and forgetting



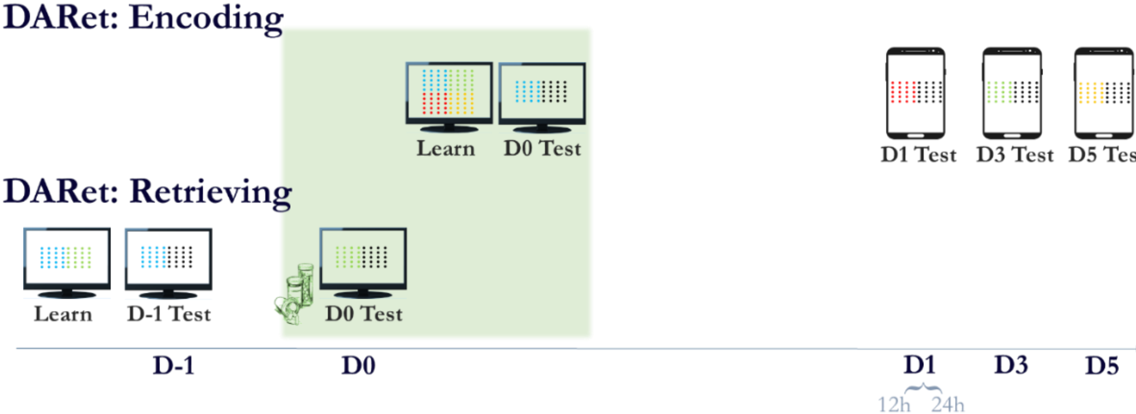


Figure 21: L-DOPA versus Placebo timing and memory for DOPAMIND and DARet studies

I targeted dopamine's effects on different memory processes by carefully timing the administration of L-DOPA (or placebo); timing shown as . Across three experiments, volunteers learnt lists of words (targets) on a computer screen. Each dotted colour (● ● ● ●) represents an individual word and colours represent lists. Their recognition memory was tested on the targets and unique distractors (●) using the Remember-Know paradigm either on-site using a computer screen of over the phone. Memory was prompter on the day of learning (D0), and/or 1, 3 and 5 days (D1, D3, D5) later. Each volunteer completed both a L-DOPA and a placebo testing session.

Remember-Know – DARet retrieval

The purpose of this test was to study L-DOPA's effect on retrieval. During learning on D-1 (day before dosing) volunteers were presented with 48 complete nouns on a computer screen. They were instructed to read the words aloud and try to memorise them for later. Each word was shown once (5s, separated by 2s fixation cross), and no responses to the words were made during learning. There were no breaks in the learning block (total duration = 5mins 36secs).

Memory was tested for half of the items 30 minutes (D-1, baseline), and for the remaining items 24 hours (D0) after learning (Figure 22). The D0 test was given when L-DOPA was at its peak concentration (~ 1h following dosing). In the test phases (D-1 and D0), participants responded using the using keyboard keys 1, 3, or 5, and they were advised to take as long as they needed to make a response. "1" corresponded to options on the left of the screen ('OLD' and 'REMEMBER'), "3" to options in the middle ('KNOW') and "5" to options on the right ('NEW' and 'GUESS).

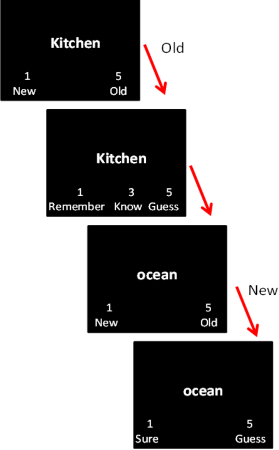


Figure 22: DARet Remember-Know paradigm

In the DARet Retrieval Experiment recognition was tested using the Remember-Know paradigm
At both tests (baseline and ON L-DOPA/ placebo), volunteers were shown 24 targets (present at learning) and 24 distractors (not present) words in a randomised order on the computer screen. For each word the volunteer had to first judge if a word was OLD (target) or NEW (distractor). They were then asked if they made a guess or if they were sure (for NEW judgements), or if they remembered, knew, or made a guess (for OLD judgements).

All text was presented in 'helvetica' font, lower case. The nouns were from four different categories (e.g. countries, cars, insects), with 12 words from each category present at learning. During test, half of the distractors (n=12) were from the same category as the learnt items and half were unrelated. The words were taken from rated publicly available word-lists (Murdock, 1976; Van Overschelde, Rawson, & Dunlosky, 2004). Different categories were used for different versions of the task, but this was not considered during analysis, see Appendix E for word lists.

Remember-Know – DARet encoding

The purpose of this verbal memory experiment was to assess whether L-DOPA enhances encoding. Learning (encoding) took place on D0 around 1.5 hours after dosing (ON L-DOPA/placebo). At learning, volunteers saw 96 complete nouns presented on a computer screen in block capital letter in 'helvetica' font (duration 5s, fix cross 2s). The words were first presented in a random order in two blocks, and then again in another random order in two blocks (n blocks = 4; n words per block = 48; n breaks =3; block duration = 5 minutes 36 seconds). Therefore, each word was shown twice to enhance learning. Volunteers were encouraged to take a break in between blocks to reduce fatigue.

Memory was prompted four times, immediately after learning (D0, ON L-DOPA/placebo), and 1, 3 and 5 days after learning (D1, D3, D5). At each test, 24 unique targets and distractors were tested, so each target was only tested once. Test on D0 followed the same procedure as the DARet retrieval experiment (Figure 22). On D1, D3 and D5 the volunteer was contacted over the phone for a recognition test following the Remember-Know procedure. Phone calls were scheduled before the participant left the study site. Each word was read aloud to the participant and then spelled out letter-by-letter. If the participant did not hear the word, this was repeated over. No definitions were given.

It is possible that fatigue from learning several lists with such short recovery periods in between affected performance. Furthermore, conducting tests over the phone when words were learnt on a computer screen may also affect performance and influence results. Memory tests were carried out over the phone in order to avoid burdening participants and researchers – several phone calls fell on weekends. To avoid confounding elements of these flaws in the study design, treatment order and word list orders were randomised and counter-balanced to avoid this influencing the study results.

Remember-Know – DOPAMIND

The main purpose of the DOPAMIND study was to examine the effect L-DOPA (compared to placebo) on consolidating and forgetting verbal information. Crucially, learning took place *before* dosing. Memory was probed four times:

Test	Delay from learning	L-DOPA active	List tested
D0	2h	ON	List A
D1	12h	OFF	List A & B
D3	3 days	OFF	List C
D5	5 days	OFF	List D

L-DOPA was active in the system during D0 test but not at other tests (Figure 21).

During learning, 80 words, drawn from 4 lists (A-D), were presented individually in a random, interleaved, order on a computer screen. Each word was presented once, and the learning session was divided into two blocks of 40 words. To enhance encoding, volunteers were asked to memorise as many of the words they could and prompted with a semantic question during learning – “Is this alive?”. Prior to learning they were given examples of alive (e.g. ‘tree’) and

non-alive (e.g. 'rock') items. They were also asked if they understood the instructions and encouraged to ask questions. Stimulus presentation time remained fixed (3.6 seconds, 1s fix cross) even if no response was made. The experimenter explained to the volunteers that they should not worry if they made a mistake, or if they did not have the time to respond to some words (Figure 23). See Appendix D for detailed instructions for experimenters administering the task.

On D0 (1.5h after learning), one of the lists (e.g. List A, 20 targets) and 20 distractors were presented to the volunteer on the computer screen. Participants responded verbally, and

responses were recorded by the experimenter. For OLD judgements, participants were asked if they REMEMBER, were FAMILIAR or made a GUESS – and for NEW judgements if they were SURE or made a GUESS. Note that instead of the word 'know' participants were presented with the word 'familiar' based on volunteer feedback during piloting. When a word was REMEMBER, the experimenter asked for justification for the answer. An example of a remember response for 'princess' could be remembering that they judged the word as alive or that they thought of a royal wedding when they learnt the word. If the volunteer gave an inadequate justification, such as 'I just remember the word was on the list, but I do not

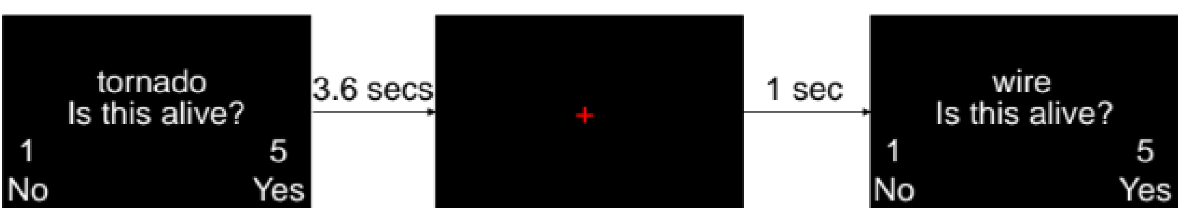


Figure 23: Memory learning phase in DOPAMIND

Each word was presented on the screen individually, and the volunteer was asked to read it aloud and decide if the word was living or not. This figure is illustrative only, in this iteration no numbers were displayed on the screen, and the stimuli is not shown to scale. Image courtesy of Dr James Selwood.

remember what I thought of when I saw it', the difference between a 'remember' and a 'know' was explained again and a 'know' judgement was recorded.

On D1, the same testing protocol was used but this time the targets presented at D0 (List A) were presented again along with 20 words learned but not presented at D0 (List B) and a matching number of unique distractors (n novel targets = 20, n re-tested targets = 20, n novel distractors = 40). On D3 and D5 volunteers were contacted over the phone and tested on the words (Lists C and D with novel distractors). Each word was first read aloud and then spelled out. No definitions of words were given to avoid biasing answers, but if the volunteer offered a definition the experimenter could either confirm it or not. At each phone call, 20 targets and 20 novel distractors were tested. The phone calls were 5-15 minutes in duration.

The D1, D3 and D5 tests for novel targets were used to assess L-DOPA's effect on consolidation or forgetting. The D1 novel and re-tested targets were assessed to study L-DOPAs effect on tagging 'important' memories with the rationale that when a word is presented a second time, it will be deemed to be more 'worthy' of being remembered later than a word that is presented once.

Note that the order of testing lists A-D was also randomised but for clarity I will refer to them in alphabetical order in this thesis.

Hardware and software

Computerised tests were carried out on Toshiba or Dell 64-bit laptops running Windows 7 pro. Volunteers made responses either laptop keys in the DARet study or using a made-to-order response pad from The Black Box ToolKit company in the DOPAMIND study. The pad was a USB 2.0 device in black (202mm x 137mm) with three white round buttons (30mm diameter)

placed a horizontal line in the middle of the box. Note that in the DOPAMIND study during testing, the experimenter recorded the responses.

All experimental tasks were programmed in the MATLAB environment (2015b or 2017a) using the Psychophysics Toolbox V3 (Kleiner, Brainard, & Pelli, 2007).

Magnetic resonance

Hardware: MRI scans were taken on a Siemens Magnetom Skyra 3T scanner with a 32-channel head-coil at the Clinical Research and Imaging Centre (CRIC) at the University of Bristol, Bristol.

Scanning procedure and sequences: Each volunteer underwent ~30 minutes of structural scanning with several sequences taken. A T1 3D magnetisation prepared rapid acquisition gradient echo (MP-RAGE) scan was taken using a standard sequence: acquired in sagittal plane, Repetition time (TR)= 2200ms; inversion time = 900ms; flip angle = 9 degrees; field of view (FOV) = 220 x 220 x 179 mm^3; acquired resolution = 0.86 x 0.86 x $0.86mm^3$ after 2-fold interpolation in k-space; scan time = 5.07min.

To image the hippocampus, I used a multi-contrast spin-echo sequence: acquired in coronal plane, TR = 4500ms; 3 echoes at TE 9.1, 72 & 136 ms, slice thickness = 1.5mm (including slice gap 15%), 34 slices (order interleaved), FOV = 220 x 220 x 34, acquired matrix size = 270 x 320 x 58; in-plane resolution = .34 x.34 following 2-fold interpolation; scan time = 5:09min. During acquisition this scan was auto-aligned perpendicular to the hippocampal long axis, and manually placed to image the space from ~1cm anterior to the head to ~1cm posterior to the tail. This scan did not therefore cover the whole brain. I also took two diffusion -weighted images (duration = 2x 3 mins 15 secs) but these scans are not discussed here.

Polysomnography

Participants in the DOPAMIND study had full night of polysomnography recorded for both study nights. A standard in-laboratory, polysomnography (PSG), including video was recorded using Embla Sleep Diagnostic at the CRICBristol, University of Bristol, Bristol, UK, sleep facility recorded on Embla RemLogic software Equipment (Natus Medical Inc., California). Recordings were taken from 12 scalp channels (F3, Fz, F4, C3, Cz, C4, M1, Pz, M2, O1, O2, and a ground electrode placed approximately between Cz/P3 and C3/Pz). Eye movements were detected by electro-oculogram recorded from E1 and E2 sites and muscle activity by electromyogram recorded below the chin. I also recorded two-lead electrocardiograms over the duration of the night.

Electrode locations were determined manually using the international standard 10/20/20 system. Electrodes were attached individually. Application time ranged from 1-2h and each recording started when I switched the lights off for the night and continued until an agreed wake-up time or until the volunteer naturally woke up. Electrodes were manually removed in the morning. All signals were sampled at 500Hz.

Paper assessments

Refer to Table 4 for a summary of all paper assessments. Further details of paper assessments are given in appendices.

	DOPAMIND	DARet	Purpose
Screening questionnaire	At screening visit	On D-1 of first session	Assess eligibility
MRI safety questionnaire	At screening and before scan	N/A	
Digit span assessment	N/A	Each visit	Assess working memory. The results are published elsewhere (J. P. Grogan et al., 2018).
Beck Depression Index	At screening visit		
Lille Apathy Rating scale (Sockeel et al., 2006)	N/A	On D-1 of first session	
Barratt impulsivity scale (Pacini & Epstein, 1999)	At screening visit	On D-1 of first session	
Rational-Experiential Inventory scale (REIS) (Pacini & Epstein, 1999)	At screening visit	On D-1 of second session	
Pittsburgh Sleep Quality Index	At screening visit	N/A	
Montreal Cognitive Assessment (MoCA) Versions 7.1, 7.2 and 7.3, (Nasreddine, Phillips, & Chertkow, 2012)	At screening visit (only one version per volunteer)	On D-1 of first session	Measures general cognitive ability and where a score less than 18 may be indicative of memory impairment.
St Mary's Hospital Sleep Questionnaire (Ellis et al., 1981)	Each morning of sleep visits	Each visit	
Depression Anxiety Stress Scales (DASS) (Boyle, 1985)	At screening visit	On D-1 of second session	Measures depression, anxiety and stress
Toronto Hospital Alertness Test (THAT) (Shapiro et al., 2006)	Each morning of sleep visits	N/A	Measure alertness
Trails A and B (Sanchez-Cubillo et al., 2009)	Each morning of sleep visits	N/A	Measure alertness
Positive and Negative Affect Schedule (PANAS) (Zevon & Tellegen, 1982)	Each morning and evening of sleep visits	N/A	Measures current mood
Leeds Sleep Evaluation Questionnaire (LSEQ)	Each morning of sleep visits	N/A	
Sleep diary	For one week prior to sleep visits	N/A	
Unified Parkinson's disease rating scale (UPDRS)	N/A	On D-1 of second session	To control for undiagnosed Parkinson's disease

Table 4: Method: Paper assessments

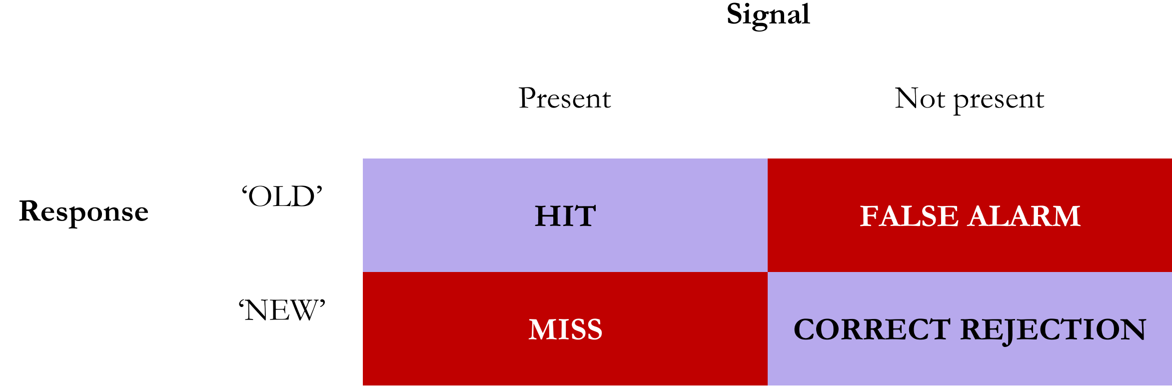
Analyses

Verbal memory

Signal detection theory

The first phase of the RKN forced-choice recognition memory task (i.e. 'OLD', 'NEW' judgements) was evaluated using the signal detection theory (SDT; (Birdsall & Roberts, 1965; Treisman, 1964)). In short, SDT can be used to explain volunteers' response strategies for discriminating between signal (targets) and noise (distractors) using the distribution of 'OLD' and 'NEW' responses. SDT assumes that the likelihood of detecting signal and noise are expressed as two often overlapping Gaussian equal variance probability distributions. The more overlap there is between the distributions, the less likely the volunteer is to discriminate signal from noise. Each response in the recognition task can therefore be classified as a Hit (*H*), a Miss (*M*), a Correct Rejection (*CR*), or a False Alarm (*FA*). *H* refers to a response where an item present at learning was correctly identified as 'OLD' while a 'NEW' response would in this case be a *M*. A word that was not present during encoding, a correct 'NEW' response would be classified as a CR while an incorrect 'OLD' response would be a FA (Figure 24).

Each volunteers' signal detection technique can be described using D' (dee-prime) and criterion (c, Figure 25). The D' is the main outcome measures throughout Chapters III to VI. *D'* describes discriminability between targets and distractors by quantifying how well a volunteer detects signal from noise, or targets from distractors – it is similar to the discrimination index and can be used to measure accuracy. *D'* is the distance between the means of the aforementioned Gaussian distributions. A large D' indicates that signal is easy to detect from noise, i.e. participants have good performance, while a small D' refers to considerable overlap between the distributions.



		Signal	
		Present	Not present
Response	'OLD'	HIT	FALSE ALARM
	'NEW'	MISS	CORRECT REJECTION

Figure 24: Signal Detection Theory: Responses possibilities

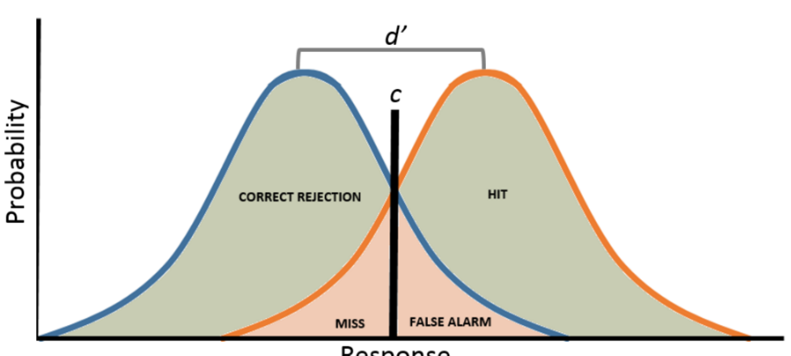


Figure 25: Signal Detection Theory: Model

D' refers to how ability to discriminate between signal (distribution on the right) and noise (distribution on the left), while c refers to response bias. Here, c = 0.

D' can be considered a measure of accuracy. However, D' does not fully explain response behaviours. For example, D' could be the same for three volunteers, one of whom responded 'OLD' each time, one of whom responded 'NEW' each time and one of whom made an equal number of random 'OLD' and 'NEW' responses. For this reason, c is also needed to explain the response tendency, as it quantifies how liberal – or likely to identify a target over uncertainty – the participant is in their responses.

C can be thought of as the difference between a "perfect" volunteer who has no internal response bias, and the measured responses. When c = 0, the volunteer has no bias (i.e. *p(FA)* = *p(M)*), while a negative value indicates they are more likely to accept a word as a target under uncertainty (i.e. *p(FA)* > *p(M),* or the c line in is shifted to the left).

They can be quantified as,

$$D' = Z(p(H)) - Z(p(FA))$$

and

$$c = \frac{(Z(p(H)) + (Z(p(FA)))}{2}$$

where $Z(p(H))$ and $Z(p(FA))$ are the z-transformed probabilities for *H* and *FA* respectively.

The primary outcome measures were D' and c. Secondary outcome measures were the number of Hs, and FAs. CRs and Ms were not considered in the analyses as they provide no additional information because *p(H)* + *p(FA)* = *p(CR)* + p(*M)* = *1*.

Remember-Know responses

When analysing REMEMBER and KNOW responses, proportions of these responses were used while incorrect and GUESS responses were disregarded;

$$p(R) = \frac{R|H}{H} \qquad p(K) = \frac{K|H}{H}$$

H; HITS

R; REMEMBER

K; KNOW

MLMS

Mixed linear models (MLMs) are an extension of simple linear models, such as analysis of variance. The main benefits of MLMs are that they allow for non-independence and missing data. These models allow both fixed and random effects as opposed to fixed effects only. Random effects are variables that allow for grouping the data, while fixed effects can either be continuous or discrete non-grouping variables. If a variable is considered a random effect, each of its levels' means are assumed to represent a sample of means drawn from a normally distributed population with a global mean and distribution. As we are dealing with only a subset of elderly adults from the entire population, I could fit the participants in as random effects.

Treating participants as random factors in mixed linear modelling is standard practice in psychological sciences (Barr, Levy, Scheepers, & Tily, 2013; Gelman, 2005; Janssen, 2012 ; Magezi, 2015).

MLMs come in varying levels of complexity. In an intercepts-only model, each participant has their own deviation from the group mean, but the slope to which memory scores decay over time or the extent to which they are affected by the treatment is assumed stable across groups. In other words, each volunteer has their unique "baseline" but shared "decay rate". The next level of complexity is a model that fits in random slopes but not intercepts. Here, the baseline is assumed the same across subjects (everyone has same level of encoding) but the rate at which their memories decay or are affected by treatment and time are assumed to vary. Other types of models assume both random slopes and intercepts, consider random effect on possible interaction terms of the fixed effects, and account for correlativity between random slopes and intercepts. Mixed linear models therefore allow fitting in varying degrees of complexity, but over-fitting the models with a modest dataset can give results that are not generalisable. Therefore, care needs to be taken in choosing the right model.

Here, I aim for a maximal model fit that can be justified by the study design – this is to say the model should still be feasible and not include unnecessary and over-complicated interactions but include all possible physiologically plausible parameters. This 'maximal' approach is demonstrably most suitable for confirmatory hypothesis testing (Barr et al., 2013). Where possible, I fitted between-participant variability in baseline memory, rate of decay over time or over types of tests, and in response to the medication in our models. Memory test scores (either D' or c) were used as predictors for each model. A caveat of this the maximal approach is the possibility of overfitting the model (Bates, 2018).

I did not account for correlations between slopes to avoid this problem. However, some overfitting still occurred. Where problems with overparameterization were encountered, I simplified the model by removing slopes in a stepwise manner. I accepted the most complex model that could be used to explain our data without overfitting. An alternative approach would be to start with a minimal model and work 'up' to maximise model complexity. The resulting final model from either approach should be the same.

When two equally complex models (e.g. slopes for one fixed effect but not the other) could be fitted as the maximal model, I chose to use the more physiologically plausible model. An alternative and possibly more robust approach would be to compare the model fits (for example the Akaike information criterions for both models) and decide on the best fit.

MLMs were conducted on R 3.5.3 using Rstudio, lme4 (LME4-Authors, 2019) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2019). These packages provide diagnostic tools to identify overfitting, and the latter uses the Kenward-Roger approximation method for obtaining degrees of freedom estimations, and F and p statistics. Once models were obtained, R^2 were calculated for models using the MuMIn version 1.42.1 (Barton´, 2018) package in R. This

function provides both marginal (R^2m) estimates of variance explained, which indicates how much of the variance in the response variable is explained by the fixed effects.

In Chapter IV, I included the participants as random effects and as fixed effects I included treatment and the memory test delay (Day 1, Day 3 and Day 5, or Day 1 tested and re-tested, depending on the analysis). In Chapter V, I also considered sleep physiology measures as fixed effects. I included the treatment as a binary variable (L-DOPA vs placebo). All variables included in the model were mean centred but not scaled.

The final mixed effects model with by-subject random intercepts and slopes was fitted using the following syntax in LmerTest. The practical application of this syntax is explained in detail elsewhere (Kuznetsova, Brockhoff, & Christensen, 2017)

*Response ~ Delay * Treatment + (Delay + Treatment || subject)*

Where the *response* variable is the relevant memory test score (e.g. D'), *Delay* is the time of the test (e.g. Day 0, 1, 3 or 5) or type of the test (e.g. 'tagged' or not), and *Treatment* is either L-DOPA or Placebo. The *(Delay + Treatment || subject)* term refers to the random intercept for each subject with random slopes for both *Delay* and *Treatment* without accounting for by-subject variability for the interaction term.

This can also be expressed as follows:

$$Y_{sjk} = \beta_{0i} + \beta_1 X_{1sj} + \beta_2 X_{2sk} + \beta_3 X_{1sj} X_{2sk} + e_{sjk}$$

Where,

β_{0i} = $\beta_0 + s_{0s}$

Y_{sjk} : The response variable for the $_s{}^{th}$ subject at the $_j{}^{th}$ time point with $_k$ dose

β_{0i} : Intercept with by-participant variation

$\beta_1 X_{1sj}$: Slope of X_1 for the $_s{}^{th}$ subject at the $_j{}^{th}$ time point

$\beta_2 X_{2sk}$: Slope of X_2 for the $_s{}^{th}$ subject with $_k$ treatment

$\beta_3 X_{1j} X_{2k}$: Time point * treatment interaction for the fixed effects. Note there is no by-subject variation included in this term.

s_{0s} : Error term

Bayesian analyses

The above tests turn a p-value, which is the probability of the observed – or a more extreme – effect given the null hypothesis. It can be used to assess the probability of the observations given the null hypothesis, not to assess the probability of the null hypothesis given the observations. Traditional null hypothesis statistical testing does not address the latter.

I addressed this issue by obtaining the Bayes Factor (BF). Table 5 gives an intuitive summary for how to interpret BFs.

Another strength of Bayesian analyses is that the posterior credible intervals are predictors for the true value of the test statistic (Quintana & Williams, 2018). Traditional confidence intervals used in null hypothesis significance testing give the average confidence for the test statistic in hypothetical replication studies in the long run (Greenland et al., 2016).

Bayesian statistics consider the observed data and prior beliefs, or priors, when assessing evidence under the following rules:

$$P(D|H_1) = \frac{P(H_1|D)*P(D)}{P(H_1)}$$

$$P(D|H_0) = \frac{P(H_0|D)*P(D)}{P(H_0)}$$

$$BF_{10} = \frac{P(D|H_1)}{P(D|H_0)}$$

Where D is the observed data, H_1 is the alternative and H_0 is the null hypothesis. $P(D|H_1)$ is the probability of the observed data under the alternative hypothesis, while $P(D|H_0)$ is the probability of the same data under the null. In short, BF quantifies uncertainty (i.e. how much more likely is the data is in one scenario compared to another).

All Bayesian analyses were performed using JASP version 0.9.2.0 (JASP, 2018). When using Bayesian approaches, a prior distribution needs to be defined. The prior and the observed data together are then used to estimate a posterior distribution. In this thesis, I use uninformed priors across analyses. For the uninformed distribution I chose a Cauchy distribution with a mean of 0 and an interquartile range of .5 [$\delta \sim$ Cauchy (0, .5)]. In other words, I predicted that the δ lies between -.5 and .5 with a 50% confidence. I selected this one as the δs in cognitive neurosciences typically are within those bounds, and as I did not have an informed prediction for the effect sizes (Jeffreys, 1961; Marsman & Wagenmakers, 2017).

BF_{10}	BF_{01}	Interpret as
>100	< 1/100	Extreme evidence for H_1
30 – 100	1/30 – 1/100	Very strong evidence for H_1
10 – 30	1/10 – 1/30	Strong evidence for H_1
3 – 10	1/10 – 1/3	Moderate evidence for H_1
1 – 3	1/3 – 1	Anecdotal evidence for H_1
1	1	H_1 and H_0 equally likely
1/3 – 1	1 – 3	Anecdotal evidence for H_0
1/10 – 1/3	3 – 10	Moderate evidence for H_0
1/10 – 1/30	10 – 30	Strong evidence for H_0
1/30 – 1/100	30 – 100	Very strong evidence for H_0
< 1/100	>100	Extreme evidence for H_0

Table 5: Method: Bayes Factors

The BF is the likelihood ratio of the observations under one hypothesis over another. This can be expressed either as BF_{10} or as BF_{01}, where the former quantifies how likely the observed data are under the alternative compared to the null hypothesis, and vice versa for the latter. BF_{01} of 3 would mean that the observed data was 3 times more likely to occur under the null compared to the alternative hypothesis. Using this method, I can utilise the observed data in assessing the likelihood of the null hypothesis (against the alternative).

Note that BF_{10} is the reverse of BF_{01}. This table is based on suggestions in (Jeffreys, 1961; M. D. Lee, Wagenmakers, E-J., , 2013).

Magnetic Resonance

First-level

Segmentation: The open access Automatic Segmentation of Hippocampal Subfields (ASHS) (version: rev103, dated 12/06/2014). procedure (Yushkevich et al., 2015) was used to mask HC subfields and adjacent regions. This toolkit uses a machine learning approach to create a labelled HC subfield atlas based on manually segmented subfields. As the training set, the ASHS working group Atlas (dated 16/04/2014) (Yushkevich et al., 2009) was used. This set contains segmented maps for healthy older adults and older adults living with mild cognitive impairment with labels for subfields CA1, CA2, CA3, dentate gyrus, and subiculum, and surrounding regions, entorhinal cortex, collateral sulcus and Brodmann areas 35 and 36 (together the perirhinal cortex). The masks for the hippocampal subfields, and entorhinal cortex are used in this thesis.

Following the learning phase, ASHS can be used to segment new hippocampi. The toolkit requires both T1 and T2 weighted scans as input with the latter having sufficient resolution for detecting subfield boundaries. I used the MP-RAGE and the multi-contrast spin echo sequences with echoes summed together to form a single T2-weighted image.

Prior to running the ASHS procedure, brain extraction was performed for the T2-weighted images in FSL bet2 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; S. M. Smith, 2002), which removes non-brain tissue from the images. The brain extractions were visually inspected and fractional intensity threshold was adjusted case-by-case where necessary. T2 maps were then created and T1-weighted images were registered to T2 space using FSL FLIRT (Jenkinson et al., 2012) rigid-body transformation.

Processing took up to 25 hours per brain, following which each ASHS mask was visually inspected to ensure high standard. An example of how the procedure performed on this dataset is given in Figure 26.

Volumetry: The main outcome for the volumetric measure was the hippocampal volume in mm^3, which was extracted from the number of voxels within each segmented medial temporal lobe subregion. Left and right subfields were collapsed together for all analyses.

T2 relaxometry: Distributions of T2 relaxation times for medial temporal lobe structures were obtained from the volumetric masks and T2 maps. The main T2 distribution measure was the standard deviation, i.e. intraindividual mask-specific standard deviation of absolute T2 relaxation

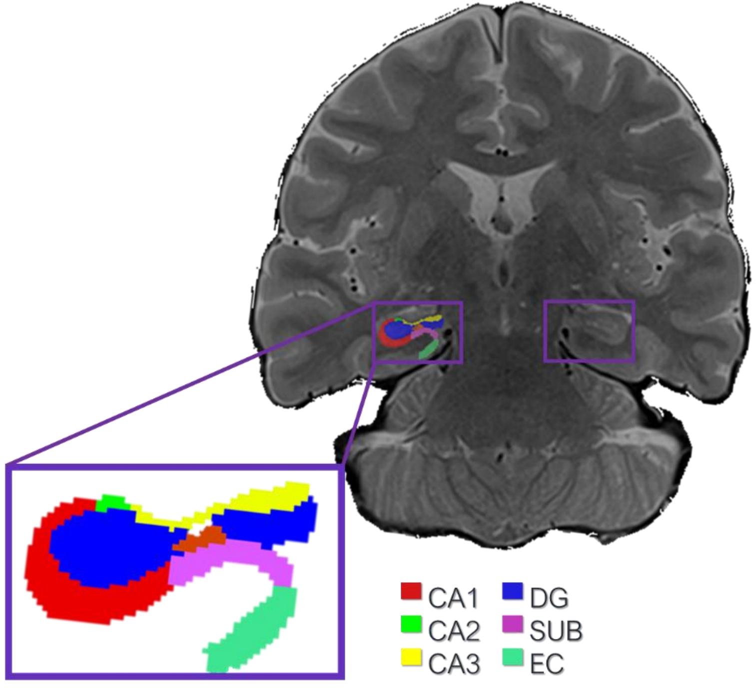


Figure 26: Hippocampal segmentation procedure

times. In other words, the standard deviation of T2 relaxation times from each voxel (derived from the T2 maps) in each mask (e.g. CA1) for every participant individually. Standard deviation was used as some attributes (e.g. increased water content) increase and others (e.g. increased iron) decrease T2. Both increased water and iron can be related to pathology, and if one is not dominant over the other, measures of central tendency can mask them out, while measures of distribution width do not.

All analyses collapse across hemispheres, this brings with I the risk of losing information to do lateralised function. While outside the scope of this thesis, future exploratory analyses on lateralised hippocampal relationships with sleep may be of interest.

Second-level

The relationship between memory and hippocampal subfields was assessed using correlation analyses. Group-level analyses were performed using R versions 3.5.2 and 3.5.3 (RCoreTeam, 2013).

Contributions

All MRI scanning was performed at CRICBristol, University of Bristol. Each scan was operated by two people: a 1st scanner who operated the scanner and a 2nd scanner who assisted during scans where needed. The 1st scanner screened participants, prepared the scanning environment and operated the scanner.

Majority of the scans were 1st operated by me (the author) and she was present at each scan. Other first operators were Aileen Wilson, Alfie Wearn, Volkan Nurdal and Michael J Knight. 2nd operators were Alfie Wearn, James Selwood, James McErlane, Volkan Nurdan, Carlos Muños, Rachel Williams, Beth Ford, Will Carr, John Grogan, and George Averill.

The scanning sequences were either standard sequences available in any SIEMENS MAGNETOM SKYRA scanner or they were adapted from standard sequences by Michael J Knight.

Polysomnography

Manual sleep scoring was performed in 30s epochs using standard criteria on REMLogic by Will Carr and Oliver Radtke, and 10% of randomly selected recordings were quality-controlled by me. Minutes in stage 1, stage 2, stage 3, REM. awake, asleep and total time in bed were extracted in minutes. Spindle characteristics were then isolated with in-house written MATLAB scripts using the EEGlab toolbox. Electrodes were re-referenced to contralateral mastoid and empty and high variance epochs were removed.

Following this, solely data from the Cz electrode was used. First, the channel was visually inspected and epochs with high noise or clear artifacts were removed manually. Data was then filtered (high pass 11Hz, low pass 17Hz) and rectified. After this it was smoothed using a moving average window of 200ms. Then, data was resampled to 100Hz (from 500Hz) for computational efficacy. An event was automatically marked as a spindle if the threshold exceeded the 90th percentile for that data set (i.e. sorting data into an ascending order and including top 10%) for .5 – 3 seconds and a minimum .5s gap to the next / previous spindle was present. The spindle detection was performed by Will Carr with the help from Ullrich Bartsch.

Contributions

All sleep studies were carried out at CRICBristol, University of Bristol. Each night was set up and operated by me. Several others assisted in aspects of setting up, such as taking scalp measurements, gluing electrodes and removing them. The following people assisted: Alfie

Wearn, Volkan Nurdal, James Selwood, James McErlane, Volkan Nurdan, Carlos Muños, Rachel Williams, Beth Ford, Will Carr, John Grogan, George Averill, Lisa Knight, Will Mears, Luke Emrich-Mills and Claire Durant.

The scanning sequences were either standard sequences available in any SIEMENS MAGNETOM SKYRA scanner or they were adapted from standard sequences by Michael J Knight.

Significance

I used the conventional two-tailed α threshold of .05. However, with multiple tests, the probability of false positives increases. I corrected for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995), which decreases the false discovery rate (FDR). This was performed using the p.adjust function in R.

In the Benjamini-Hochberg procedure, dependent tests are considered together. Here, I took families of tests – for example each subfield correlation against a single memory score – to correct p-values so, that the α threshold for all the test considered together is .05.

Chapter III: Dopamine in encoding and retrieval

Data reported in this chapter was collected as a part of the DARet study.

Introduction

During encoding, a specific neural representation, or an engram, of learnt information is created (Semon, 1909; Tonegawa, Liu, et al., 2015). When the memory engram is later retrieved, at least in the context of explicit memories, the same patterns of neural activation that were present at encoding are reconstructed (Nyberg, Habib, McIntosh, & Tulving, 2000; Schacter & Wagner, 1999). Attention, reward and previous experiences may tag memories encountered at encoding

for preferential storage and retrieval (Redondo & Morris, 2011). I stipulate, and I will provide evidence in Chapters IV and V that dopamine plays a role in selecting memories to-be-kept, and that it does so during tagging and over periods of consolidation during sleep. Does dopamine only affect memory in the time *after* learning, or does it bias other memory stages too?

Evidence in animals suggests that dopamine plays a role in encoding. For example, in drosophila, dopamine at encoding enhances learning (Berry et al., 2012; Berry et al., 2018). In mice, optogenetic stimulation of dopamine during encoding increases memory by enhancing subsequent memory reactivation during sleep (McNamara, Tejero-Cantero, Trouche, Campo-Urriza, & Dupret, 2014). Memory reactivation is well known to support memory persistence.

Memory reactivation can also be studied using depth electrodes in humans. In human intracranial recordings, the dopaminergic connections between the nucleus accumbens and the hippocampus, along the VTA-hippocampal loop have been shown to activate in response to encoding unexpected stimuli (Axmacher et al., 2010). This encoding effect was not directly associated with enhanced memory, but it was associated with later hippocampal activity that predicted memory performance. Note that these findings align well with what was seen in mice (McNamara et al., 2014). While in humans this was not associated with memory performance directly, this suggests that dopamine has the potential to bias memory.

Using dopamine-like medications in healthy volunteers to study dopamine's effects in modulating encoding has yielded further supporting evidence. Administration of dopamine precursor L-DOPA prior to learning, has been shown to increase theta coherence, working memory and memory persistence in humans (Eckart, Fuentemilla, Bauch, & Bunzeck, 2014). Theta activity both in the cortex and in the hippocampus supports memory encoding and biases spindle activity during sleep (Hasselmo, 2005; Jarovi et al., 2018) . Sleep spindle activity supports both memory consolidation and strategic forgetting (Cairney et al., 2018; Dehnavi, Moghimi,

Sadrabadi Haghighi, Safaie, & Ghorbani, 2019). Therefore, the findings from these two studies suggest that, similar to mice, in humans dopamine at encoding may tag memories to be later captures for consolidation.

However, others have found disparate effects. In Parkinson's disease patients being ON their usual dopaminergic medication during encoding impaired memory compared to being OFF medication (J.P. Grogan et al., 2015). When taken OFF dopamine replacement therapies Parkinson's disease patients' motor symptoms return quickly. It is therefore interesting that these patients perform better when taken off their usual medications. Overall, evidence in support of dopamine biasing encoding in humans is patchy and findings so far have been inconsistent. Most studies that have shown dopamine medication to attenuate memory do not target encoding specifically (Chowdhury et al., 2012; J.P. Grogan et al., 2015; Knecht et al., 2004; Shellshear et al., 2015). Therefore, dopamine's role on encoding and consolidation remains unclear.

Yet, dopamine's effects on retrieval are likely the least studied. In rats, the D2 agonist haloperidol has been associated with enhanced retrieval. Sara (1986) showed that haloperidol had no effect on memory when injected during training, but after a 25-day retention interval, when injected prior to retrieval, rats produced fewer errors than rats given saline. Larger doses of haloperidol were associated with less forgetting (Sara, 1986). Similar effects of haloperidol in rats have also been found in other studies (Chugh, Saha, Sankaranarayanan, & Sharma, 1991). Therefore, dopamine may enhance retrieval in rats.

To our awareness there are not many studies that specifically investigate dopamine's involvement on retrieval in humans. Dopaminergic striatal regions activate during recognition retrieval (Clos, Schwarze, Gluth, Bunzeck, & Sommer, 2015; Han, Huettel, Raposo, Adcock, & Dobbins, 2010; Kim, 2013; Schwarze, Bingel, Badre, & Sommer, 2013; Spaniol et al., 2009). This suggests that dopamine may be involved in retrieval, particularly in recognition memory settings.

Supporting evidence of dopamine enhancing recognition comes from a trial where healthy volunteers were dosed with haloperidol. In an fMRI study, Clos and colleagues (2019) showed that haloperidol increased recognition memory when administered to target retrieval 24 hours after learning. The dopamine-driven boost in retrieval was also associated with increases in activity in the dopaminergic midbrain (Clos, Bunzeck, & Sommer, 2019). Therefore, dopamine during retrieval may enhance recruiting brain regions that support recognition memory.

To illuminate dopamine's effects on encoding and retrieval, I composed a second double-blind placebo-controlled randomised trial, where exogenous dopamine administration was times carefully to target encoding or retrieval. I specifically sought to investigate if L-DOPA biases memory during encoding or retrieval.

Method

The method is reported in Chapter II (pg. 77). A short reiteration of the method is given here.

Design and procedure

In this placebo-controlled double-blind treatment-order randomised within-subjects study, healthy elderly participants were dosed both with 150mg of L-DOPA and placebo but on separate sessions. The volunteers completed two testing sessions, each spread over 1 week. The visits were identical except for treatment allocation (Figure 27).

Encoding

To test dopamine's effects on encoding, volunteers were given L-DOPA approximately 1.5h before learning a word list. After learning, participants were offered a short break and refreshments before their memory on a quarter of the items was tested (baseline) using the Remember-Know paradigm (pg. 90). The rest of the items were tested 1, 3, or 5 days later, using the same paradigm. Therefore, L-DOPA was active in the system at learning and at the first test, but not at Day 1, 3, and 5 tests. As the findings from the first test would be difficult to interpret in light of our research aims, I did not analyse results from the immediate test.

Retrieval

In order to study dopamine's effects on retrieval, participants learnt a list of 48 words one day before receiving L-DOPA (Day -1). Their memory was tested for half the items 30 minutes later (baseline) and the other half 24 hours after learning. Crucially, the 24 h test (D0) was completed when L-DOPA was at its peak concentration.

Analyses

Encoding: Mixed linear models were used to assess D' (i.e. a measure of accuracy) and criterion (same as response bias) across time. For each model the predictor variable was D' or criterion, the fixed effects were time (Day 1, Day 3 and Day 5) and treatment. I then conducted confirmatory pairwise comparisons (either t-tests or Wilcoxon's signed ranks together with Bayesian analyses) for all measures of interest.

Retrieval: Pairwise comparisons (as above) were carried out for all measures of interest for the Day -1 (baseline day, before dosing) and Day 0 tests.

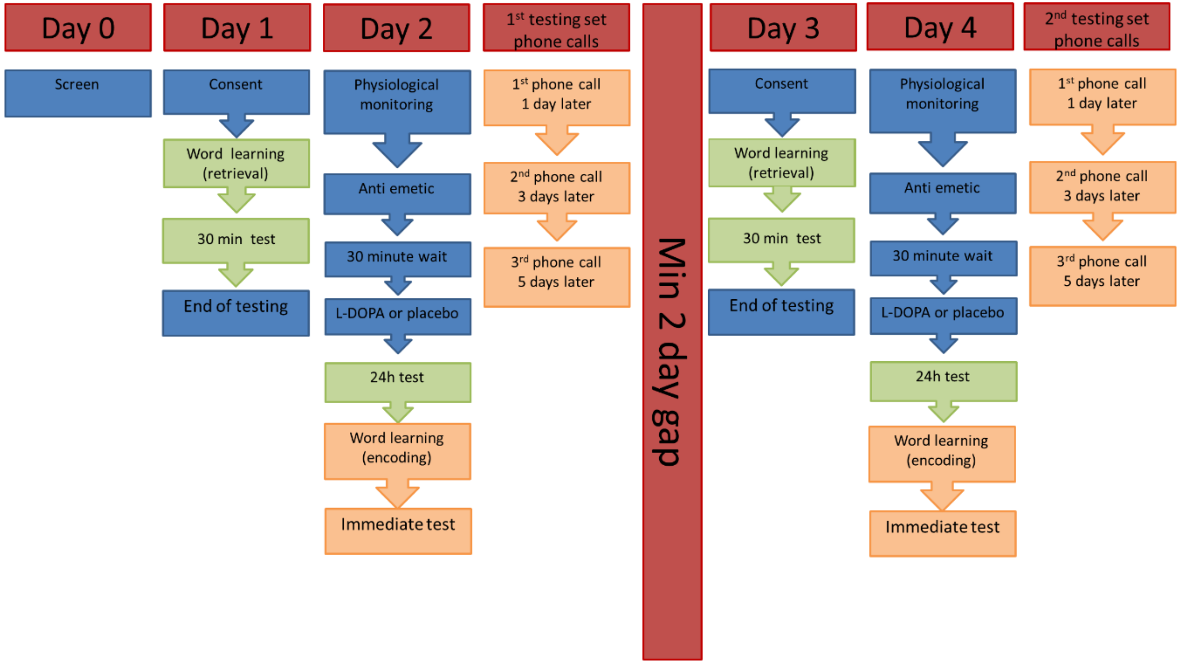


Figure 27: Study timeline for testing dopamine's effects on encoding and retrieval

Participants were invited for two identical testing sessions (left and right of the 2-day gap). The aim of the study was to test L-DOPA's effect on memory encoding (orange) and retrieval (green). L-DOPA or placebo was given in a random order on days 2 and 4. Therefore, for the encoding experiment Dopamine was active at learning and immediate test, but not at Day 1-5 tests. In the retrieval experiment dopamine was not active at learning or subsequent sleep (and consolidation) but it was active at the subsequent 24 hours later.
These timelines are not to scale.

Bayesian comparisons: The absence of a 'significant' p-value cannot be used as evidence of the absence of an effect. However, the Bayes Factor gives the probability of the given data having occurred under null compared to alternative hypothesis. Here, I use the Bayes Factor to demonstrate our confidence in the null hypothesis.

Results

Final sample

Thirty-seven healthy elderly (65+ years) volunteers were recruited to take part in this study. Two were excluded prior to dosing; one due to a contraindication, and one for participating in another drug trial simultaneously. Three volunteers did not complete the study; two withdrew consent for personal reasons and one experienced significant nausea and vomiting as a side effect on their second testing session. In the encoding experiment, data was missing partially for a further 8 volunteers either due to missed phone calls or experimenter error, and one volunteer was excluded entirely as their accuracy was below chance level suggesting they had misunderstood the task ($n_{encoding-} = 32$). Further, wrong test versions were used for two volunteers for the retrieval experiment ($n_{retrieval} = 28$). Demographic characteristics of the final sample are reported in **Table 6** and **Table 7**. Note that one volunteer scored 18 on the MoCA, which may indicate heightened risk of having mild cognitive impairment or dementia (Carson, Leach, & Murphy, 2018). As this volunteer was not an outlier on any of the other tests, including the verbal memory test and a test of working memory (results published in (J. P. Grogan et al., 2018)), they were included in the analyses and considered healthy.

	Test day							
	L-DOPA				Placebo			
	0	1	3	5	0	1	3	5
# of volunteers								
1	X	X	X	X				
2		X		X				
1		X			X			
1				X		X	X	X
1					X	X	X	X
1						X	X	
1							X	
1								X
2					X	X	X	X

Table 6: Encoding and Retrieval: Missing data

Crosses (X) denote missing data points for each test session with the left column denoting the number of volunteers affected. Remaining data for each volunteer was used except where the volunteer only completed one test session (n = 3, top and bottom rows)

	Encoding $n = 32$		Retrieving $n = 28$	
	Mean (SD)	Range	Mean (SD)	Range
Age	**71.1** (7.1)	65 – 92	**70.9** (6.9)	65 – 92
Years of education	**14.7** (3.5)	10 – 24	**14.5** (3.5)	10 – 24
MoCA	**26.1** (3.3)	18 – 30	**26.1** (3.2)	18 – 30
Height (cm)	**170.0** (10.3)	152 – 186	**170.0** (10.6)	152 – 186
Weight (kg)	**75.2** (15.4)	51 – 105	**75.0** (15.6)	51 – 105
Body mass index (kg/cm^2)	**25.8** (3.7)	18.1 – 35.1	**25.8** (3.7)	18.1 – 35.1
L-DOPA concentration (mg/kg)	**2.08** (0.44)	1.43 – 2.95	**2.09** (0.45)	1.43 – 2.95
Gender (f/m)		**16** / **16**		**14** / **14**
Treatment order (L-DOPA/ Placebo first)		**17** / **15**		**13** / **15**
Blinding (accurate / inaccurate / missing**)**		**17** / **12** / 3		**16** / **10** / 2
Rationality Experientiality index (REI) scores				
Overall rationality	**3.6** (0.8)	1.5 – 5.0	**3.7** (0.9)	1.5 – 5.0
Rational Engagement	**3.8** (0.9)	1.0 – 5.0	**3.8** (0.9)	1.0 – 5.0
Rational Ability	**3.5** (1.0)	1.0 – 5.0	**3.5** (1.0)	1.0 – 5.0
Overall Experientiality	**3.1** (0.6)	2.0 – 4.2	**3.1** (0.6)	2.0 – 4.2
Experiential Engagement	**2.9** (0.7)	1.0 – 4.2	**2.9** (0.8)	1.0 – 4.2
Experiential Ability	**3.3** (0.6)	2.0 – 4.5	**3.3** (0.6)	2.0 – 4.3
Depression, Anxiety and Stress Scale (DASS)				
Depression	**4.2** (4.9)	0 – 18	**4.0** (4.7)	0 – 18
Anxiety	**2.0** (2.4)	0 – 11	**2.0** (2.6)	0 – 11
Stress	**5.4** (4.1)	0 – 14	**5.4** (4.3)	0 – 14
Barratt Impulsivity Scale (BIS)				
Motor impulsiveness	**21.3** (3.7)	14 – 29	**20.9** (3.5)	14 – 29
Non-planning	**22.1** (5.1)	11 – 30	**21.6** (4.7)	11 – 30
Attentional	**14.6** (2.5)	10 – 19	**14.4** (2.5)	10 – 19

Table 7: Encoding and Retrieval: Demographics
L-DOPA concentration was calculated as drug dose (mg) / body weight (kg). Volunteers were dosed with 150mg of L-DOPA CR in the form of co-beneldopa. The volunteers for both experiments are the same with unique iterations of missing data, as described previously.

Encoding

L-DOPA at encoding did not affect subsequent memory (Figure 28**Error! Reference source not found.**). I probed recognition memory 4 times testing unique items each time: once on-site shortly after learning (~5 minutes later), then 1, 3 or 5 days later over the phone.

The mixed linear model (excluding random effects) explained 15.0% of the variation in D' (t(30) = -5.525, p = < .001), which was driven by the effect of time. There was no significant effect of treatment for neither D' nor criterion (**Table 8**).

I also found substantial evidence that L-DOPA during encoding does not affect subsequent verbal memory performance a day later (t(28) = - .352, p = .728, BF_{01}= 4.6) with evidence for days 3 (t(25) = -2.128, $p_{uncorrected}$ = .043, $p_{corrected}$ = .129 / BF_{01}= .7) and 5 being inconclusive (t(26) = - .325, p = .748 / BF_{01}= 2.6). See **Table 9**.

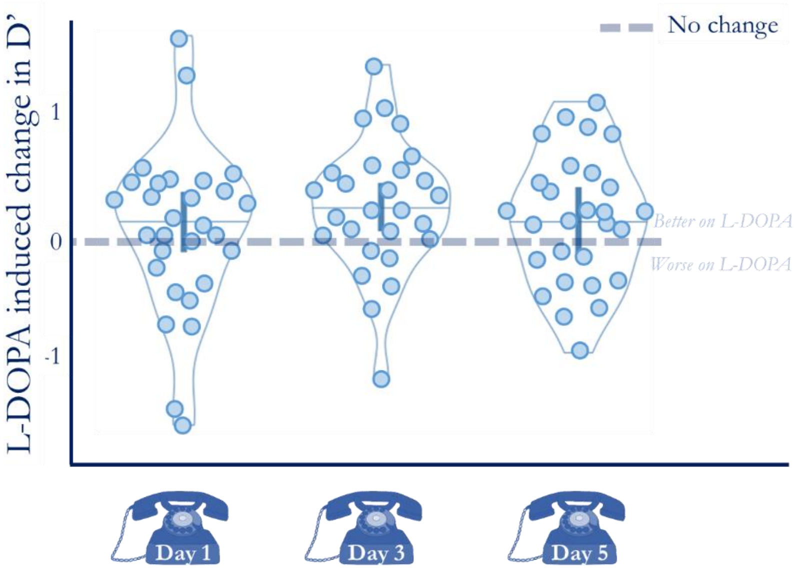


Figure 28: L-DOPA and encoding

L-DOPA during encoding does not affect subsequent memory. A violin plot showing the kernel density for L-DOPA mediated change in D'. The change in accuracy was calculated for each volunteer as:
D' [L-DOPA induced effect] = D' [L-DOPA] – D' [Placebo]
Therefore positive values (above the dashed line) denote better performance when L-DOPA was given at encoding compared to placebo, zero (dashed line) denotes no difference and negative values poorer performance. Note that at the time of test L-DOPA was not active in the system.

Encoding

	Estimate (std error)	t	df	p	R²m
D'	D' ~ Delay * Treatment + (Delay + Treatment | participant)				
Intercept	.975 (.10)	9.356	3 .3	<.001	.150
Delay	-.545 (.18)	- 3.082	114.1	.003	
Treatment	.108 (.08)	1.311	29.4	.200	
Delay*Treatment	.002 (.15)	.016	91.3	.987	
Criterion	Criterion ~ Delay * Treatment + (Treatment || participant)				
Intercept	- .083 (.06)	- 1.326	51.2	.191	.009
Delay	- .149 (.10)	- 1.438	107.9	.153	
Treatment	.012 (.05)	.235	43.8	.815	
Delay* Treatment	- .059 (.09)	- .626	107.5	.532	

Table 8: Encoding: Mixed linear models

The delay (time of test; D1, D3 or D5) explained variation in D' but not in criterion. L-DOPA status explained none of the variability in responses. Specific models are specified above each model's results. R^2m shows how much of the variance in the response variable is explained by the fixed effects, their interactions and the intercept.
As none of the main effects of interest (i.e. effects of L-DOPA) were significant, these analyses were not corrected for multiple comparisons.

Encoding

	Mean (SD) L-DOPA	Mean (SD) Placebo	Credible interval δ	t	p	BF_{01}	H_0 vs H_1
Day 1				df = 28			
D'	1.257 (.76)	1.149 (.61)	[- .295 – .426]	- .352	.728	4.6	
Criterion	- .027 (.35)	- .083 (.39)	[- .310 – .414]	114*	.197	4.7	
Recollect	.569 (.25)	.606 (.22)	[- .447 – .277]	.495	.625	4.3	
Familiar	.220 (.21)	.161 (.15)	[- .128 – .612]	93.5*	.291	2.2	
Day 3				df = 25			
D'	.865 (.47)	.710 (.50))	[- .001 – .763]	-2.128	.043	.7**	
Criterion	- .107 (.40)	- .126 (.46)	[- .352 – .368]	- .040	.968	4.8	
Recollect	.442 (.25)	.461 (.26)	[- .410 – .308]	.289	.775	4.6	
Familiar	.221 (.16)	.204 (.18)	[- .306 – .419]	130*	.820	4.6	
Day 5				df = 26			
D'	.692 (.45)	.592 (.40)	[- .317 – .588]	- .325	.748	2.4	
Criterion	- .147 (.38)	- .167 (.33)	[- .334 – .378]	- .148	.884	4.9	
Recollect	.414 (.25)	.427 (.30)	[- .414 – .301]	197.5*	.585	4.7	
Familiar	.234 (.20)	.232 (.22)	[- .313 – .402]	91*	.614	4.8	

Table 9: Encoding: Pairwise comparisons

L-DOPA during encoding did not affect verbal memory performance later. δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. Where credible intervals are the 95% intervals overlapping zero denote no difference. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (blue) hypothesis. All error % < .024

* Wilcoxon test used due to non-parametric data. All samples contained zero values for paired differences. P-values for Wilcoxon tests for such data are less reliable
** BF_{10} = 1.4

Retrieval

Across the board I show moderate evidence that L-DOPA does not affect retrieval of previously learnt information, Figure 29. When memory was probed an hour after administration of L-DOPA, I found no effect of L-DOPA on D' or criterion. Instead, I found moderate evidence to suggest L-DOPA does not affect D' or criterion (BF_{01} =4.6, BF_{01} = 4.9). If anything, the difference between the L-DOPA and placebo was more pronounced on Day -1, i.e. on the baseline test taken the day before treatment (top of **Table 10**).

Retrieval

	Mean (SD)		Credible interval δ	T	p	BF_{01}	H_0 vs H_1
	Day preceding						
Day -1 (Before L-DOPA / Placebo given)	L-DOPA	Placebo		df = 27			
D'	2.842 (.72)	2.721 (.76)	[- .180 – .538]	-1.041	.307	3.1	
Criterion	.114 (.34)	.196 (.35)	[- .564 – .149]	-1.198	.241	2.6	
Recollect	.592 (.22)	.615 (.29)	[- .445 – .264]	178*	.959	4.4	
Familiar	.298 (.18)	.230 (.23)	[- .095 – .618]	189*	.741	1.9	
Day 0	L-DOPA	Placebo		df = 27			
D'	1.658 (.55)	1.609 (.56)	[- .278 – .417]	.393	.698	4.6	
Criterion	- .216 (.39)	- .192 (.47)	[- .384 – .316]	- .224	.968	4.9	
Recollect	.592 (.22)	.615 (.29)	[- .421 – .261]	.289	.825	4.4	
Familiar	.298 (.18)	.230 (.23)	[- .102 – .611]	1.491	.147	1.9	

Table 10: Retrieval: Pairwise comparisons
L-DOPA does not affect verbal memory retrieval. δ is Cohen's delta – the standardised effect size – for the paired differences derived from the Bayesian posterior distribution. BF_{01} and H_0 vs H_1 show the likelihood of our data having been observed under the null (white) as opposed to the alternative (purple) hypothesis. D -1 denotes baseline performance for a recognition verbal memory test conducted 30 minutes after learning and prior to drug administration. All errors % < .022
* Wilcoxon test used due to non-parametric data. All samples contained zero values for paired differences. P-values for Wilcoxon tests for such data are less reliable.

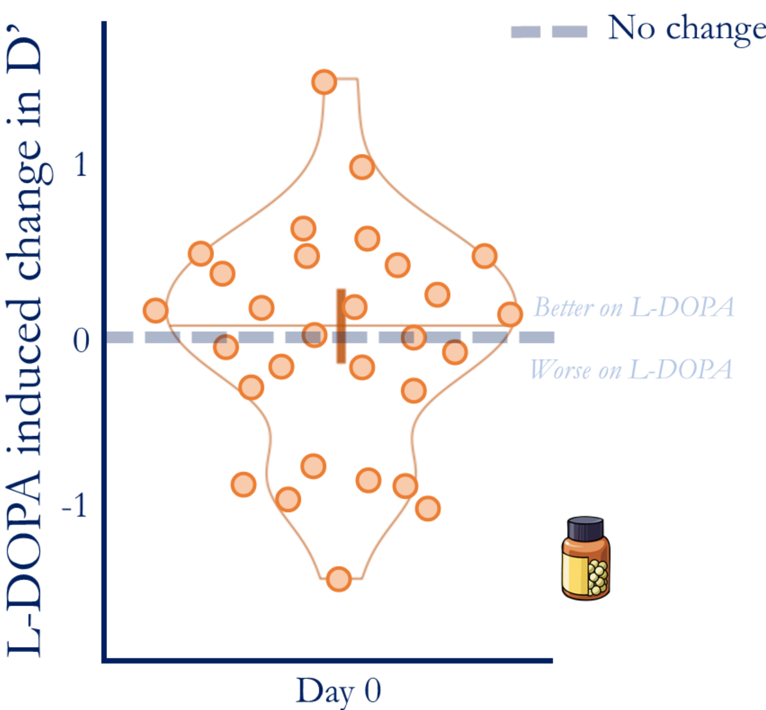


Figure 29: L-DOPA and retrieval

L-DOPA on Day 0 did not affect recognition memory performance on items learnt a day previously.

Discussion

In this chapter I provide evidence that dopamine does not alter memory performance at encoding or retrieval when memory is tested 24 hours later. Using a Bayesian approach to analyse these data, I found moderate evidence that L-DOPA active during retrieval does *not* affect memory. When L-DOPA was given before learning, I found moderate evidence that it does not affect recognition memory when tested immediately, the following day or five days after learning. However, when tested 3 days after learning I observed a small enhancement in the L-DOPA condition.

Therefore, I found weak evidence that dopamine may alter encoding for information that is retained at longer delays. However, this effect did not survive family-wise correction for multiple comparison, and Bayesian analyses for day 3 were inconclusive. Dopamine may therefore weakly enhance encoding when tested 3 days after learning. However, there is a high possibility that this finding was a false positive as it did not survive multiplicity corrections and the Bayesian analyses were inconclusive either way.

If true, perhaps L-DOPA increased early consolidation processes. Dopamine was active at periods of time following L-DOPA administration, possibly enhancing processes of early consolidation during this time. Second, there was no effect of dopamine on Day 1, so if dopamine enhances memory at encoding it is likely that the items retained on dopamine at Day 3 were ones that, on placebo, would have been lost between Days 1 and 3. However I did not specifically test this hypothesis.

The observed effect on Day 3 did not persist on Day 5 but many volunteers were performing at chance-level by this point, which may have masked drug effects. Therefore, if this effect on Day 3 is real, it may be that L-DOPA at encoding, or shortly after encoding, promotes persistence of strongly encoded or salient information. While speculative, this interpretation also fits in with the drosophila literature, where dopamine has been shown to both enhance encoding and accelerate strategic forgetting (Berry et al., 2012; Berry et al., 2018). While this would be in line with our previous findings, further work is required to address this possibility.

Previously, Clos and colleagues (2019) have found a beneficiary effect of a D2 agonist on encoding. This study had many key differences to ours. First, I used L-DOPA which increases both D1 and D2 type activity compared to a D2 agonist. Second, our participants were elderly as opposed to healthy young people. Age-related differences in L-DOPA responses in relation to memory (Morcom et al., 2010) have been previously observed, so therefore these **pre-existing**

differences between the studies may explain discrepancies in results. Third, their participants were performing close to baseline suggesting the task they used was much more difficult. Finally, I used a verbal memory task as opposed to pictures. I cannot rule out the possibility that in a different setting L-DOPA would enhance recognition memory.

Others have also found that while there was no difference on subsequent memory when the D2 agonist Bromocriptine or the D2 antagonist Sulpiride were compared against placebo, memory performance was enhanced in the D2 agonist compared to the antagonist condition (Morcom et al., 2010). Therefore, there may be a small effect whereby dopamine at encoding enhances memory, possibly via modulation of D2 receptors.

I also showed that dopamine at neither encoding nor retrieval affected recollection, while findings for familiarity were inconclusive. In this study, the way the remember-know task was delivered was not consistent. Some members of the research group gave instructions that could have promoted volunteers to judge their confidence rather than true recollection. It is possible this influenced our findings and when the task is delivered more robustly dopamine may modulate these processes at encoding or retrieval. However, when volunteers were dosed with a dopamine agonist or antagonist during encoding in another study, no differences were found between recollection or familiarity at a later test either (Morcom et al., 2010). Therefore, it is unlikely that dopamine during retrieval or encoding affects recollection or familiarity.

Findings of other aspects of this study are reported elsewhere. I showed that in this study L-DOPA affected neither working memory (digit span) nor retrieval in a reinforcement learning task (J.P. Grogan et al., 2019; J. P. Grogan et al., 2018).

Conclusion

Here, I tested the effect of L-DOPA on encoding and retrieval. I found that while L-DOPA compared to placebo did not enhance encoding or retrieval when tested a day later, it may modulate persistence for strongly encoded items when tested 3 days later. However, the evidence for this was weak and inconclusive.

Chapter IV: Dopamine and memory persistence

Data reported in this chapter was collected as a part of the DOPAMIND study.

Introduction

Seemingly effortlessly, the human brain selectively stores salient details of our daily events, while disregarding irrelevant information – you have probably forgotten where you parked your car while shopping last week, but you will remember your parking slot in an airport carpark after a week's holiday. When memories are made, past experiences are encoded by physical traces, or engrams, in the brain (Semon, 1909; Tonegawa, Liu, et al., 2015). Instead of being an analogue,

one-to-one match with information encountered in the environment, memories are shaped and moulded at different stages depending on complementary information, and then reconstructed at retrieval (Anderson, Bjork, & Bjork, 2000; J. L. C. Lee, 2010; Nader, 2015; Nader & Hardt, 2009).

During memory encoding and consolidation, engrams of salient information can be tagged (prioritised) for storage, based either on previous knowledge, repeated exposure, or other associations, such as financial or emotional reward or cost (Frey & Morris, 1997a; Redondo & Morris, 2011). Passive re-exposure alone is unlikely to be enough. For example, repeated re-study after learning does not enhance subsequent memory, but testing does (Karpicke & Roediger, 2008). Contextual information encountered at a later time-point can also retroactively tag previous memories (Patil et al., 2017). Tagging usually occurs within hours of encountering information and it increases the longevity of the tagged memory (Frey & Morris, 1997a; Pu & Yu, 2019).

During sleep, spontaneous hippocampal replay prioritises tagged memories and supports offline memory consolidation (Clemens et al., 2007; Heib et al., 2015; Sadowski et al., 2016; van de Ven et al., 2016). Evidence that similar mechanisms act as a fast route for online consolidation during wakeful re-activation comes from several studies (Antony et al., 2017; Jadhav, Kemere, German, & Frank, 2012). Disrupting these events either during sleep (Ego-Stengel & Wilson, 2010) or awake (Jadhav et al., 2012) impairs memory retention. As the neurotransmitter dopamine is involved in detecting behaviourally important information (Eban-Rothschild et al., 2016; Schultz, 1998), it might promote processes leading to memory selection.

Dopamine is released from neurons that connect reward and memory systems within the brain and modulate synaptic connections and memory persistence (J. Lisman et al., 2011). Models of memory based on drosophila point to dual effects – dopamine enhances encoding of new

information at the cost of triggering forgetting of competing information (Berry et al., 2012; Berry et al., 2018). This dopamine-induced strategic forgetting might be selective to weakly encoded memories. In humans with dopamine depletion, the timing of dopamine-like medication administration relative to learning critically determines its effects on memory (Coulthard et al., 2012; J.P. Grogan et al., 2015).

Dopamine increase after learning, to target consolidation and forgetting processes, enhances memory persistence (Feld et al., 2014; J.P. Grogan et al., 2015). In drosophila, dopamine during sleep actively accelerates forgetting (Berry et al., 2015). In rats, dopamine in the hippocampus enhances consolidation 12h after learning, but not immediately (Rossato et al., 2009). In healthy young adults overnight dopamine enhances persistence of low-reward items (Feld et al., 2014) and in elderly dopamine during encoding and consolidation enhances memory persistence and recollection for weakly encoded information at a 6h delay but not at a 2h delay (Chowdhury et al., 2012). Together these findings suggest that dopamine plays a role in memory persistence over long periods but not in the short-term.

I sought to test if dopamine biases human memory storage to maximise retention of behaviourally relevant information. To study the relationship between dopamine, tagging, forgetting and long-term memory persistence, I carefully timed administration of L-DOPA to increase dopamine availability within the brains of healthy older adults in a placebo-controlled double-blind randomised crossover experiment. Critically, L-DOPA or placebo was given *after* participants had learnt information. Here, I report two set of analyses from this clinical trial.

In the first part (memory persistence), memory was probed for words 1, 3 or 5 days after learning. In the second part (tagging) I tagged a quarter of the items as salient by repeated exposure (recognition test with no feedback) shortly after L-DOPA administration. Memory for the tagged items was tested the following day together with a matched number of non-tagged

items. Therefore, exogenous dopamine was not active in the system during learning or during memory tests. Dopamine was instead active during tagging and subsequent sleep.

I hypothesised that:

- Dopamine during sleep will enhance memory persistence particularly 3 and 5 days later.
- Saliency-tagged information will be better retained than non-tagged information on both L-DOPA and placebo
- Dopamine will increase the tagging effect by enhancing retention of salient information

Method

Comprehensive overview of the method is given in Chapter II (pg. 77). A short overview is given here.

Note that in this chapter, I discuss data from the same study separately – crucially, some data between the two analyses are shared (i.e. List B).

Design and procedure

The second and third visits (sleep visits) were identical except for drug allocation. Volunteers arrived on site in the evening and they were re-consented and screened for eligibility. They then learnt 4 lists (**A**, **B**, **C**, and **D**) of 20 target words (n = 80 targets) presented on a computer screen one at a time (Figure 30). 30 minutes after learning they were given either an oral dose of 200mg L-DOPA controlled release or placebo (at different visits) to be active overnight. All evening

events were calculated backward from usual bedtime so that L-DOPA was administered 115 minutes prior to switching the lights off for the night. Volunteers slept on-site for a full night, and they were

woken up at their usual wake-up time. Below is a short outline of the study procedures relevant for Parts 1 and 2.

Figure 30: DOPAMIND: Verbal memory learning phase

Volunteers learnt four lists of target words (List A-D, 80 words), one at a time, in a random order. During learning they were asked whether the item was 'alive' in order to enhance learning. Each dot represents a word.

Part 1: Dopamine in tagging memories

An hour after dosing, one of the lists (List **A**) was tagged as salient by a recognition test whilst ON L-DOPA (/placebo). No feedback was given. An hour after the test, and two hours after L-DOPA was given, the volunteers went to bed. ~2h after waking up whilst OFF L-DOPA volunteers' verbal memory was tested for Lists **A** and **B** together with a matched number of novel distractors. The tagged and untagged (List A and List B) targets were used to study L-

DOPAs effect on saliency-tagging 'important' memories with the rationale that when a word is tested, it will be deemed to be more important, and it will be preferentially remembered.

Part 2: Forgetting

The aim here was to assess the role of nocturnal dopamine in modulating memory persistence. Volunteers went to bed ~2h after receiving L-DOPA (Figure 31A). The following morning (~ 12h after dosing), volunteers' memory was tested on-site on one of the lists (Figure 31B). 2 and 4 days later (3 and 5 days after learning) the volunteers were contacted over the phone for follow-up recognition memory tests. At test, volunteers' memory was probes using the remember-know paradigm (pg 90). Crucially, volunteers were OFF L-DOPA during learning, ON L-DOPA over the subsequent night and OFF L-DOPA at each testing instance.

Analyses

The primary outcome measure was the D'. I also analysed the criterion, recollection and familiarity separately. Drug dose was calculated as mg/kg.

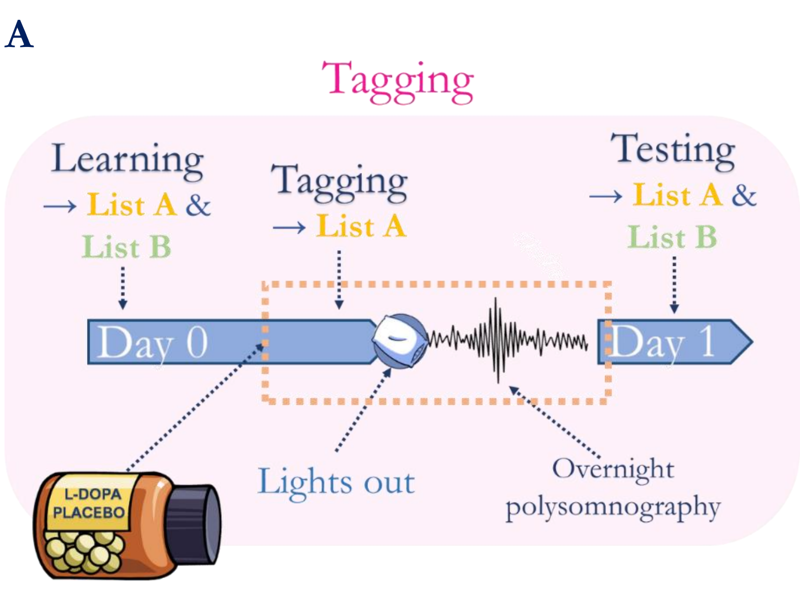
Part 1: The main outcome measure was the difference between tagged and untagged items (i.e. tagged minus untagged) for D', hereafter referred to as the tagging effect. The tagging effect was also calculated for the criterion and the remember-know judgements.

Part 2: Mixed linear modelling was used as described on pg. 105. I also assessed each day's (1, 3, and 5) performance separately using paired comparisons, see pages 104 and 108.

Control measures: To ascertain that any effects of L-DOPA reflected dopamine's effects on memory rather than mood or alertness, participants completed the Positive and Negative

Affective Score (PANAS) questionnaire and the Trail Making Tests A and B. The former is a measure of current mood while the latter is a broad measure of executive control.

A



B

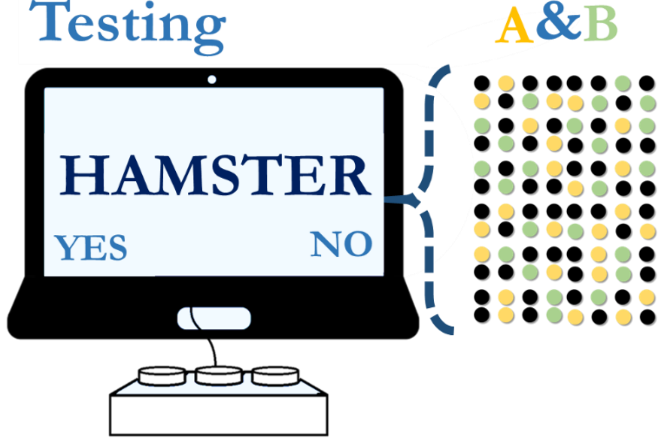


Figure 31: Part 1 Timeline and recognition test

Summary of the timeline for Part 1. Volunteers learnt lists A&B in the evening ***before*** dosing. ***After*** dosing, List A was tagged as salient by a recognition test. L-DOPA was given to target tagging and overnight sleep. The orange area denotes when L-DOPA was active in the system. Memory was tested the following day. Lists A and B were tested together. Each dot represents a word. The lists were tested in a random order with a matched number of distractors (black dots).
These figures are not to scale.

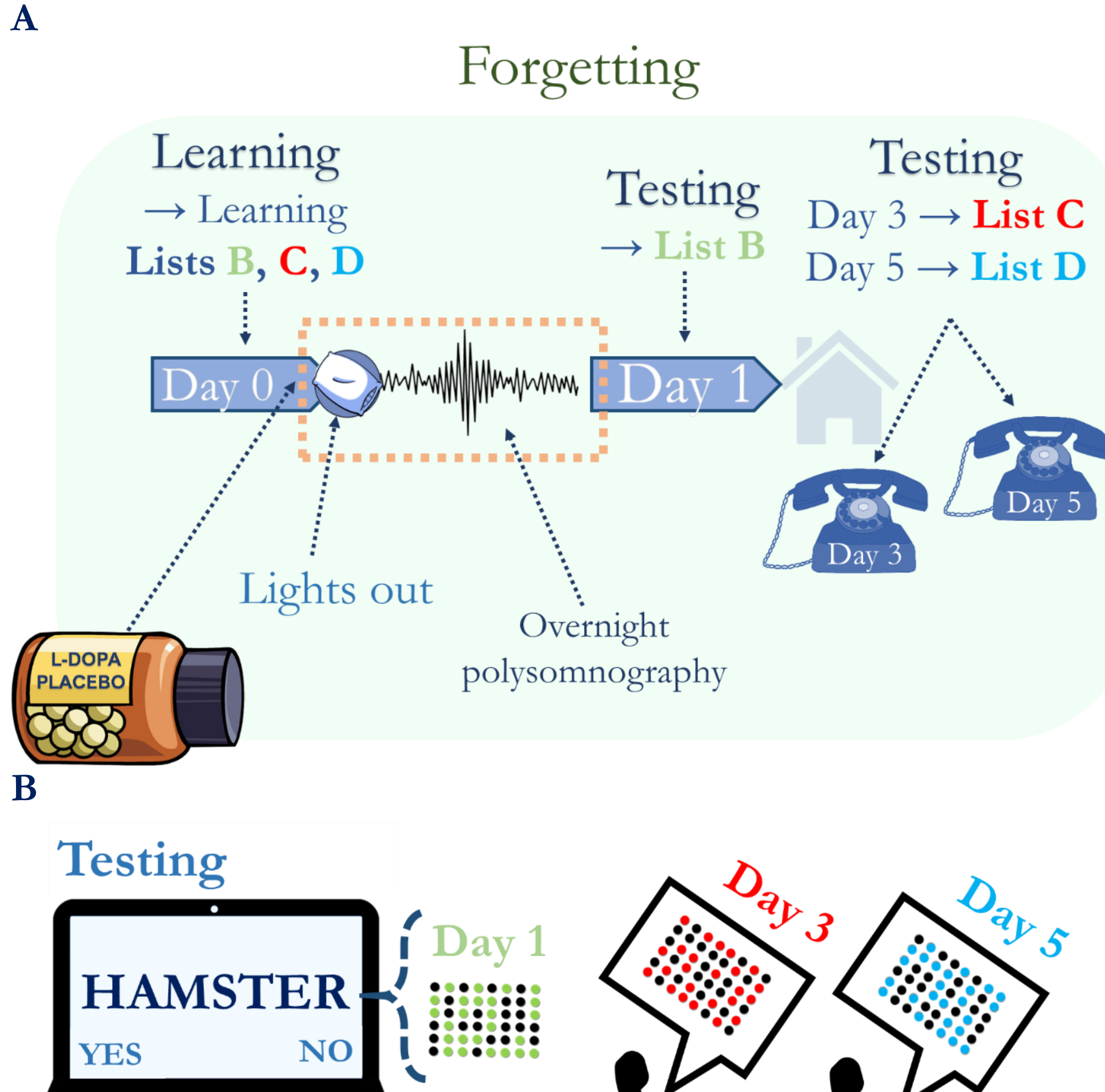


Figure 32: Part 2: Timeline and recognition test

Summary of the timeline for Part 2. Volunteers learnt lists B-D in the evening ***before*** dosing. L-DOPA was given to target memory processes overnight. Memory was tested for each list separately at a 1, 3, or 5-day delay. The orange dotted box denotes when L-DOPA was active in the system.
Different word lists were tested on different days using a recognition memory procedure. On days 3 and 5 testing was performed over the phone. Each dot represents a unique word. Black dots represent distractor words.
These figures are not to scale.

Results

Participants

I recruited healthy elderly (65+ years) native or fluent English speakers with normal or corrected-to-normal vision completed the study. Forty-five volunteers completed screening: five could not take part due to diary clashes, 2 due to incidental findings that were also contraindications, and 3 due to existing cardiac or medicinal contraindications. Further 14 were initially screened p/rior to study halt (Appendix A) and were either not eligible when the trial restarted, could not be contacted, refused consent or could not be booked in due to diary clashes. Demographic variables for the final sample (n = 35) are shown in Table 11. Where not otherwise stated, all available data (n=35) was used for analyses.

Further, data for one follow-up phone call (placebo, ✆ Day 5) had to be excluded due to researcher error (same set of distractors and targets were used as on ✆ Day 3).

	Mean (SD)	Range
Age	**68.9** (3.5)	65 – 79
Height (cm)	**166.1** (7.4)	152 – 181
Weight (kg)	**70.28** (13.0)	48 – 94
Body mass index (kg/cm²)	**25.2** (3.2)	18.5 – 32.7
L-DOPA concentration (mg/kg)	**2.94** (0.54)	2.13 – 4.17
Gender (f/m)	**22 / 13**	
Treatment (L-DOPA/ Placebo first)	**18 / 17**	
Blinding (accurate / inaccurate) *	**21 / 13**	
MoCA (Montreal cognitive assessment)	**27.5** (2.5)	21 – 30
Rationality Experientiality index (REI) scores		
Overall rationality	**3.5** (0.7)	2.3 – 4.7
Rational Engagement	**3.4** (0.7)	2.1 – 4.6
Rational Ability	**3.5** (0.7)	2.1 – 4.7
Overall Experientiality	**3.4** (0.7)	2.1 – 5.0
Experiential Engagement	**3.3** (0.6)	2.2 – 5
Experiential Ability	**3.5** (0.7)	1.9 – 4.9
Depression, Anxiety and Stress Scale (DASS)		
Depression	**2.1** (3.2)	0 – 11
Anxiety	**1.8** (2.3)	0 – 8
Stress	**6.2** (5.9)	0 – 21
Barratt Impulsivity Scale (BIS) *		
Motor impulsiveness	**21.1** (3.6)	12 – 27
Non-planning	**21.7** (5.3)	14 – 35
Attentional	**14.5** (3.4)	9 – 23
	35 (10.4)	20 – 59
Pittsburgh sleep quality index		
Sleep efficiency	**1.4** (1.5)	**1.4** (1.5)
Sleep Quality	**1.7** (1.6)	**1.7** (1.6)
Daily disturbance	**1.4** (0.7)	**1.4** (0.7)

Table 11: Chapters IV to VI demographics

L-DOPA concentration was calculated as drug dose (200 milligrams divided by body weight in kilograms).
SD = standard deviation; cm = centimetre; kg = kilogram; mg = milligram;
f = female; m = male
* missing data from one volunteer

Part 1: Dopamine in tagging memories

A proportion of the items were saliency-tagged (List A) by a recognition test after L-DOPA had been given. Here, I report results for the comparisons between tagged and non-tagged items.

As expected, the tagging effect (i.e. relative benefit of tagging, List A > List B) was seen both on L-DOPA ($t(34) = -8.42$, $p < .001$, $BF_{10} = 14\ 300\ 000$, $n = 35$) and on placebo ($t(34) = -6.76$, $p<.001$, $BF_{10} = 165\ 589$, $n = 35$) for accuracy (D'). In other words, tagged items were better retained (List A; $m_{L\text{-}DOPA} = 2.20 \pm .78$, $m_{placebo} = 2.19 \pm .77$) than other items (List B; $m_{L\text{-}DOPA} = 1.25 \pm 0.59$, $m_{placebo} = 1.54 \pm .11$.). Note that it is possible that this effect is not driven by "tagging" but by having a second opportunity to encode information.

As was hypothesised, L-DOPA increased the tagging effect ($t(34) = 2.48$, $p = .018$, $BF_{10} = 2.6$, Figure 33) relative to placebo, n = 35. If both tagged and non-tagged items were considered together, there was no difference between the treatments ($m_{L\text{-}DOPA} = 1.7 \pm .61$, $m_{placebo} = 1.9 \pm .61$; $t(33) = 1.98$, $p = .056$, BF10 = 1.0). This suggests that dopamine plays a role in selecting

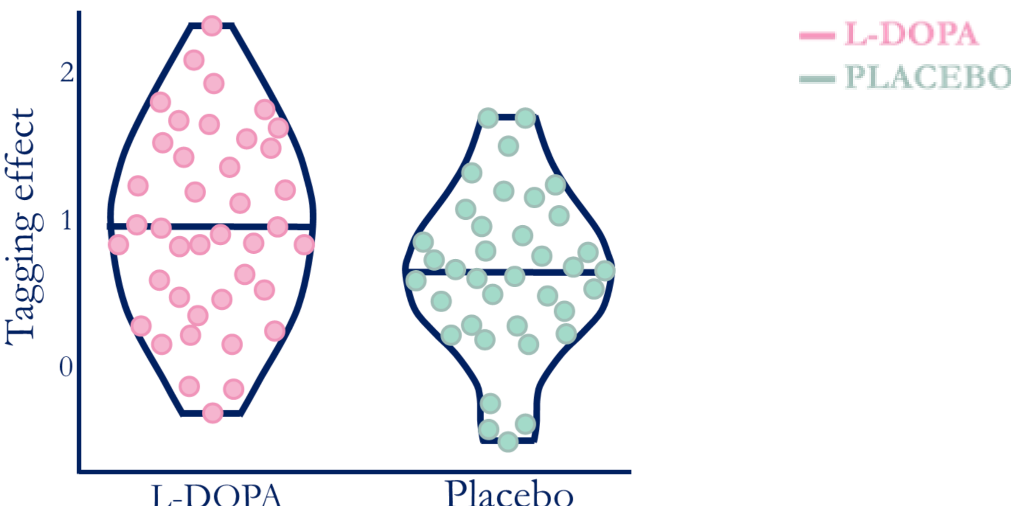


Figure 33: Dopamine and the tagging effect

The tagging effect, or relative benefit of memory tagging on subsequent memory, is increased by dopamine when L-DOPA is given after learning but before tagging and subsequent sleep.

salient memories for later. Dopamine did not impact the tagging effect for the criterion, or for recollected or familiar items (Table 12).

I used parametric and Bayesian repeated measures 2x2 ANOVAs to observe the effect of tagging (List A vs B) and treatment (L-DOPA vs placebo) on accuracy (Figure 34, Table 12, n = 35) and criterion (Table 13). I found a main effect of tagging and a treatment * tagging interaction ($F(34, 1) = 6.15$, $p = .018$, $BF_{01} = .7$ / $BF_{10} = 1.4$) for accuracy. There was a main effect of tagging on the criterion but no effect of treatment.

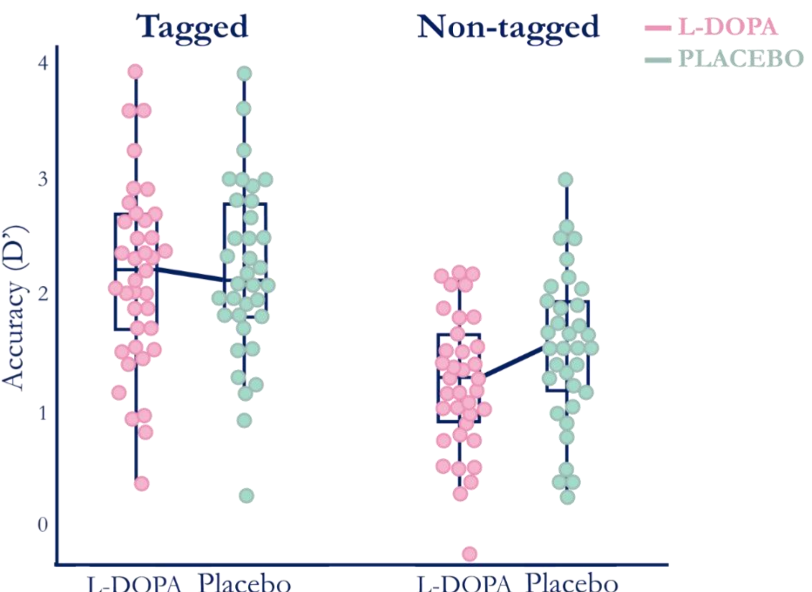


Figure 34: L-DOPA and memory for tagged and non-tagged items

Dopamine accelerates tagging by modulating persistence of tagged and non-tagged items Dopamine both increases memory for tagged (left, difference not significant) and decreases memory for other (right) items. The paired differences between tagged (left) and non-tagged (right) items are plotted in Figure 33. This figure demonstrates that L-DOPA is affecting both tagged and non-tagged items but in disparate ways. This study has low power and for this reason the effect on either on their own is small, and the effect on tagged information is not significant. However, the magnitude of the effect is larger when both are considered together, as in Figure 34.

Tagging effect

	Mean (SD)		Credible interval δ	t	p	BF_{01}	H_0 vs H_1
	L-DOPA	Placebo		df = 34			
D'	.95 (.67)	.64 (.56)	[.052 – .728]	-2.48	.018	.39**	
Criterion	-.40 (.43)	.-.35 (.35)	[- .390 – .240]	226*	.611	4.9	
Recollect	.19 (.16)	.23 (.19)	[- .487 – .158]	291*	.704	3.6	
Familiar	-.16 (.18)	-.13 (.18)	[- .425 – .201]	-.741	.464	4.3	

Table 12: Behavioural analyses Part 1: Pairwise comparisons

L-DOPA administered before tagging and subsequent sleep enhances accuracy (D') but does not affect response bias (Criterion) or recollection. δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (purple) hypothesis. All p-values are uncorrected. All errors <.001%

* Wilcoxon test used due to non-parametric data. All samples contained zero values for paired differences. P-values for Wilcoxon tests for such data are less reliable

** BF_{10} = 2.6

Tagging

Criterion	Mean (sd) L-DOPA	Mean (sd) Placebo
Non-tagged	.06 (.55)	.01 (.52)
Tagged	- .34 (.43)	- .34 (.45)

Variation source	Sum of squares	Mean square	Mean diff (std error)	Cohen's δ	F	p	BF_{01}	H_0 vs H_1
Tagging	4.81	4.81	.37 (.05)	1.36	64.57	< .001	$2.1 * 10^{-9}$	
error	*2.53*	*.08*						
Treatment	.019	.019	.023 (.05)	.07	.19	.665	5.1	
error	*3.39*	*.10*						
Interaction	.021	.021			.26	.611	3.6	
error	*2.69*	*.08*						

Table 13: Behavioural analyses Part 1: Repeated measures 2x2 ANOVA for criterion

Parametric and Bayesian ANOVAs revealed a main effect of tagging and evidence against an effect of treatment or treatment * encoding interaction for criterion. BF_{01} represents likelihoods of collecting our data under models that do not include the given source for variation (H_0 in white) compared to models that include the variation source (H_1 in dark).
All df = 34, 1

Control measures

In the morning after tests for lists A and B, participants completed Trail Making A and B and the Positive and Negative Affective Scale questionnaire (PANAS). Participants completed PANAS in the evening and morning PANAS (n_{PANAS} = 35). For the Trail Making, one volunteer had Trail A missing for the placebo visit and two had Trail B missing (n_{TRAILA} = 34, n_{TRAILA} = 33). Both were incorrectly administered. No differences were found in any of the measures. I found strong evidence that L-DOPA did not modulate neither Trail Making task nor negative affective scale

($BF_{01} > 5$), while the positive affective scale yielded no evidence in favour for or against an effect ($BF_{01} > 1.1$).

Tagging

	Estimate (std error)	t	df	p	R^2m
D'	D' ~ Tagging * Treatment + (Tagging + Treatment || participant)				
Intercept	1.796 (.10)	18.373	34.0	< .001	
Tagging	.798 (.08)	9.530	34.0	< .001	
Treatment	.139 (.08)	9.530	34.0	.107	.258 .
Tagging * Treatment	- .310 (.13)	2.480	34.0	.018	
Criterion	Criterion ~ Tagging * Treatment + (Tagging + Treatment | participant)				
Intercept	- .154 (.07)	- 2.175	34.0	.037	
Tagging	- .371 (.05)	- 7.919	68.0	< .001	
Treatment	- .023 (.05)	- .436	34.0	.665	.128
Tagging * Treatment	.049 (.09)	.521	68.0	.604	

Table 14: Behavioural analyses Part 1: Mixed linear models

Response variables (D' and criterion) were predicted using tagging (tagged versus not) and dose (top), and tagging and treatment (L-DOPA versus placebo, bottom) as fixed, and individual participants as random effects. Tagging influenced D' and criterion, for D' there was also a treatment*tagging interaction, but no main effect of treatment. Models are specified above each model's results. R^2m shows how much of the variance in the response variable is explained by the fixed effects, their interactions and the intercept. Note that estimates are mean-centred.

Part 2: Dopamine and memory persistence

To assess L-DOPAs influence on memory persistence, I included delay (1, 3 or 5 days), treatment and the delay * treatment interaction as fixed effects, with participants as random effects (including slopes and intercepts) in a mixed linear model (Table 15). The effect of treatment was not significant in this model. While the model yielded non-significant results, on visual inspection a trend on Day 1 toward accelerated forgetting was apparent (Figure 35A, left). Note that the direction of this effect was the opposite from what I hypothesised. Therefore, planned paired comparisons were performed next.

Pairwise Bayesian comparisons showed strong evidence that L-DOPA lowered performance on Day 1 (BF_{10} = 16.6) and substantial evidence that L-DOPA did not affect performance on Days 3 and 5 ($BF_{01} > 5$). A paired t-test for D' on Day 1 aligned with these results $t(34) = -3.33$, $p = 0.002$) and remained significant after adjusting for false discovery rate ($p_{corrected} = 0.006$, including tests for Days 1, 2 and 3). There was no effect on the other measures (Table 16).

Next, to assess the effect of dose (mg/kg) on memory, I performed a series of correlation for each day's performance, n = 35 each. **Contrary to my hypothesis**, on Day 1 *higher* doses were associated with poorer performance following L-DOPA (Day 1: Spearman's $\rho_{L\text{-}DOPA} = -.56$, $p < .001$) but not placebo ($\rho_{placebo} = -.23$, $p = .18$), and that these two relationships were different ($z = -2.634$, $p = .008$, (K. Pearson & Filon, 1898). This effect was significant following FDR correction including the correlational analyses for each day (i.e. Day 1, 3, and 5 correlations against dose first on L-DOPA, $n_{tests} = 3$, $p_{corrected} < .001$).

Dose was not associated with performance on other days (Day 3: $\rho_{L\text{-}DOPA} = -.06$, $p = .73$, $\rho_{placebo} = -.15$, $p = .40$; Day 5: $\rho_{L\text{-}DOPA} = -.02$, $p = .92$, $\rho_{placebo} = -.16$, $p = .36$, Figure 35B). As dose was calculated using bodyweight, it is noteworthy that it was not associated with performance on

placebo. Therefore, these effects are likely to be drug-related rather than driven by differences in body size.

L-DOPA therefore accelerated early forgetting possibly by only affecting information that would be lost in the long term anyway but did not affect strongly encoded information that would be remembered following a longer delay.

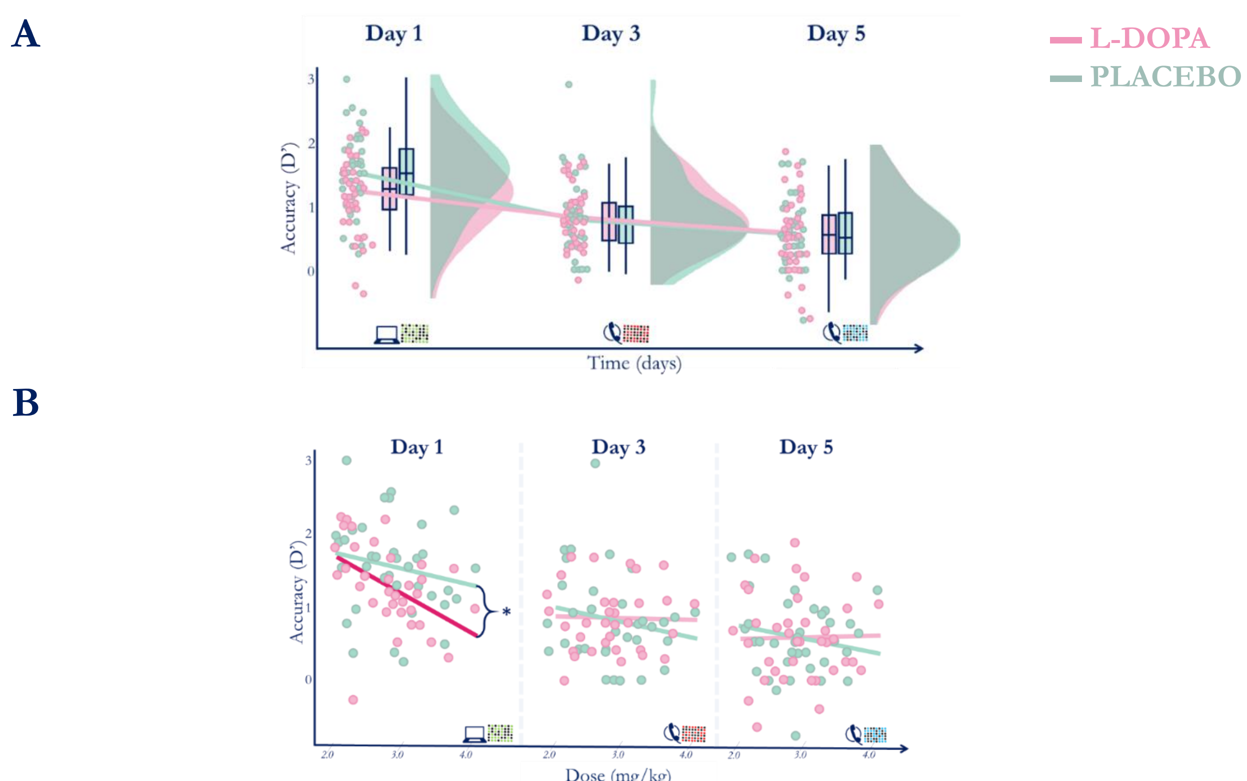


Figure 35: L-DOPA and memory persistence

L-DOPA accelerates early forgetting in a dose-dependent fashion
L-DOPA (pink) accelerated the rate of forgetting between learning and day 1 but it did not affect subsequent memory (days 3 and 5).
This effect was dose dependent: Higher doses were associated with accelerated forgetting on L-DOPA (pink) on day 1 but not any other days. This dose-dependent effect was different from the relationship between body weight and memory on placebo. There was no dose (or bodyweight) driven effect on placebo (green). Note that where the accuracy scores for a participant did not change between L-DOPA and placebo the dots overlap.

Note that y-axes on figures A and B are the same.

Forgetting – MLMs

	Estimate (std error)	t	df	p	R^2m
D'	D' ~ Delay * Treatment + (Delay + Treatment || participant)				
Intercept	.940 (.06)	14.862	34.1	<.001	
Delay	- .809 (.09)	-8.992	33.6	<.001	
Treatment	- .031 (.07)	1.215	34.2	.233	.252
Delay*Treatment	.187 (.16)	-1.817	103.9	.072	
Criterion	Criterion ~ Delay * Treatment + (Delay + Treatment || participant)				
Intercept	- .137 (.08)	1.761	34.0	.087	
Delay	- .122 (.06)	2.042	34.1	.049	
Treatment	- .013 (.05)	-.0269	34.1	.789	.009 .
Delay* Treatment	.033 (.10)	.329	104.5	.743	

Table 15: Behavioural analyses Part 2: Mixed linear models

Both the test delay and L-DOPA status explained variability in D' while neither explained variability in criterion. Specific models are specified above each model's results. R^2m shows how much of the variance in the response variable is explained by the fixed effects, their interactions and the intercept. The delay (time of test; D1, D3 or D5) explained variation in D' but not in criterion. Note that estimates are mean-centred.

Forgetting – Paired comparisons

	Mean (SD) L-DOPA	Mean (SD) Placebo	Credible interval δ	t	p (*p-corrected*)	BF_{01}	H_0 vs H_1
Day 1				df = 34			
D’	1.249 (.59)	1.544 (.65)	[- 1.202 – - .232]	- 3.333	.002 *.006*	.1**	
Criterion	.056 (.55)	.008 (.52)	[- .293 – .569]	351.0*	.211	4.5	
Recollect	.428 (.25)	.410 (.25)	[- .253 – .375]	.423	.675	5.1	
Familiar	.437 (.27)	.400 (.18)	[- .182 – .441]	.805	.426	4.1	
Day 3				df = 34			
D’	.855 (.46)	.818 (.63))	[- .360 – .508]	337.5	.313	5.2	
Criterion	.212 (.56)	.237 (.56)	[- .501 – .365]	228.0*	.353	5.2	
Recollect	.246 (.23)	.254 (.25)	[- .348 – .280]	- .248	.806	5.4	
Familiar	.495 (.21)	.473 (.27)	[- .239 – .369]	344.0*	.584	5.0	
Day 5				df = 33			
D’	.584 (.58)	.593 (.55)	[- .434 – .428]	- .023	.982	5.4	
Criterion	.161 (.55)	.138 (.49)	[- .349 – .515]	.382	.705	5.1	
Recollect	.197 (.21)	.218 (.22)	[- .368 – .269]	- .352	.727	5.1	
Familiar	.476 (.26)	.441 (.26)	[- .209 – .440]	.739	.465	4.2	

Table 16: Behavioural analyses Part 2: Pairwise comparisons.

Paired comparisons between placebo and L-DOPA conditions for verbal memory performance across different days. Either a paired t-test or a Wilcoxon signed rank test was used depending on how data were distributed. The different measures refer to different signal detection theory metrics which are explained in detail in Chapter II (General Method).
L-DOPA administered after learning impairs memory accuracy on day 1 but does not affect accuracy at later times.
δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. Credible intervals overlapping zero denote no difference. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (green) hypothesis. All p-values are uncorrected. All errors <.001%
* Wilcoxon test used due to non-parametric data. All samples contained zero values for paired differences. P-values for Wilcoxon tests for such data are less reliable.
** BF_{10} = 16.6

Discussion

Surprisingly, exogenous dopamine active after learning, but before and during subsequent sleep, accelerated routine forgetting when memory was tested the following day, but not after 3 or 5 days. I expected to see a dopamine-driven enhancement on memory following delays. The magnitude of the accelerated forgetting was dependent on the dose: higher doses were associated with lower subsequent memory scores. This effect was only present after a 1-day delay but not at later timepoints. This suggests that dopamine accelerates the forgetting of low importance information that would inevitably be lost over time.

Traditionally, forgetting was considered a passive process where information was "lost". However, newer models strongly support an active, forgetting process influenced by dopamine (Berry et al., 2012; Davis & Zhong, 2017). These results suggest an analogous dopamine-dependent active forgetting process in humans. Strategic forgetting may be necessary to give space for salient memories to become preferentially consolidated.

The finding that L-DOPA accelerated routine forgetting on Day 1 was clear from paired comparison (t-tests and Bayesian tests), and rank correlations revealed that this effect was dose-dependent. These findings were robust and survived false discovery rate adjustments. However, in a mixed linear model I could not see a significant effect. One possible explanation is that the mixed linear model structure was too complex for a small data set.

I administered dopamine after learning but before wakeful reactivation and subsequent sleep. The purpose of the reactivation was to create a saliency-tag for the re-activated information. As hypothesised, both on dopamine and placebo, the saliency-tag enhanced recognition memory, and this difference was further enhanced by dopamine. I propose that this is due to a dual effect,

where (1) online reactivation acts as a fast route for immediate and wakeful consolidation (as previously suggested by others (Antony et al., 2017)), and// (2), salient information is preferentially consolidated during sleep (Oudiette, Antony, Creery, & Paller, 2013). Dopamine has been shown to play a role in both of these processes: first by enhancing learning and then by actively accelerating routine forgetting during sleep, while sparing salient information (Berry et al., 2015; Berry et al., 2012).

While our data are in keeping with previous work where dopamine's effects on memory have been paradoxical depending on the timing in relation to the different memory processes (Berry et al., 2012; Coulthard et al., 2012; J.P. Grogan et al., 2015; Yoshinori & Rubin, 2016), other human studies have demonstrated disparate effects. Below I will outline some of the paradoxical or discrepant findings reported in the literature. While I offer some possible explanations as to why findings are so discrepant, due to differences in experimental setups, doses and participant characteristics, it might not be possible to disentangle what exactly explains these discrepancies.

Nocturnal pramipexole enhanced memory for low but not high reward items in healthy adults (Feld et al., 2014). High reward information may already be tagged strongly rendering any beneficial effect of exogenous dopamine minute, while low-reward items may have a relatively larger benefit from dopamine.

In contrast, in our study, participants did not learn to associate words with rewards. Yet it is possible that motivated participants deem correctly recognised words rewarding. While I did not provide feedback during tagging for this reason, participants were likely to be confident in at least some of their 'hit' responses. I cannot out rule that the tagging effect is explained by this rewarding "confidence-boost", which is unlikely to be as strong a reward as a purposefully manipulated monetary prize. Our observed effects may be due to dopamine increasing consolidation for low-rewarded and accelerating forgetting for non-rewarded information. In line

with this reasoning, there may be no discrepancy between our findings and those of Feld et al (2014), as saliency tagging may yield a reward analogous to their low reward.

Other explanations related to the study designs, such as participant demographics, behavioural tasks and dopaminergic agents used may also explain disparate findings. Pramipexole targets D2, unlike L-DOPA which is available to all dopamine receptors. To my awareness there are no studies in humans directly assessing disparate effects of different receptor types on different memory functions, but it is likely that pharmacological agents that act on different receptor sites impact memory and sleep in different ways.

The dose used by Feld et al (2014) was also equivalent to a much lower dose corresponding to a 50mg L-DOPA-dose-equivalence compared to 200mg used here.

When elderly participants were given 150mg of L-DOPA, memory was enhanced at a 6h delay (Chowdhury et al., 2012). While these findings are seemingly in stark contrast with ours, the effect was dose-dependent following an inverted U-shaped curve. Those with a ~2mg bodyweight-dependent dose benefited from the treatment while those with higher or lower doses did not. Doses in our study were in the region of 2.1 to 4.2 mg/kg, with an average dose of 2.9mg, and I found a decrease in performance with higher doses. In line with this, in young adults, 100mg of L-DOPA has also been found to enhance verbal learning, and this effect was positively associated with dose (Knecht et al., 2004). Considering these findings together suggests that L-DOPA may have a biphasic effect where high doses accelerate routine forgetting while lower doses support persistence.

An alternative explanation for the disparate findings between these studies is the medication timing. The dopamine-induced memory enhancement in Chowdhury et al (2012) and Knecht et al (2004) could have been explained by dopamine's actions on encoding rather than

consolidating. It is also possible that the tagging effect reported here reflects differences related to re-encoding rather than saliency tagging.

Response bias, recollection and familiarity may also be affected by dopamine during different memory stages or at different doses. Chowdhury et al (2012) found an increase in the rate of recollection (remember-hits) following 150mg of L-DOPA at encoding and consolidation. In Parkinson's disease, patients' subsequent response bias increases when they are off their usual medication during learning and early consolidation, to resemble the response bias seen in age-matched non-medicated controls (J.P. Grogan et al., 2015). This suggests that dopamine during encoding promotes more a more conservative signal detection. This change was seen at a 1-day delay from learning but not at a 30-minute delay. The lack of effect in our study may be explained by drug timing in relation to learning, or by participant demographics. Responses to dopamine replacement therapies change over time, offering a possible explanation to the discrepancy.

Limitations and caveats

While I carefully timed the administration of L-DOPA to target sleep, I did not record plasma concentrations of dopamine in this study. It is possible that residual amounts of dopamine were present in the system at retrieval, and that dopamine during retrieval biases memory.

L-DOPA clearance is slower in old compared to young adults (Robertson et al., 1989), but it is not clear how absorption changes between ages 65 and 79 (our sample age range). L-DOPA absorption can also be biased by physical activity or food ingestion (Baruzzi et al., 1987; Carter, Nutt, & Woodward, 1992). While I encouraged volunteers to not engage in vigorous physical activity in the two days preceding the sleep visits and I provided evening meals and breakfasts, I

did not measure activity or food intake. Without plasma concentration levels, I could not account for individual effects in drug absorption and clearance.

There are several other limitations I attempt to address in subsequent chapters. Dopaminergic forgetting in drosophila occurs during wakefulness (Berry et al., 2015). I administered dopamine while participants were awake, and they fell asleep around 2 hours later. It is possible that at least a portion of the dopaminergic enhancement of forgetting occurred during wakefulness, before sleep, and that this was triggered by saliency-tagging contextually relevant items. In Chapter V I will examine how nocturnal dopamine modulates sleep to address this concern.

Conclusion

Dopamine accelerates routine forgetting and saliency-tagging increases memory compared to untagged items. Dopamine during tagging further increases this effect. Dopamine may select memories for storage during sleep. Increasing the efficiency of forgetting may allow the prioritisation of effective consolidation of high importance items. This might be explained by saliency-tagging of to-be-stored information having a protective effect against forgetting, as found in drosophila (Berry et al., 2015).

I found no evidence that dopamine modulates recollection, familiarity or response bias during tagging or subsequent sleep. Exogenous dopamine may pose a potential clinical benefit in boosting memory for salient information – if the optimal timing of administration can be established.

Chapter V: Sleep

Data reported in this chapter was collected as a part of the DOPAMIND study.

Introduction

During sleep, the brain performs several restorative actions while cycling through sleep stages. REM, or paradoxical sleep, is a stage during which electrophysiological measures of brain activity look remarkably similar to wakefulness and is closely associated with dopamine levels. Increase in dopamine is thought to be a key player in regulating wakefulness and REM (Eban-Rothschild et al., 2016; Lena et al., 2005), and, conversely, REM deprivation also elevates dopamine levels (Proenca et al., 2014), which in turn induces subsequent REM (M. M. Lima, Andersen, et al., 2008).

In Chapter IV I found that nocturnal L-DOPA plays a role in memory selection. Evidence from the literature suggests that REM-related dopaminergic activity may provide a physiological basis for this. During REM, dopamine in the ventral tegmentum and nucleus accumbens is increased, mimicking the response to novelty and reward during wakefulness (Bunzeck & Duzel, 2006;

Dahan et al., 2007; Lena et al., 2005; Maloney, Mainville, & Jones, 2002; Wittmann et al., 2007) which suggests that similar processes may be occurring during REM and during learning from novelty and reward. Moreover, there is a direct link between REM and memory: reduced REM is associated with a subsequent reduction in long term potentiation and memory (Proenca et al., 2014; Ravassard et al., 2009). Both non-REM and REM benefit memory in humans in humans (Schapiro et al., 2017; Siegel, 2001; M. P. Walker & Stickgold, 2004).

Non-REM sleep is also implicated in memory processes. At and after learning, memory engrams can become tagged as salient based on contextual information (Kaminski et al., 2018; R. G. M. Morris, 2006; Pu & Yu, 2019; Redondo & Morris, 2011). Thereafter, particularly while asleep, newly acquired memories are strengthened for long term storage by spontaneous repetition (Molle et al., 2006; Stickgold, 2005; van de Ven et al., 2016; M. Y. Yang et al., 2019). Patterns of activation of neuronal assemblies at encoding are selectively replayed during slow wave sleep spindles (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). The likelihood of replay is increased for salient information (Singer & Frank, 2009). Spindles during slow wave sleep are thought to provide an accessible electrophysiological marker that relates to memory storage during sleep (Cairney et al., 2018; Dehnavi et al., 2019). This raises the question: neurotransmitters might play a role in these slow wave sleep and memory over night?

Models of memory based on drosophila point to dual effects of dopamine – dopamine enhances encoding of new information at the cost of triggering forgetting of competing information (Berry et al., 2012; Berry et al., 2018). This dopamine-induced strategic forgetting is selective to weakly encoded memories: during sleep, dopamine increases strategic forgetting of non-salient information but spares important memories. Our behavioural findings in Chapter III align well with the drosophila literature.

The increased tagging effect I found ON L-DOPA may be caused by enhanced or spared consolidation of saliently tagged information during REM and slow wave sleep, combined with forgetting which could be caused by L-DOPA potentially affecting memory-related events during slow wave sleep. With the tagging effect, I refer to the relative benefit in persistence for salient over non-salient information. The aim of this chapter is to examine the relationship between L-DOPA and sleep, and how they may interplay in contributing to memory persistence.

First, I aimed to test if different sleep stages are disparately modulated by L-DOPA. I hypothesised that following administration of nocturnal L-DOPA, compared to placebo, (H1) REM duration will increase and (H2) slow wave sleep duration will decrease. Second, utilising data reported in Chapter IV, I explored the relationship between different sleep stages and spindle characteristics and memory. I hypothesised that (H3) slow wave sleep duration and (H4) spindle characteristics will be correlated with better memory both on L-DOPA and placebo. I also explored if any L-DOPA mediated changes in spindles will be correlated with the L-DOPA mediated increase in the tagging effect.

Method

The materials and data used in this chapter were collected as a part of the DOPAMIND study. The method is comprehensively reported in Chapter II (pg. 77). A short reiteration of is given here.

Design and procedure

In this double-blind placebo-controlled study, elderly participants completed two study nights: one with L-DOPA and one with placebo administration, in a random order (Figure 36).

Materials

Sleep EEG was recorded overnight using standard in-laboratory polysomnography with 12 scalp-channels.

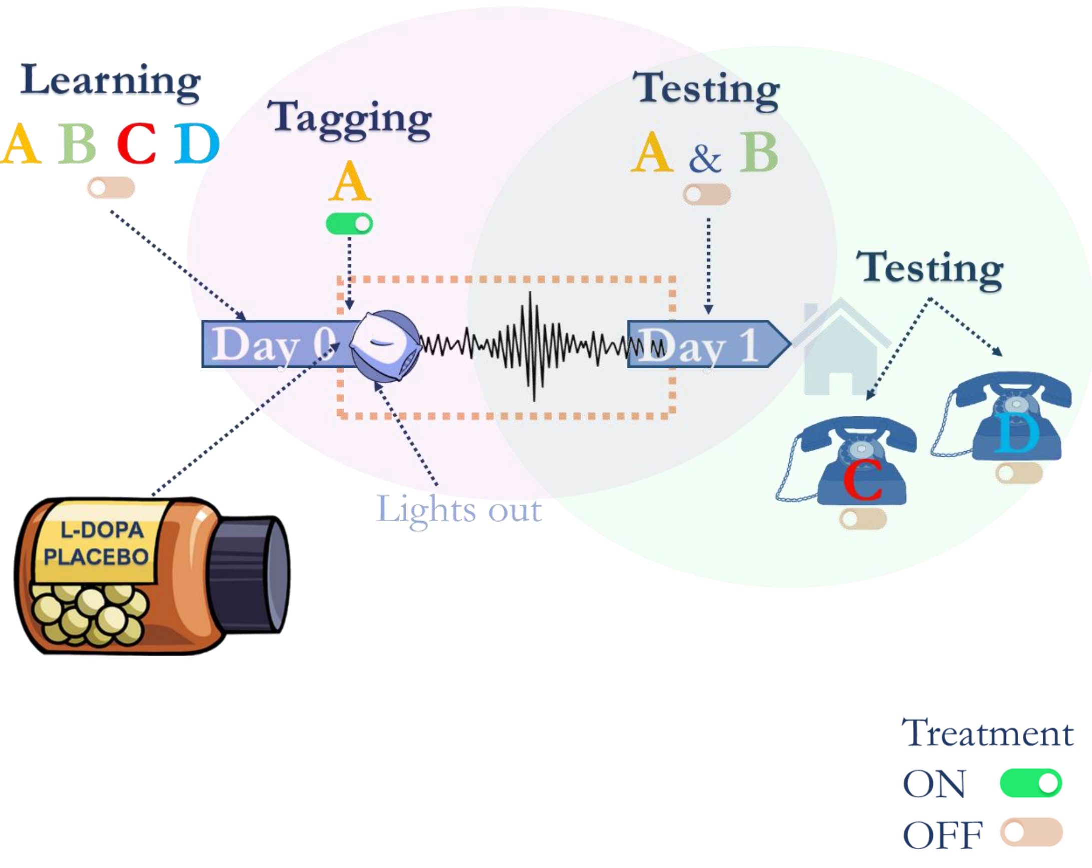


Figure 36: DOPAMIND study visit timeline

Volunteers learnt a memory task and received either 200mg L-DOPA or placebo (depending on visit), prior to having sleep recorded. The orange square denotes time when L-DOPA was active in the system.

In the morning after the polysomnography, participants filled questionnaires, two of which are reported in this chapter: The St Mary's Hospital Sleep Questionnaire (SMHSQ) (Ellis et al., 1981) and the Leeds Sleep Evaluation Questionnaire (LSEQ). The SMHSQ gives six scores:

1. **Sleep efficiency:** Proportion of time in bed spent asleep as a percentage
2. **Sleep onset latency:** Time between settling down for the night and falling asleep
3. **Sleep latency:** Overall time spent asleep
4. **Sleep maintenance:** Number of times awoken at night
5. **Wakefulness after sleep onset:** Sleep fragmentation, reported as minutes awake between falling asleep and finally waking up.
6. **Sleep satisfaction:** As a percentage where 100% is extremely satisfied.

This questionnaire has been shown to have good test/re-test reliability in patients cohorts and healthy volunteers (Ellis et al., 1981). In the LSEQ, participants rate previous night's sleep compared to usual on questions relating to four sub-scales: getting to sleep (GTS), quality of sleep (QOS), ease of awakening from sleep (AFS), and early morning behaviour following wakefulness (BFW). Scores below 5 indicate worse and scores above 5 improved sleep and alertness compared to usual. The LSEQ is sensitive to changes induced by pharmacological agents (Parrott & Hindmarch, 1980).

Analyses

The first level analyses are outlined on page 114. In short, sleep stages were determined manually using standard criteria to extract the duration of each sleep stage (awake, stage 1, stage 2, slow wave sleep and REM).

Using the trace from the Cz channel only, spindle characteristics (amplitude, mean duration, frequency and density) were extracted for stages 2 and slow wave.

Effect of L-DOPA on sleep architecture: To determine the effect of dopamine on sleep architecture, I performed pairwise comparisons between the L-DOPA and placebo nights. L-DOPA mediated change was calculated as the paired difference between L-DOPA and placebo conditions such that positive scores indicate a relative benefit or increase on the drug.

Effect of sleep on memory: To assess the effect of L-DOPA mediated differences in the relation between sleep and memory, I performed a mixed linear model together with correlational analyses on L-DOPA mediated changes on sleep architecture and memory.

Results

Participants

Thirty-five volunteers were tested (Table 11, page 142). An outline of missing data is given here.

Behavioural data: Data was entirely missing for the St Mary's Hospital Sleep Questionnaire (SMHSQ) for two volunteers following the L-DOPA and one volunteer following the placebo night ($n_{SMHSQ} = 32$). One volunteer on placebo, and two on L-DOPA, had omitted answers on the SMHSQ, so their sleep satisfaction score (SSS) could not be determined ($n_{SSS}=29$). These questionnaires were otherwise scored. The wakefulness after sleep onset (WASO) score was calculated as the difference between self-reported sleep onset time and final wake up time, and self-reported sleep latency. Some volunteers reported less time between sleep onset and waking than spent asleep. For these nights, wakefulness after sleep onset was changed to 0 minutes to avoid negative values. There was no missing data for the Leeds Sleep Evaluation Questionnaire.

Polysomnography: Data was partially or entirely missing for five volunteers: two due to technical errors at recording, neither of the nights were scored for them (n_{PSG} = 33), and a further 3 could not be reliably analysed on the automated spindle detection tools ($n_{PSGSPIN}$= 30).

Sleep questionnaires

There were no differences in between L-DOPA and placebo nights in subjective measures (Table 17). Sleep onset latency was an average ~ 10min shorter on L-DOPA but this tendency was not significant. Overall, volunteers reported worse sleep than usual (all LSEQ subscale mean scores <5) for both visits – but there was no effect of treatment.

L-DOPA and sleep architecture

Sleep stages: Sleep architecture on L-DOPA and on placebo followed a typical pattern where stages 2 and slow wave sleep were the most prominent across the nights (Figure 37A), with slow wave sleep typically dominating the first half of the night, and REM dominating in the latter half.

L-DOPA increased slow wave sleep duration by an average of 11% (Figure 37B, Figure 38A). This effect was significant following Benjamini-Hochberg false discovery rate correction ($p_{corrected}$ = .044), where sleep stages (stages 1, 2, slow wave, and REM) were included. L-DOPA did not modulate the duration of stages 1 or 2, total sleep time or wakefulness (BF_{01}>3), while evidence against L-DOPA affecting REM duration was anecdotal (Table 18).

	Mean (SD)		Credible interval	t	p	BF_{01}	H_0 vs H_1
	L-DOPA	Placebo	δ	df = 31			
St Mary's Hospital Sleep Questionnaire							
Efficiency (%)	75.8 (15.6)	77.0 (22.9)	[- .378 – .275]	163*	*.156*	5.1	
Onset latency (min)	17.1 (20.7)	27.4 (31.6)	[- .641 – .045]	61.0*	*.060*	1.2	
Latency (min)	390.5 (84.2)	384.5 (95.1)	[- .442 – .223]	186*	*.952*	4.3	
Maintenance score	3.1 (1.3)	2.8 (1.2)	[- .117 – .636]	174*	*.270*	2.4	
Sleep satisfaction**	62.1 (19.4)	67.5 (15.4)	[- .468 – .229]	- .694	*.494*	4.1	
Wakefulness (WASO)	31.8 (38.7)	27.8 (40.7)	[- .282 – .378]	.302	*.765*	5.1	
Leeds Sleep Evaluation Questionnaire				df = 34			
Total	4.2 (.86)	4.3 (.96)	[- .432 – .221]	- .668	*.509*	4.5	
GTS	3.9 (1.7)	3.9 (.1.2)	[- .432 – .221]	.109	*.914*	5.5	
QOS	3.5 (1.8)	3.7 (1.8)	[- .432 – .221]	- .677	*.503*	4.5	
AFS	4.8 (1.6)	4.9 (4.9)	[- .432 – .221]	304.5	*.911*	4.9	
BFW	4.8 (1.6)	4.9 (1.4)	[- .432 – .221]	282.5	*.600*	4.8	

Table 17: Sleep: Self-evaluation

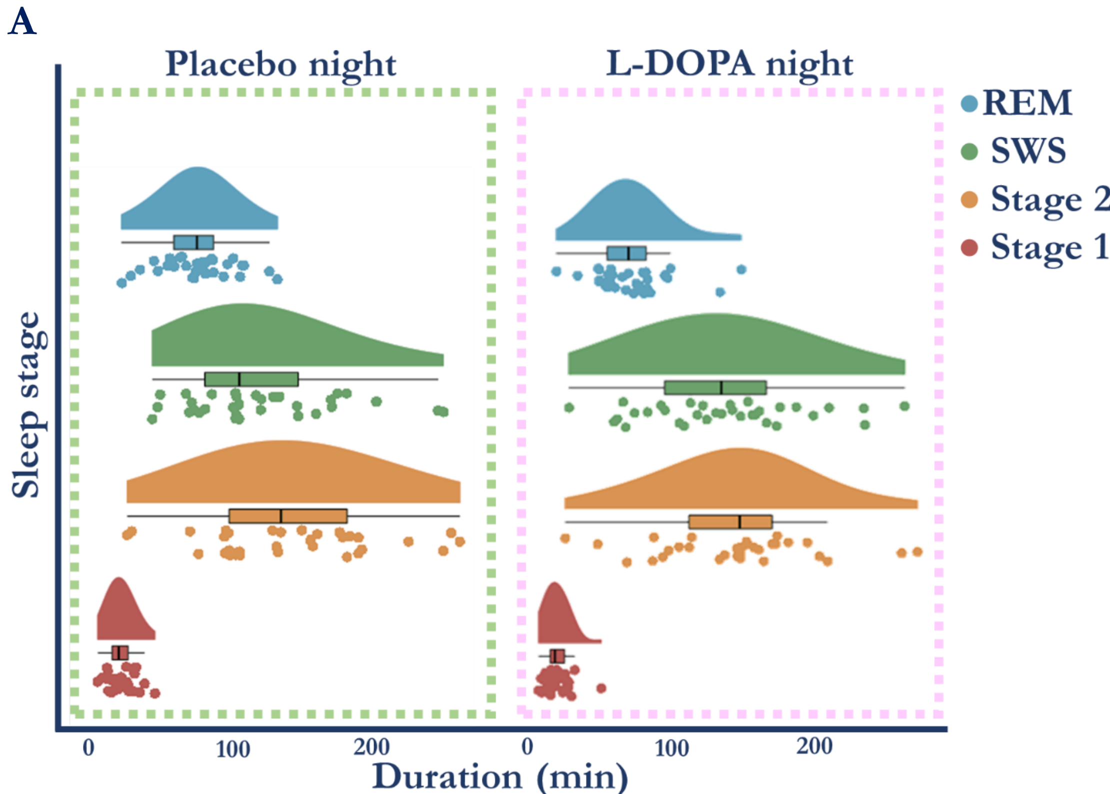
We found no differences in self-reported measures of sleep quality. δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (blue) hypothesis. All p-values are uncorrected. All errors <.06.
GTS = getting to sleep, QOS = quality of sleep, AFS = awakening from sleep, BFW =behaviour following waking.
*Wilcoxon's test; ** df = 28

Time in minutes	Mean (SD) L-DOPA	Mean (SD) Placebo	Credible interval δ	t (df = 31)	p *p-corrected*	BF_{01}	H_0 vs H_1
Asleep	363.7 (60.4)	358.0 (53.8)	[- .368 – .543]	.402	*.691*	4.9	
Awake	116.6 (46.6)	110.5 (50.9)	[- .272 – .636]	.838	*.408*	3.8	
Stage 1	20.0 (8.4)	21.9 (8.8)	[- .712 – .210]	- 1.160	*.255*	2.9	
Stage 2	140.9 (51.1)	140.6 (58.2)	[- .574 – .327]	- .576	*.569*	4.5	
SWS	132.8 (54.0)	120.0 (51.3)	[.116 – 1.104]	2.702	*.011* *.044*	.2**	
REM	70.0 (24.7)	75.5 (25.1)	[- .768 – .142]	- 1.426	*.164*	2.1	

Table 18: Sleep: Sleep stages

L-DOPA increased time spent in slow wave sleep, but it did not affect light sleep (stages 1 and 2), wakefulness, REM or total time spent asleep. δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (black) hypothesis.
SWS = slow wave sleep; REM = Rapid Eye Movement
* Wilcoxon test used due to non-parametric data; ** BF_{10} = 4.0
False discovery rate adjustment was performed using each sleep stage (n_{tests} = 4) and corrected p-values were determined using the Benjamini-Hochberg procedure.

A



B

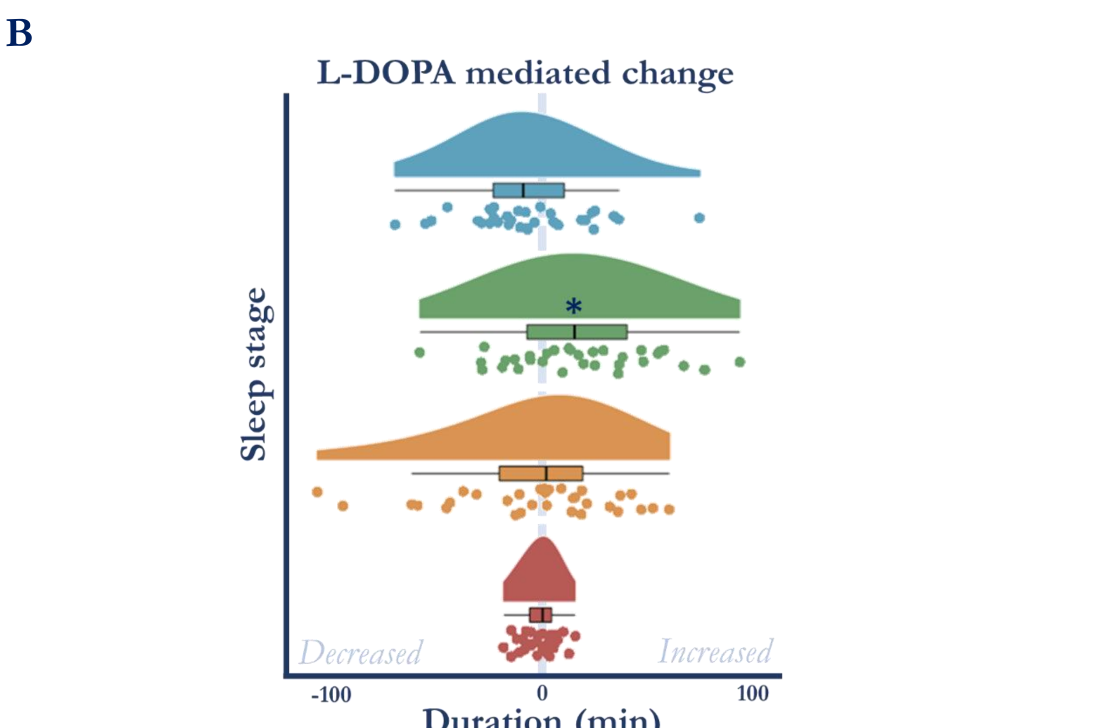


Figure 37: Sleep stage durations

Durations of different sleep stages across placebo (left) and L-DOPA (right) nights.
L-DOPA mediated pairwise changes showed that slow wave sleep duration was increased on L-DOPA. The light blue line denotes no change (change = 0); datapoints to the left had shorter and to the right longer durations on L-DOPA

A **B**

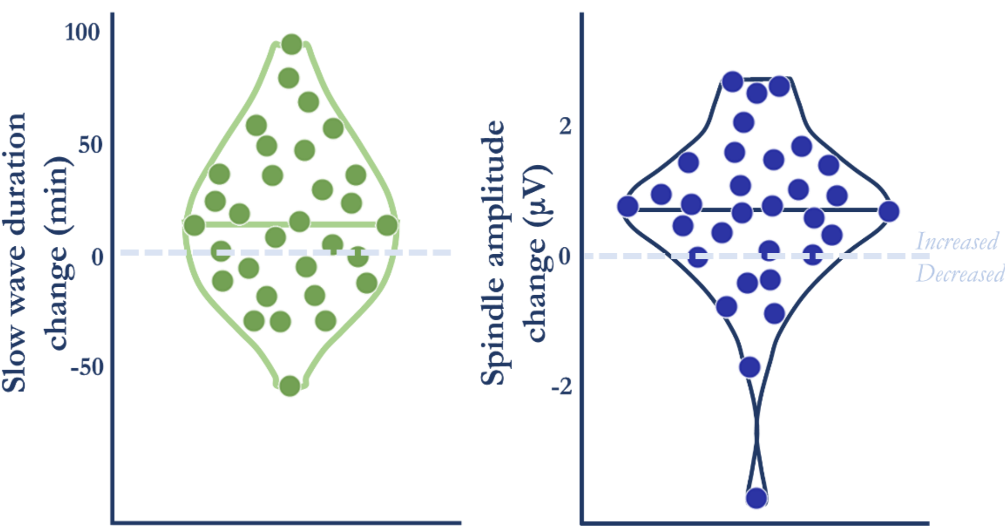


Figure 38: L-DOPA and slow wave architecture

L-DOPA mediated effects on slow wave sleep duration and spindle amplitude L-DOPA increased slow wave sleep duration (A) and spindle amplitude (B).

Panel B: When accounting for the paired nature of the data and the non-normal distribution in the change – as is done by the rank test that is reported in Table 14 – the difference is clear: the change in spindle amplitude is observed in 25 our of 31 individuals but the mean is distorted by a single outlier. However, there is no difference in means in spindle amplitude between the two conditions.

Sleep spindles: Spindles during both slow wave sleep and stage 2 have been associated with memory (Dehnavi et al., 2019; Sirota et al., 2003), but I did not find evidence to suggest that L-DOPA affects stage 2 spindles in the pairwise comparisons Spindle characteristics were analysed for spindles occurring during stage 2 and slow wave sleep separately.

During slow wave sleep, spindle amplitude was increased on L-DOPA compared to placebo (Figure 38B, Table 19). This effect remained following false discovery rate correction including density, amplitude, frequency and duration ($p_{corrected}$ = .008). L-DOPA also mediated spindle amplitude change during stage 2, but this effect did not survive multiple comparison correction ($p_{corrected}$ = .060). Given the consistent trend with the spindle amplitude change in slow wave sleep, the lack of effect may reflect inadequate power.

	Mean (SD) L-DOPA	Mean (SD) Placebo	Credible interval δ	t (df = 30)	p *p-corrected*	BF_{01}	H_0 vs H_1
Slow wave sleep spindles							
Count	686.2 (265)	641.0 (284)	[- .168 – .512]	1.03	*.312*	3.2	
Density	5.7 (2.2)	5.8 (2.5)	[- .353 – .313]	249*	*.992*	5.2	
Amplitude (µA)	28.9 (8.3)	28.2 (8.5)	[.082 – .799]	401*	*.002* *.008*	.3**	
Frequency (Hz)	13.6 (.26)	13.6 (.26)	[- .422 – .242]	197*	*.327*	4.6	
Duration (sec)	.93 (.05)	.94 (.06)	[- .632 – .049]	-1.76	*.088*	1.3	
Stage 2 spindles							
Count	434 (301)	475 (269)	[- .500 – .174]	-1.02	*.317*	3.3	
Density	.94 (.06)	.95 (.06)	[- .531 – .152]	-1.15	*.260*	2.9	
Amplitude (µA)	29.5 (8.2)	29.0 (8.7)	[.068 – .793]	2.60	*.015* *.060*	.3***	
Frequency (Hz)	13.6 (.24)	13.6 (.26)	[- .376 – .287]	-.287	*.776*	5.0	
Duration (sec)	4.8 (1.6)	4.9 (1.4)	[- .545 – .132]	-1.25	*.221*	2.6	

Table 19: Sleep: Spindle characteristics

L-DOPA increased spindle amplitude. δ denotes effect size for the paired differences derived from the Bayesian posterior distribution. BF_{01} and H_0 vs H_1 show the probability of our data having been observed under the null (white) as opposed to the alternative (blue) hypothesis. All p-values are uncorrected. All errors <.04.
Note that the slow wave sleep amplitude effect survived multiple comparisons.
*Wilcoxon; **BF_{10} = 3.6; ***BF_{10} = 3.2
False discovery rate adjustment was performed using each spindle characteristic (n_{tests} = 5) and corrected p-values were determined using the Benjamini-Hochberg procedure.

L-DOPA, sleep and memory

To study the effect of L-DOPA on memory, I concentrated on Day 1 D' (which is a measure of accuracy) as this is where the largest L-DOPA mediated effects were found (Chapter IV). The focus was on the relationship between memory and slow wave duration and slow wave spindle amplitude.

Sleep stage durations: To assess the relation between sleep stage duration and memory, I conducted a series of Spearman's correlations between stage 2, slow wave sleep and REM durations against memory for tagged and non-tagged items (Figure 39, Table 20). Complementing the findings from the mixed linear models, slow wave sleep duration was associated with memory performance for the salient items only. When L-DOPA was given this effect disappeared ($z = 1.99$ $p = .046$), as tested using a Pearson's r-to-z transform.

Spindles: Our main interest was on the L-DOPA mediated *change* in spindle amplitude during stage 2 and slow wave sleep against memory performance. While all memory scores (D' for tagged, untagged and tagging effect) and stage 2 spindle amplitude followed a normal distribution, slow wave spindle amplitude had high kurtosis (>2). The L-DOPA mediated spindle amplitude change was associated with the L-DOPA mediated change in the tagging effect during slow wave sleep (Figure 40)– but not during Stage 2 (Table 21). This effect also survived false discovery rate adjustments ($p_{corrected} = .045$), adjusting for all comparisons between slow wave sleep duration and memory.

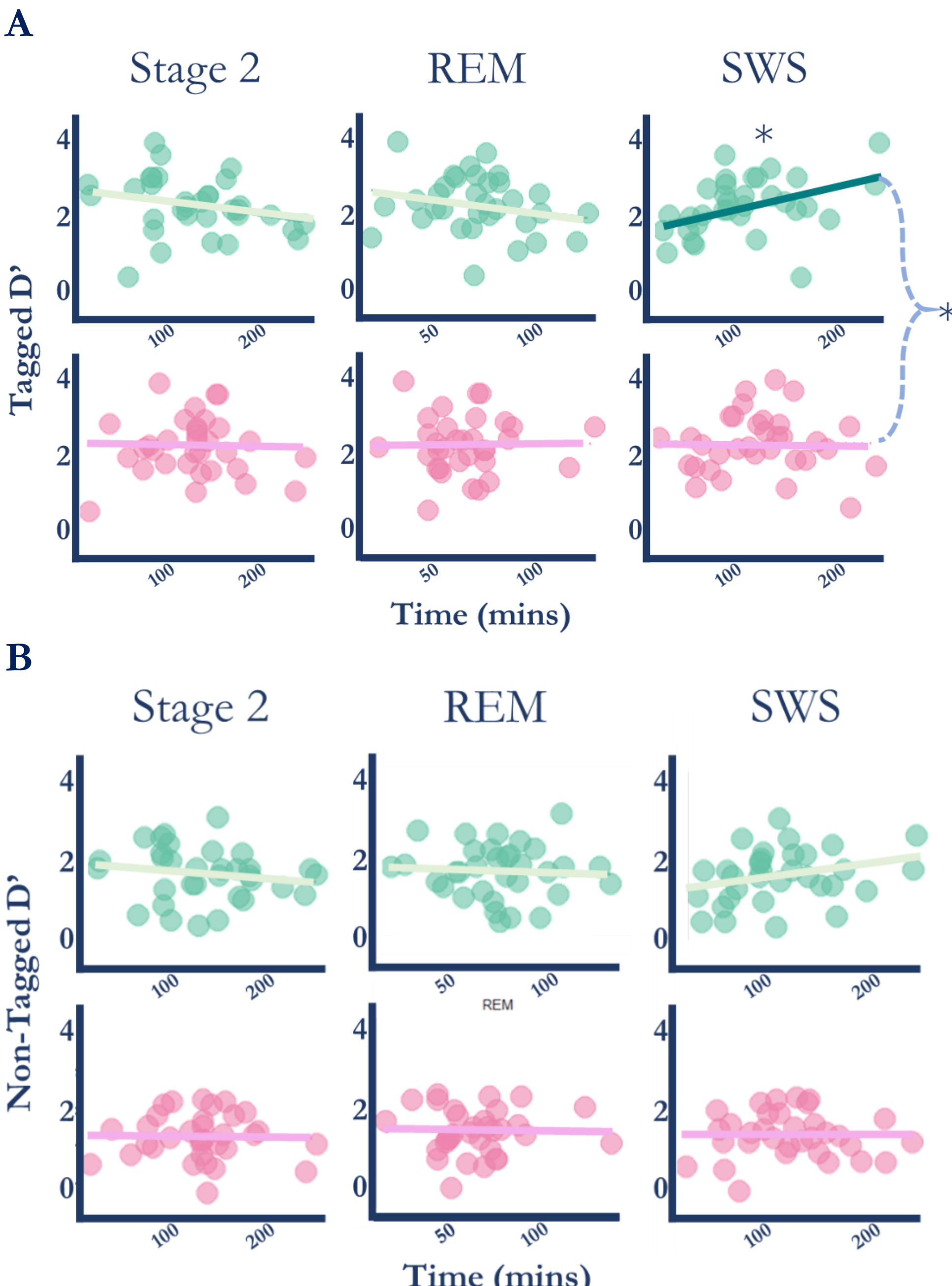


Figure 39: Memory and sleep stage durations

Slow wave sleep duration was associated with memory for the re-exposed / strongly encoded items on placebo. However, this relationship disappeared when L-DOPA was given. No other relationships between memory and sleep duration were observed.
Note that all relationships were assessed using Spearman's rank correlations and the fitted lines are for illustration only. The only correlation that was found was between slow wave sleep duration and memory for the tagged items. The relationship between slow wave sleep duration and tagged information was wiped out on L-DOPA ($z = 1.99$ $p = .046$).
REM = rapid eye movement; SWS = slow wave sleep.

L-DOPA Placebo

	Non-tagged (D’)			
Sleep stage (min)	ϱ	p	ϱ	p
Stage 2	.005	*.980*	-.260	*.150*
REM	.008	*.970*	-.036	*.840*
Slow wave sleep	.065	*.720*	.320	*.071*
	Tagged (D’)			
	ϱ	p	ϱ	p *p-corrected*
Stage 2	-.041	*.820*	-.360	*.040* *.120*
REM	.026	*.890*	-.200	*.250*
Slow wave sleep	.043	*.810*	.450	*.009* *.054*

Table 20: Sleep: Sleep stage duration and memory correlations

Slow wave sleep and memory for the tagged items were correlated on placebo but not on L-DOPA. Corrected p-values shown in purple were obtained using the Benjamini-Hochberg procedure and including each analysis in the bottom portion of this table. The top portion was not corrected for as there were no statistically significant observations.

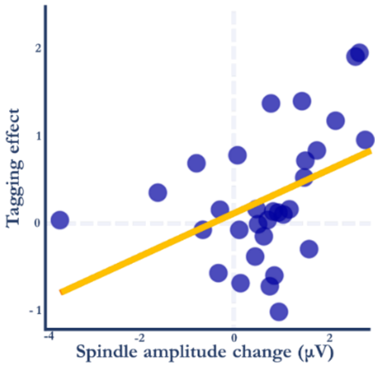


Figure 40: L-DOPA, the tagging effect and spindle amplitude

L-DOPA mediated change in the tagging effect against spindle amplitude The light blue line denotes no difference on L-DOPA. Negative values were lower on L-DOPA than on placebo. Both tagging and spindle amplitude increased on L-DOPA, and these two increases were associated with one another. Note that a Spearman’s rank correlation was used to analyse these data and the line is fitted only for illustration purposes. Correlations between spindle amplitude and re-exposed and other items (not change) are shown in Table 17.

In post-hoc analyses, I also looked at the relationship between spindle density (number of spindles per minute), duration and frequency against the three different memory scores (tagged, untagged and tagging effect). The complete analyses are reported in Appendix M. In short, I found no relationship between L-DOPA mediated changes in density, duration or frequency of spindles in either stage 2 or slow wave sleep and memory measures (tagged, untagged or tagging effect for D').

Inclusion of stage 1: I did not account for the relation between stage 1 sleep and memory for various reasons:

1. Stage 1 sleep duration is short and there is little variability in this stage
2. No spindles take place during stage 1
3. There were no L-DOPA mediated changes in the duration

There is no theoretical reason to think this stage is important for memory

Spindle amplitude (µA)	Tagged (D')		Non-tagged (D')		Tagging effect	
	ϱ	p	ϱ	P	ϱ	p *p-corrected*
Stage 2	.270	*.142*	.006	*.973*	.230	*.195*
Slow wave sleep	.312	*.088*	-.231	*.211*	.438	*.015* *.045*

Table 21: Sleep: L-DOPA mediated changes and memory

Corrected p-values shown in purple were obtained correcting for each Slow wave sleep analysis reported in this table ($n_{tests} = 3$) using the Benjamini-Hochberg procedure

Discussion

I found several interesting L-DOPA mediated effects on sleep characteristics and their relationships with memory. First, in this sample, L-DOPA increased slow wave sleep duration and slow wave spindle amplitude. Second, slow wave sleep duration was associated with memory performance for salient information, but the relationship was lost on L-DOPA nights. Third, L-DOPA mediated changes in spindle amplitude were associated with the L-DOPA-induced increase in the tagging effect.

L-DOPA and sleep

I hypothesised that L-DOPA would increase REM and decrease slow wave sleep duration. Instead, L-DOPA increased slow wave and had no effect on REM duration in an elderly sample. This preliminary finding is interesting because advanced age reduces slow wave sleep and disrupts sleep (Redline et al., 2004). Multiple protective and adaptive processes take place during sleep, many of which are associated with deep sleep (L. Xie et al., 2013).

One of these restorative actions is the flushing of the β-amyloid peptide out of the central nervous system into the cerebrospinal fluid flow (CSF) (Y. E. Ju et al., 2017). During the day, levels of central β-amyloid increase. In turn, at night – particularly during slow wave sleep – the levels are decreased. β-amyloid becomes pathological when the homeostasis of clearance is disrupted (H. A. Pearson & Peers, 2006). In old age, both slow wave sleep and the nocturnal β-amyloid clearance are reduced (Y. F. Huang et al., 2012). When these peptides are not sufficiently cleared, they accumulate in the extra cellular space, disrupting the chat between neurons and

eventually causing cellular death (Benilova, Karran, & De Strooper, 2012; Murphy & LeVine, 2010; Selkoe & Hardy, 2016).

To complete the vicious cycle, plaques of accumulated β-amyloid disrupt hippocampal memory consolidation by reducing slow wave sleep (Mander et al., 2015). As the β-amyloid load increases, the restorative benefit of sleep decreases (Bateman, Wen, Morris, & Holtzman, 2007). Human PET imaging has shown that even a single night of sleep deprivation increases β-amyloid in the hippocampus and thalamus (Shokri-Kojori et al., 2018). In healthy elderly, chronic poor sleep is associated with reduced CSF β-amyloid (Sprecher et al., 2017), and with increased cortical amyloid burden (Sprecher et al., 2015). Reduced CSF β-amyloid is a marker of poorer amyloid clearance and increased central build-up.

It is not clear whether poor sleep causes for β-amyloid to accumulate, if β-amyloid causes poor sleep, or if both are influenced by some other age-related or pathological process. However, more recent evidence shows that specifically disrupting slow wave sleep increases amyloid burden (Y.-E. S. Ju et al., 2017). In one study (Y.-E. S. Ju et al., 2017), CSF β-amyloid samples were taken from healthy adults in the morning after a sleep of targeted slow wave activity disruption or a sham. Participants whose slow wave activity had been disrupted specifically showed a reduction in CSF β-amyloid – this effect could not be seen following sham sleep disturbance. This evidence strongly indicate that not only does sleep have a causal role in increasing amyloid burden – at least transiently – this effect is also specific to processes that take place, at least predominately, during slow wave sleep.

Accumulation of cortical β-amyloid is a key marker of Alzheimer's disease pathology (Selkoe & Hardy, 2016). Impaired sleep increases risk and severity of Alzheimer's disease, likely by disrupting β-amyloid clearance (Lucey & Bateman, 2014). This suggests that pharmacological agents targeting slow wave sleep to enhance cognition may be most useful in healthy elderly or

those at risk of developing memory impairment. Here, I showed that L-DOPA increases slow wave sleep – the stage of sleep most important in clearing β-amyloid. The benefit of L-DOPA in reducing amyloid load remains to be seen, however.

Few studies have attempted to disentangle the effect of dopamine-medication on sleep in healthy individuals. In contrast to our findings, in young healthy men, a single dose of the D2 agonist pramipexole delivered nocturnally reduces slow wave sleep and REM, and increased Stage 1-2 durations, without affecting total sleep time (Feld et al., 2014). In other words, deep sleep and REM decreased while participants spent more time in light sleep. Others have shown that in healthy young adults, the D2 agonist ropinirole reduces sleep onset time (Ferreira et al., 2002) and pramipexole increases daytime somnolence at 3.5h and 5.5h delays from administration (Micallef et al., 2009), suggesting that D2 agonists may promote sleep.

While there were no significant differences in the sleep questionnaire measures, sleep onset latency was on average reported to be shorter on the L-DOPA night. This coupled with the increase in slow wave sleep suggests that L-DOPA at this dose promoted somnolence. On the Leeds Sleep Evaluation Questionnaire, participants tended to report worse-than-usual sleep across visits. This finding was not surprising, as participants were sleeping in an unfamiliar hospital environment with electrodes attached to their scalps.

L-DOPA also increased slow wave sleep spindle amplitude. Spindle amplitude is shaped by the interplay between the thalamus and the cortex (Contreras, Destexhe, Sejnowski, & Steriade, 1997) and increased spindle amplitude is associated with a wider topographical expression of spindles (Nir et al., 2011). Measuring spindles by amplitude has been shown to have better test-retest reliability than spindle density (Cox, Schapiro, Manoach, & Stickgold, 2017). In a sample of over 11 000 individuals, spindle amplitude was shown to be stable across individuals overall, but to also decline with age (Purcell et al., 2017). Spindle amplitude in this study was more closely

linked to sigma power than other spindle characteristics. Sigma power is often referred to as a sleep "fingerprint" because it is highly stable within individuals (De Gennaro, Ferrara, Vecchio, Curcio, & Bertini, 2005).

Spindle amplitude also correlates positively with subsequent memory (Mednick et al., 2013). Therefore, spindle amplitude is a useful metric likely to convey information about how multiple brain regions are working synchronously to forget irrelevant and retain important memories.

L-DOPA, sleep and memory

I hypothesised that slow wave sleep duration will be positively correlated with memory both on L-DOPA and placebo. Instead, I found a relationship between slow wave sleep duration and memory only for saliently tagged information, but this effect disappeared with L-DOPA. There was no relationship between slow wave sleep duration and memory for non-tagged items in either conditions.

Spindles during slow wave sleep are indirectly associated with hippocampal replay, the anatomical underpinning for memory storage (Molle et al., 2006; M. Y. Yang et al., 2019). Different spindle characteristics, such as amplitude, are associated with enhanced memory persistence (Mednick et al., 2013). It may not be possible or worthwhile to replay all memories during sleep.

Instead, memories are selectively replayed causing selective memory retention (Blaskovich, Szollosi, Gombos, Racsmany, & Simor, 2017).

L-DOPA-mediated change in slow wave sleep spindle amplitude was associated with the tagging effect. In Chapter IV I showed that both on dopamine and placebo, information tagged as salient was better retained than non-salient information, and that this difference was enhanced by

dopamine. I proposed that this is due to dopamine increasing both wakeful reactivation – that acts as a fast route for online consolidation (Antony et al., 2017) – and salient information being preferentially consolidated during sleep (Oudiette et al., 2013). The relationship between spindle amplitude change and the tagging effect suggest that dopamine may mediate this effect overnight. Dopamine has been shown to play a role in both of these processes: first by enhancing learning and then by actively accelerating forgetting during sleep (Berry et al., 2015; Berry et al., 2012).

While sleep spindles during slow wave sleep are well linked to memory, this study is the first to illuminate the behavioural relevance of spindle characteristics and their relationship to dopamine. The magnitude of the difference between the effect of dopamine on memory performance for salient and non-salient information was associated with an increase in spindle amplitude.

There are two possible explanations for this. First, dopamine generally enhances spindle amplitude which in turn enhances the way in which memory is biased in favour of salient information. Second, tagging before sleep alters spindle amplitude to reflect the changes that have taken place during tagging.

Learning alone is sufficient to change both sleep architecture and spindle characteristics. For example, in after intense motor memory training, both stage 2 duration and spindle density have been reported to increase (S. M. Fogel & Smith, 2006). These changes have also been shown to be associated with theta coherence during learning (S. M. Fogel, Smith, & Beninger, 2009). Therefore, it is plausible that dopamine during repeat exposure to the stimuli "tagged" or earmarked the word-list to be preferentially repeated in sleep. Therefore, the spindle amplitude change may be a secondary effect to dopamine's effects during wakeful re-learning.

Spindle amplitude has also been associated with enhanced memory retention in a motivated forgetting task (Blaskovich et al., 2017) and a tagging paradigm involving re-test (Heib et al.,

2015) suggesting that spindle amplitude may be associated with selecting memories for later retention. During tagging by re-test, theta coherence – which is associated with enhanced encoding – is increased. The change in theta coherence during the memory, compared to a control, task predicted increased spindle amplitude and subsequent memory (Heib et al., 2015). Therefore, learning can not only influence later spindle density but also amplitude of spindles.

In line with this it is possible that dopamine during tagging enhanced encoding-or-retrieval-related theta activity which triggered changes in sleep architecture and subsequent memory. In the absence of EEG recordings from the tagging phase, I cannot ascertain if dopamine enhanced theta. It is possible that here, I manipulated processes during learning that then had a subsequent effect on sleep. The data presented in this thesis is not sufficient in disentangling whether dopamine is **directly** influencing learning, sleep, or both.

In humans, evidence that behaviourally salient information benefits from sleep is abundant (Hu, Stylos-Allan, & Walker, 2006; Oudiette et al., 2013; Rauchs et al., 2011; Saletin, Goldstein, & Walker, 2011; Sterpenich et al., 2009; Wilhelm et al., 2011). While there are fewer studies examining the role of dopamine in slow wave sleep, rewarded information is preferentially replayed during this slow wave sleep but not during REM (Lansink, Goltstein, Lankelma, McNaughton, & Pennartz, 2009; Pennartz et al., 2004). Salient memories are also preferentially consolidated by replay (Fischer & Born, 2009).

This suggests that either dopamine plays a role in slow wave sleep regulation or that dopamine during learning tags memories, and these tags are utilised later in a process that no longer requires dopamine release. It is unlikely that tagging of 20 words would have influenced slow wave sleep with an average 11% increase.

Dose

Dose may introduce a biphasic effect of dopamine on sleep. Smaller doses of central D2 agonists apomorphine or bromocriptine are associated with increased REM and slow wave sleep in rats, while larger doses have the opposite effect. The D2 agonist pergolide also has a biphasic effect on slow wave sleep and wakefulness but it inhibits REM at all given doses (Monti, Hawkins, Jantos, Dangelo, et al., 1988; Monti et al., 1989). A word of caution is required when interpreting rodent studies. Rodent sleep is ultradian rather than circadian in nature. Differences in human and rodent sleep/wake cycle regulation are likely. Sleep disturbances in human Parkinson's disease typically precede disease onset, often by decades (Videnovic & Golombek, 2013). This suggests that either too much or too little dopamine can have a detrimental effect on sleep integrity, which may affect cognition and memory.

The disparate findings may be explained by dose-dependent effects, differences in tasks and study paradigms.

Limitations and future directions

Age-related changes in sleep architecture are well reported. Ageing decreases the duration of deep sleep, and the number and amplitude of spindles (Nicolas, Petit, Rompre, & Montplaisir, 2001) – with some reporting a nearly 50% reduction in spindle amplitude with advanced age (Crowley, Trinder, Kim, Carrington, & Colrain, 2002). The reasons for age-related decrease in spindle amplitude is not fully known. Whatever the cause, enhancement of slow wave sleep duration or architecture could yield clinical benefits.

Our current findings may have implications for prevention of Alzheimer's disease and other age-related diseases affecting sleep. However, the potential of L-DOPA in augmenting disease progression in neurodegenerative diseases affecting sleep and memory needs to be further assessed in longitudinal clinical trials in at-risk individuals. Slow wave sleep is reduced through

ageing and may be affected early in Alzheimer's Disease (Matthew P. Walker, 2009). Interrupting slow wave sleep is proposed to hinder clearance of β-amyloid from the brain and β-amyloid plaques are one of the pathological changes in Alzheimer's Disease (Y.-E. S. Ju et al., 2017; L. Xie et al., 2013). Through increasing slow wave sleep duration and spindle amplitude with nocturnal dopamine, I open up a new therapeutic avenue for Alzheimer's disease prevention – repurposing L-DOPA to prevent Alzheimer's. Future clinical trials with longer term interventions in healthy elderly and those at risk are needed to ascertain any potential clinical benefit of L-DOPA on amyloid clearance.

It is not clear whether the association between L-DOPA mediated increase in spindle amplitude and the tagging effect is mediated by dopamine's influence on tagging, sleep, or both. Future studies are needed to ascertain L-DOPAs effects on tagging by recording EEG during tagging and encoding, and by manipulating L-DOPA timing to target tagging.

It is possible that sleep-mediated effects other than L-DOPA affected performance here. Participants were sleeping in an environment that was novel to them and several participants reported that their sleep was worse than usual during the study nights. It is likely that this would have had an impact on memory related processes, particularly on the first night.

This could have been counteracted somewhat by introducing an acclimatisation night. It could be argued that an added acclimatisation night would have made this study more robust by making sleep more naturalistic. However, the participants were already asked to spend two nights in a hospital to take part and it is not clear if the benefit of including an acclimatisation night would have outweighed the cost on participants and the research budget.

Furthermore, in this trial, treatment order was counterbalanced and randomised: half of the participants received L-DOPA and half received placebo on their first visit. While not including an acclimatisation night likely reduced sleep quality during study visits, the effect would have

been similar on L-DOPA and placebo conditions. Therefore, it is unlikely the dopamine-induced changes on sleep physiology and memory can be explained by these other sleep-mediated effects on memory.

Furthermore, while unlikely, it is possible that discrepancies in sleep scoring affected some my findings in this chapter. Manual sleep staging requires intimate knowledge of sleep physiology but even with expert raters using the AASM scoring system, the inter-rater agreeability is only around 80% (Danker-Hopfe et al., 2009). Here, manual scoring was performed first by two raters (Will Carr and Oliver Radtke), neither of whom are experts. Then, I manually quality-controlled 10% of nights rated by each rater by visual inspection. Even when precautions are taken, there is a large margin of error in both inter- and intra-rater reliability.

Furthermore, each participant was rated by the same person in batches. In other words, each individual participants' L-DOPA and placebo nights were rated together in one go. While this approach makes rating easier – as the rater's eye will adapt to the participant's individual sleep "fingerprint" – this also introduces possible bias. As L-DOPA altered sleep architecture and characteristics, it is possible that the raters were trying to guess which treatment was allocated to which night.

Reflecting to earlier Chapters

Taking these findings together with the findings from Chapters III and IV, suggest that dopamine's effect on memory may be reliant on sleep. In Chapter III I did not find reduced memory for the encoding condition a day after learning. Therefore, the finding that L-DOPA accelerated forgetting during sleep is unlikely to have been driven by dopamine during the tagging phase in that experiment. In Chapter III (DARet), I tested a proportion of the items following learning, proposedly tagging those items as relevant. While these items were not re-tested in the DARet study, had the 'tagging' phase biased accelerated forgetting in the

DOPAMIND study, I would expect to see poorer memory for the tested items on Day 1 in the encoding experiment. Instead, I saw no effect of L-DOPA.

However, I did not have a control experiment in which the setting would have been the same except for the involvement of sleep. This question could be addressed using a different experimental setup in which participants receive L-DOPA both during a 90-minute nap and during an equal amount of time spent awake. If dopamine's memory modulating effects are specific to sleep, one would expect to see L-DOPA-driven changes in consolidation and/or forgetting processes in the nap condition but not in the awake condition.

Conclusion

Together, our findings suggest that saliency-tagging increases the likelihood that an item is remembered. Dopamine during tagging further increases this effect. During subsequent sleep, dopamine increases both the duration of slow wave sleep and the coherence of thalamocortical communication and causes more synchronous cortical firing during spindles. I propose that this increase in coherence is associated with more selective hippocampal replay. However, to my awareness, this hypothesis is yet to be directly studied using direct recordings from the hippocampus in animals or humans.

Chapter VI: Sleep, memory and the medial lobe

Data reported in this chapter was collected as a part of the DOPAMIND study.

Introduction

During sleep, memories are processed through interactions between the medial temporal lobe structures and the cortex (Squire et al., 2015). In the absence of these structures, including the hippocampus, memories do not become consolidated (Scoville & Milner, 1957). There is a great deal of fluctuation in healthy brain morphology between individuals while longitudinal changes within participants are small and slow in comparison. Similarly, compared to the large variability

between individuals in sleep electrophysiology (Tucker, Dinges, & Van Dongen, 2007; Werth, Achermann, Dijk, & Borbely, 1997), there is relatively little fluctuation in night-to-night sleep physiology within individuals (De Gennaro et al., 2005; Lewandowski, Rosipal, & Dorffner, 2013). It is likely that interpersonal variations in sleep physiology are interlinked with the underlying brain anatomy.

During sleep, structures of the medial temporal lobe play a role in supporting memory longevity (Cairney et al., 2018; M. Y. Yang et al., 2019). Long term memory persistence relies on efficient communication between brain structures that support encoding, consolidation, strategic forgetting, and retrieval. The medial temporal lobe in particular plays a key role in memory, with interconnected substructures having unique roles along the stages of the memory processing pathway. The medial temporal memory system includes of the hippocampus (comprised of subfields CA1, CA2, CA3, dentate gyrus and subiculum), and adjacent cortical regions including the entorhinal, perirhinal and parahippocampal cortices (Squire, 1992). A hallmark symptom of damage to these structures, particularly the hippocampus, is profound memory loss (i.e., amnesia).

A classic case is patient H.M. (Scoville & Milner, 1957), whose hippocampi were removed bilaterally to treat severe epilepsy. His surgery led to a severe amnesia and almost complete inability to form long lasting memories (Squire, 2009). While short-term (immediate) memory was unaffected, this damage caused severe anterograde amnesia with little or no effect on cognition otherwise. Since, similar symptoms in patients with hippocampal damage have been described by others (Annese et al., 2014; Rempel-Clower, Zola, Squire, & Amaral, 1996; Zola-Morgan, Squire, & Amaral, 1986). In rats with hippocampal lesions, immediate memory is intact while delayed memory is impaired (Clark, West, Zola, & Squire, 2001). Typically, hippocampal damage mediated memory impairment generalises across sensory modalities and affects

declarative memory selectively (Squire, Stark, & Clark, 2004). Medial temporal lobe structures therefore play a key role in episodic memory persistence over long delays.

Recognition memory tests can be used to assess memory persistence. Recognition memories are thought to be the outcome of two separable processes – recollection (remembering) and familiarity (knowing). The former refers to conscious recollection of the learning context, while familiarity refers to recognising information as familiar in the absence of recollection. These are thought to be analogous to episodic and semantic memories (Brown & Aggleton, 2001). For example, you may *know* that the capital of Finland is Helsinki in the absence of conscious recollection of having learnt this information. The type of information recognised is unimportant – rather what matters is whether a recollection of the learning context is present.

Familiarity and recollection memories are thought to be supported by different medial temporal lobe structures. The perirhinal and lateral entorhinal cortices have been associated with the familiarity component of recognition memory, whereas the hippocampus is associated with the recollection component (Bird, 2017; Yonelinas et al., 1998). The hippocampus is intimately involved in binding together associations, such as learnt items and learning contexts. Meanwhile, the adjacent perirhinal and entorhinal cortices can recognise familiar items in the absence of recollections for associated information. Note that there is some controversy as to whether these two processes are truly separate and some argue that both familiarity and recollection require the hippocampus, e.g. (C. N. Smith, Wixted, & Squire, 2011; Wixted & Squire, 2010).

Attempts to illuminate the role of medial temporal lobe structures in the different recognition memory processes have been made using structural MRI. In one study the total volume of the hippocampus and entorhinal cortex was not associated with recognition overall. Yet, 25% of age-related changes in recollection memory could be explained by differences in total hippocampal volume (Schoemaker et al., 2017). This study did not isolate different substructures of the

hippocampus, so it is not clear which age-related hippocampal volumetric changes are driving this finding.

The hippocampus consists of several subfields with dissociable functions (de Wael et al., 2018; Dillon et al., 2017). Structurally, different domains of memory and cognitive ability are more strongly associated with individual subfield than total hippocampal function (Travis et al., 2014; Uribe et al., 2018). In fMRI, activity within the CA1 and CA2/CA3/dentate gyrus (as one subregion) was associated with processes that bind memories and contextual associations together (Dimsdale-Zucker, Ritchey, Ekstrom, Yonelinas, & Ranganath, 2018), suggesting that these subfields may support the recollection component. Yet, the role of individual hippocampal subfield volumes and integrity remains unclear. It is also not clear how age-related differences in the structure and integrity of hippocampus and its subfields contribute to those sleep-related events that support memory persistence.

Only a handful of studies to date have examined the relationship between sleep and medial temporal lobe morphology in healthy human subjects. In children, self-reported overall sleep duration has been positively associated with total hippocampal volume (Taki et al., 2012). In healthy young adults sleep architecture was associated with anterior corpus callosum volume (Buchmann et al., 2011), while slow wave sleep amplitude has been associated with grey matter morphology (Reinhard et al., 2014; Saletin, van der Helm, & Walker, 2013). In healthy ageing, cortical thinning mediates the effect of age-related changes in spindle amplitude (Dube et al., 2015) and in young adults hippocampal volume has been associated with memory-related spindle activity (Saletin et al., 2013). Decreased spindle amplitude likely reflects a reduction in cortical connectivity during sleep. While volumes of different subfields are associated disparately with different cognitive functions their involvement with sleep is not well established.

Chapter VI: Sleep, memory and the medial lobe

Introduction

Whereas earlier work has focused on hippocampus as a whole, more recent studies have focused on subfield-specific effects of sleep. Medial temporal lobe structures are known to be associated with memory-related sleep events during both REM and slow wave sleep (Louie & Wilson, 2001; Rauchs et al., 2005; Stickgold, 2005; M. A. Wilson & McNaughton, 1994). Sharp wave ripples, which mediate memory, are seen primarily in the CA1 and CA3 regions. Temporally CA3-1 ripples are preceded by CA2 activity, while during ripples CA2 activity is suppressed (Kay et al., 2016). During wakefulness, entorhinal cortex primes CA2 enhancing likelihood of replay – while during sleep ripples are predominately triggered by activity in the CA3 (Oliva, Fernandez-Ruiz, Buzsaki, & Berenyi, 2016). In vitro, activity in the subiculum can also precede ripples (Norimoto, Matsumoto, Miyawaki, Matsuki, & Ikegaya, 2013). These regions are therefore likely to be associated with sleep spindles. The volume of CA2-CA3-dentate gyrus (grouped as one subfield) can also predict cognitive vulnerability in response to sleep deprivation in humans (Saletin et al., 2016). Ultimately during sleep, CA1, CA2, CA3, the subiculum and the entorhinal are all associated with ripple and replay.

The volumes of different hippocampal subfields can be studied using magnetic resonance. In addition to volumetry, T2 relaxometry can be used to assess subfield tissue integrity. The T2 time is influenced by the presence of different molecules in the tissue. Importantly, it is increased when with more water in the tissue. As an intuitive analogue, a bruised apple will have faster T2 relaxation due to increased water content, yet the volume is unaffected by bruising. T2 relaxation times are sensitive to early stages of dementia pathology, and they can predict disease progression (Callaghan et al., 2014; Knight et al., 2019; Tang et al., 2018). Early neurobiological changes associated with age and pathology that are missed out by looking at the volumes alone can thus be detected with T2 relaxometry.

The aim of this chapter is to examine the volumetry and relaxometry of the hippocampal subfields and the entorhinal cortex in relation to memory, sleep and the dopamine-induced effects on memory and sleep architecture. To that end, I hypothesised that;

- Volumes and T2 relaxometry measures of hippocampal subfields and entorhinal cortex are correlated with recognition memory accuracy
- Volumes and T2 relaxometry measures of CA1, CA3, dentate gyrus, and subiculum are correlated with recollection
- Volume and T2 relaxometry measures of Entorhinal cortex are correlated with familiarity
- CA1, CA2, CA3 and subiculum will be associated with sleep spindle densities
- Subfield volumes will be associated with the L-DOPA mediated changes in memory and sleep characteristics.

I also explored the relationship between sleep stage durations and spindle characteristics and volumetry and T2 relaxometry.

Method

The method is reported comprehensively in Chapter II. In short, volunteers took part in a double-blind randomised controlled trial where single doses of L-DOPA 200mg and placebo were given nocturnally in a random order. Polysomnographic recordings were obtained both from the L-DOPA and the placebo nights. Participants also completed memory tests as described on page 90. On one of the visits, a structural magnetic resonance scan was taken in order to assess hippocampal subfield volumes and relaxometry, as described on page 99.

Analyses

The first objective was to assess the relationship between medial temporal lobe subregional volumes and relaxometry. Volumes were measured in cubic millimetres and relaxometry was measured as the width of the intraregional T2 relaxation times (standard deviations). I ran a series of correlations between subfield volumes and relaxometry and accuracy, familiarity and recollection on days 1, 3, and 5 in the placebo condition. Next, I examined the relationship between sleep stage durations and spindle densities, and subfield volumes. I included time spent in Stages 2, slow wave and REM sleep in minutes – but not stage 1 – and I included spindle densities during stage 2 and slow wave sleep. Spindle densities were calculated as the average number of spindles per minute.

Finally, I wanted to see if hippocampal subfield volumes were associated with the dopamine mediated changes in memory scores or sleep characteristics. To achieve this, I assessed the relationship between subfield volumes and L-DOPA-mediated changes in day 1 forgetting (D' for non-tagged items), the tagging effect, slow wave sleep duration and spindle amplitude.

Any findings were false discovery rate adjusted, considering all subfields, using the Benjamini-Hochberg procedure.

Note that the volumes used in this chapter are raw volumes and they were not corrected for intracranial or total brain volume.

Results

Participants and descriptive statistics

Two of the 35 participants who completed the study could not have MRIs due to metal implants (n_{MRI} = 33). Visual inspection of subfield segmentation confirmed that the algorithm performed well. Two scans could not be segmented to a good standard from initial scans – one due to movement and another due to poor scan alignment around the hippocampal /axis during scanning. These two volunteers were re-scanned 6 and 7 months after testing. All other volunteers were scanned between the screening visit and end of final sleep visit. All analyses in this chapter include 33 participants. For descriptive statistics see Table 11 on page 142, and Table 11, Figure 41.

Distributions CA1, CA2, subiculum and the entorhinal cortex volumes were normally distributed. CA3 and dentate gyrus were not, so non-parametric tests were used. In the behavioural measures accuracy (D') and recollection (remember-HITs) on Day 3 and recollection on Day 5 were not normally distributed. From sleep measures minutes in sleep stages (Stage 2, slow wave sleep and REM) were normally distributed. Spindle density during stage 2 and the L-DOPA mediated effect on stage 3 spindle amplitude were both had high kurtosis. All T2 relaxometry distributions (standard deviations) were considered non-normal.

I note that volume distributions varied between subfields. I do not know the explanation for this, but possibilities include anatomical variability of the effects of age and disease on subfield volume or a technical anomaly (which we have done our best to eliminate). Exploring this could be the basis of future work.

	Mean (SD)	Range
Volumetry (mm^3)		
CA1	**2241** (304)	1756 – 3099
CA2	**31** (9)	13 – 52
CA3	**122** (37)	77 – 231
Dentate gyrus	**1560** (252)	1204 – 2620
Subiculum	**699** (79)	569 – 880
Entorhinal cortex	**888** (118)	651 – 1126
T2 relaxometry (SD)		
CA1	**22.6** (2.1)	19.2 – 30.7
CA2	**19.6** (3.2)	14.6 – 30.3
CA3	**22.6** (2.9)	15.8 – 32.0
Dentate gyrus	**22.2** (2.0)	19.7 – 29.3
Subiculum	**25.1** (1.8)	21.2 – 29.8
Entorhinal cortex	**23.9** (2.1)	21.0 – 29.1

Table 22: MRI: Descriptives

N = 33; CA1-3 = Cornu Ammonis SD = standard deviation

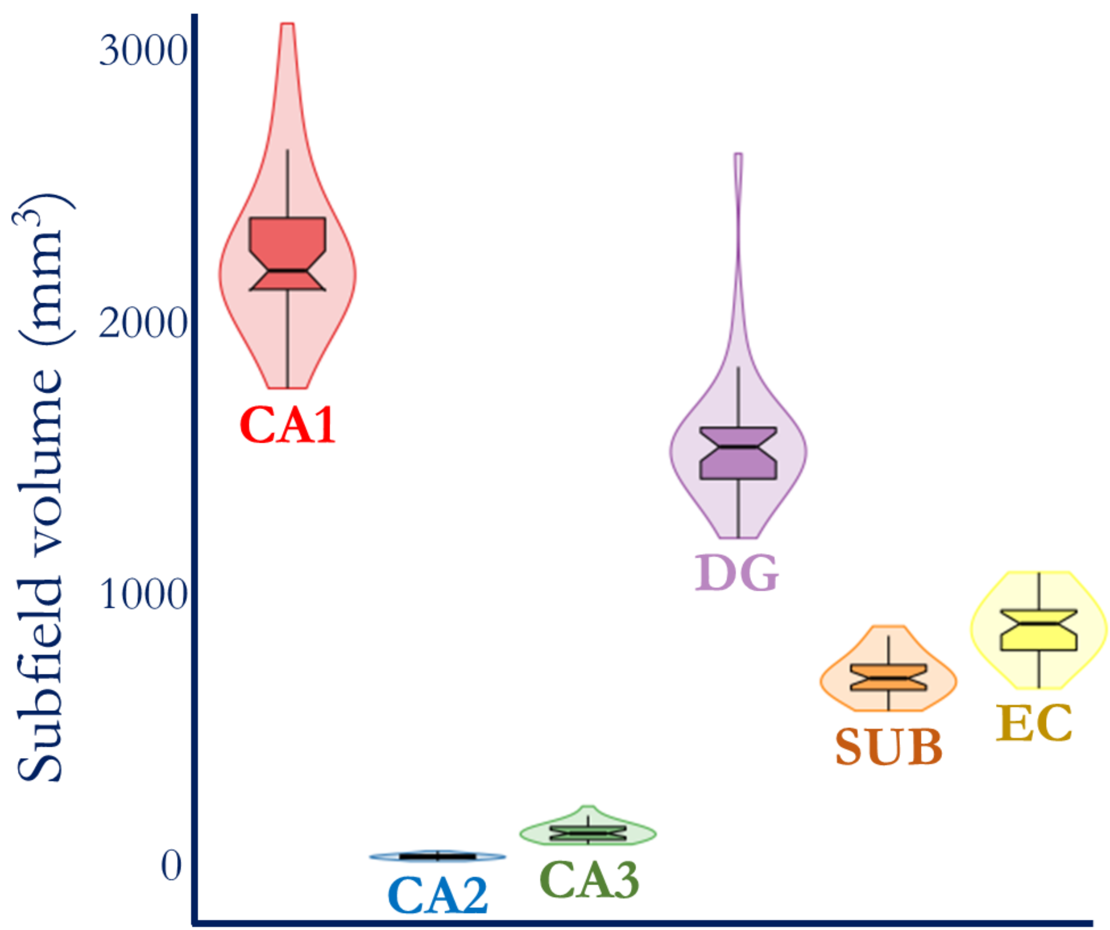


Figure 41: Medial temporal lobes substructure volumes

Medial temporal lobe structures and memory

Correlations between subfield volumes and memory initially revealed a negative relationships between CA2 volume and day 1 memory both for non-tagged and tagged items (Lists A and B, Table 23, Figure 42). These correlations disappeared upon correcting for multiple comparisons (tagged $p_{corrected}$ = .288; non-tagged $p_{corrected}$ = .096).

VOLUMES	CA1	CA2	CA3*	DG*	SUB	EC
			r *p*			
Day 1						
Non-tagged (D')	-.26 *.140*	-.42 *.016*	-.19 *.300*	-.34 *.054*	-.27 *.120*	-.06 *.740*
Tagged (D')	-.09 *.630*	-.36 *.038*	-.30 *.092*	.01 *.960*	-.05 *.800*	-.18 *.330*
Tagging effect (D')	.18 *.310*	-.04 *.830*	.26 *.150*	.04 *.820*	.25 *.160*	-.18 *.310*
Familiarity	<.001 *.996*	-.08 *.679*	<.001 *>.999*	-.10 *.569*	-.22 *.211*	.09 *.638*
Recollection	.003 *.990*	.04 *.830*	.07 *.700*	-.04 *.840*	.01 *.970*	-.06 *.760*
Day 3						
Accuracy (D') *	-.08 *.660*	-.11 *.540*	.08 *.660*	-.01 *.950*	-.14 *.440*	-.06 *.760*
Familiarity	.17 *.356*	.27 *.127*	.21 *.240*	.02 *.218*	.21 *.907*	.06 *.754*
Recollection*	-.10 *.590*	-.11 *.530*	-.11 *.530*	-.16 *.380*	-.25 *.160*	-.11 *.540*
Day 5 **						
Accuracy (D')	-.24 *.190*	-.30 *.097*	.06 *.740*	-.13 *.490*	-.20 *.270*	-.15 *.410*
Familiarity	.06 *.743*	.12 *.497*	.28 *.123*	.13 *.474*	.02 *.918*	-.01 *.698*
Recollection*	.07 *.690*	.01 *.940*	-.01 *.960*	-.04 *.810*	.01 *.970*	.11 *.550*

Table 23: MRI: Subfield volumes and memory.

Correlation coefficients and *p-values*. Note that red-shaded associations did not survive false discovery rate correction.

*Spearman's test used
**One memory score missing.

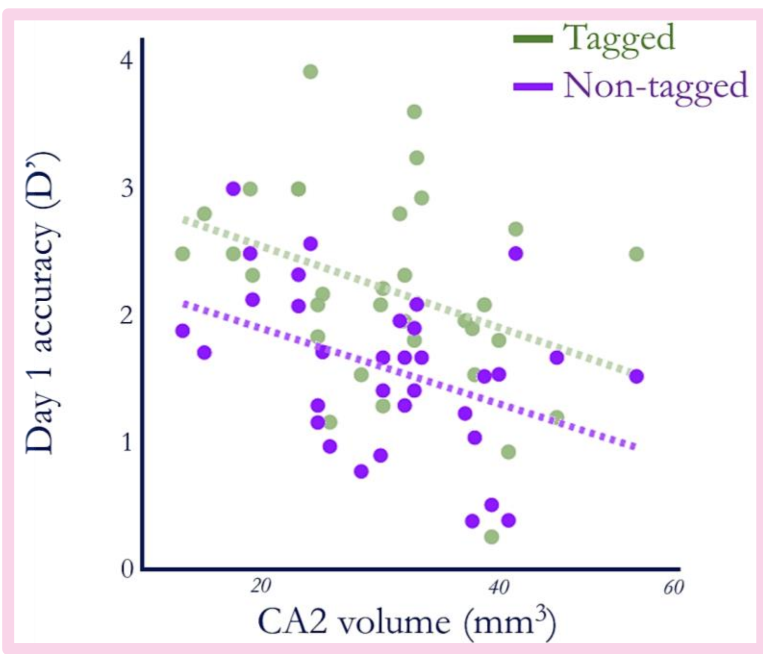


Figure 42: Memory accuracy on Day 1 and CA2 volume

The relationship for Day 1 accuracy plotted separately for tagged and non-tagged information. Note that neither of these relationships remained following false discovery rate correction.

Medial temporal lobe and sleep

Sleep stage durations: Correlations between subfield volumes and sleep revealed several relationships (Table 24). After correcting for false discovery rate, slow wave sleep duration was *negatively* associated with the CA2 ($p_{corrected}$ = .040), the dentate gyrus ($p_{corrected}$ = .004) and the entorhinal cortex ($p_{corrected}$ = .040) volumes (Figure 43A). As both hippocampal subfields and sleep architecture can be affected by body size, I performed partial correlations controlling for body weight (kg). I found that controlling for body weight did not affect the results for slow wave sleep duration against CA2 (r = -44, p = .017), dentate gyrus (r = -.38, p = 0.44) or entorhinal cortex (r = -.37, p = .048) volumes.

	CA1	CA2	CA3*	DG*	SUB	EC
			r *p*			
VOLUMES						
Duration n = 30						
Stage 2	-.02 *.907*	.30 *.109*	.19 *.306*	.16 *.406*	.10 .587	.13 *.490*
Slow wave sleep	-.35 *.059*	-.44 *.014*	-.35 *.062*	-.63 <.001	.27 *.156*	-.42 *.020*
REM	.14 *.456*	.06 *.741*	-.003 .988	.22 .236	.19 *.314*	-.21 *.277*
Spindle density n = 28						
Stage 2*	.01 *.962*	-.21 *.276*	.11 *.587*	.22 *.264*	-.07 *.727*	.08 *.683*
Slow wave sleep	.06 *.766*	.52 *.004*	.02 *.915*	-.07 *.716*	.46 *.014*	.22 *.257*
T2 RELAX						
Duration n = 30						
Stage 2*	-.19 *. 308*	-.19 *.322*	.14 *. 469*	-.27 *. 145*	-.29 *. 115*	-.31 *.088*
Slow wave sleep*	-.30 *.107*	-.20 *.287*	-.28 *.131*	-.32 *.209*	-.19 *.296*	.12 *.533*
REM	-.07 *. 711*	-.16 *. 401*	-.03 *.856*	.01 *.960*	-.34 *. 058*	-.37 *.039*
Spindle density n =28						
Stage 2*	-.12 *.535*	-.16 *.404*	.02 *.920*	-.44 *.018*	-.31 *.103*	-.40 *.031*
Slow wave sleep*	.01 *.950*	.12 *.521*	.12 *.533*	.18 *348*	.14 *.466*	.07 *.735*

Table 24: MRI: Subfield volumes and sleep

Correlation coefficients and *p-values*. Note that red-shaded associations did not survive false discovery rate correction.

*Spearman's test used

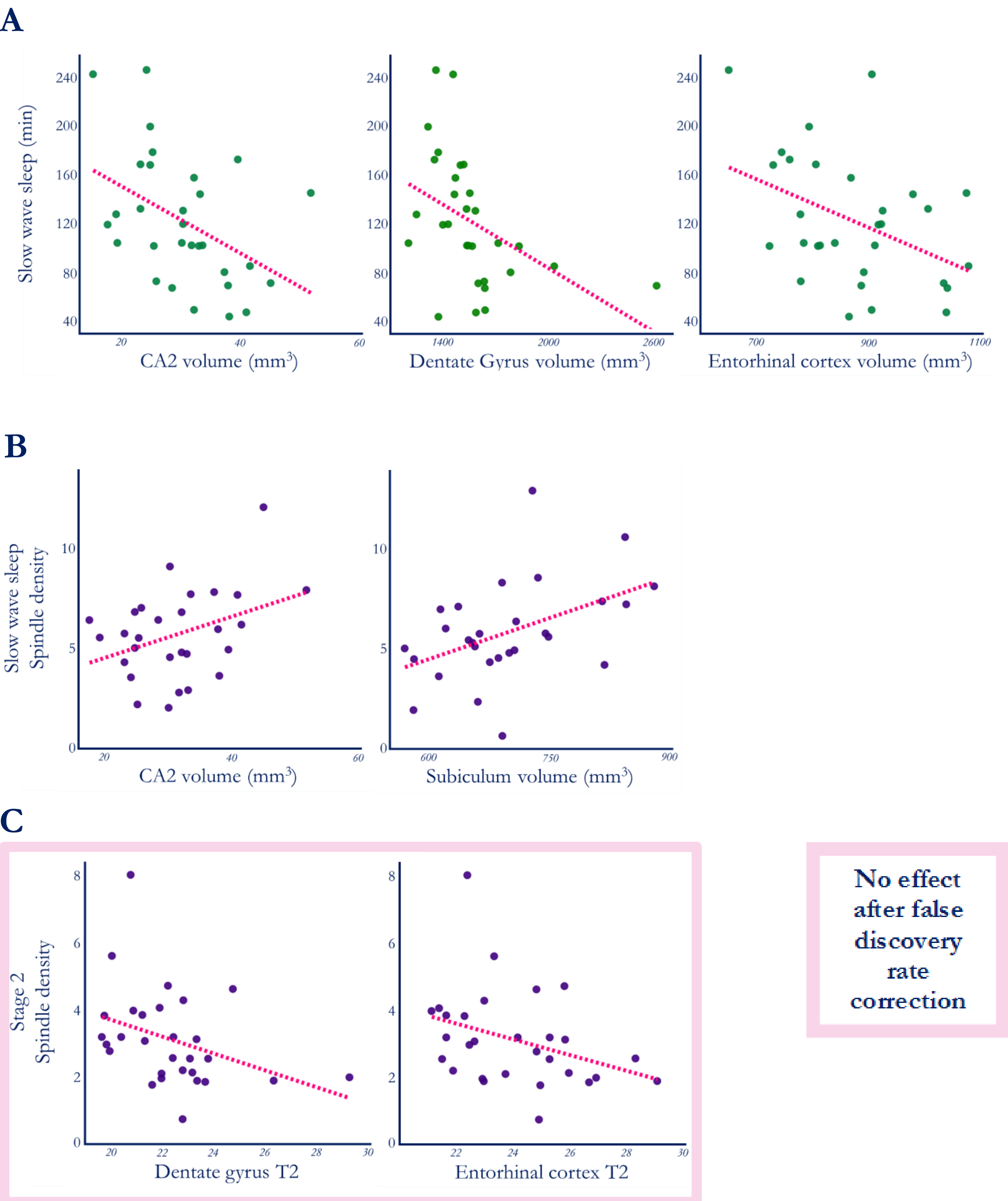


Figure 43: Slow wave sleep and medial temporal lobe

A: Slow wave sleep duration was associated with volumes of CA2, dentate gyrus and entorhinal cortex. Note that the correlation between deep sleep duration and dentate gyrus was calculated using a Spearman correlation. The line is fitted as an illustration and does not reflect the underlying analyses.
B: Spindle density in slow wave sleep was associated with CA2 and subiculum volumes.
C: Note that neither of these relationships remained following false discovery rate correction.

Multiple stepwise linear regression was then calculated to predict slow wave duration from subfield volumes using the following formula:

$$SWS_{duration} = C + \beta * BW + \beta * CA2v + \beta * DGv + \beta * ECv$$

Where SWS duration : slow wave sleep duration in minutes

C : constant

BW : Body weight (kilograms)

v : volume (mm^3)

DG : Dentate gyrus

EC : Entorhinal cortex

The end equation ($F(1, 28) = 6.81$, $p = .014$, $R^2 = .17$) included CA2 volume, but not body weight, entorhinal cortex or dentate gyrus. Slow wave sleep duration reduced by 2.8 minutes with each mm^3 decrease in CA2 volume. It is possible that the other subfields were filtered out of the model due to intercorrelations between subfield volumes. Note that the dentate gyrus volume was not normally distributed. This could also affect the outcome as the above regression model is linear.

Mediation analysis: Slow wave sleep duration was associated with both CA2 volume and memory for the tagged items, and CA2 volume was associated – albeit weakly and only before correcting for comparisons – with memory for the tagged items. I explored if CA2 volume mediates the effect between slow wave sleep duration and memory accuracy for the salient items. For the following analysis, I only included participants who had all three variables (MRI, polysomnography and memory scores) leading to a sample size of 31. Note that this is a small

sample for mediation analyses yielding is possibly underpowered. The following regression analyses were first performed:

D' ~ SWS; $R^2 = .12$, $p = .033$

CA2v ~ SWS; $R^2 = .17$, $p = .012$

D' ~ CA2v; $R^2 = .10$, $p = .049$

D' ~ SWS + CA2v; $R^2 = .13$, $p = .052$

Where *CA2v* is the CA2 volume in mm^3, *SWS* is slow wave sleep duration in minutes, and D' is the day 1 memory performance for tagged memories (List A, on placebo). The relationship between slow wave sleep duration and the tagged accuracy disappeared when CA2 volume was included in a regression model (p = .152). Next, using the 'mediate' toolbox on R, I performed a bootstrapped causal mediation analysis using 1000 samples, I found no average causal mediation effect (ACME; β = .002, p = .260), average direct effect (ADE; β =.004, p = .310) or total effect (β = .006, p = .070). In other words, despite finding a trend, I did not find that the relationship between slow wave sleep duration and memory for salient information was reliably mediated by CA2 volume.

Spindle characteristics: As spindle densities during stages 2 and slow wave sleep are frequently associated with memory consolidation, relationships between spindle densities in both stages were analysed against hippocampal subfield volumes. There were no associations between stage 2 spindle densities and subfield volumes. After false discovery date, slow wave spindle density and CA2 ($p_{corrected} = .024$) and subiculum ($p_{corrected} = .042$) volumes were positively correlated (Figure 43B). After correcting for body weight, the associations between slow wave sleep spindle density remained for both CA2 (r = .537, p = .004) and subiculum (r = .507, p = .007) remained.

Multiple stepwise linear regression was then calculated to predict slow wave spindle density from subfield volumes using the following formula:

$$Spindle_{density} = C + \beta{*}BW + \beta{*}CA2v + \beta{*}SUBv$$

Where $SWS_{density}$: number of spindles per minute during slow wave sleep

C : constant

BW : Body weight (kilograms)

v : volume (mm^3)

SUB : Subiculum

The end equation ($F(1, 28) = 9.8$, $p = .004$, $R^2 = .25$) included CA2 volume. Slow wave spindle density increased by .17 spindles per minute with each mm^3 decrease in CA2 volume. The subiculum volume and body weight were not included in the model. It is possible that subiculum volume was not included due to being correlated with CA2 volume.

T2 relaxometry: I analysed T2 relaxometry against Stage 2, slow wave sleep and REM durations and spindle densities during both stage 2 and slow wave sleep. Initially, I found *negative* relationships between REM duration and T2 relaxometry distribution in the entorhinal cortex, and between Stage 2 slow wave spindle density for the dentate gyrus and the entorhinal cortex (Figure 43C). These associations did not survive false discovery rate correction ($p_{corrected} = .174$; $p_{corrected} = .606$; $p_{corrected} = .093$, respectively).

Medial temporal lobe and L-DOPA mediated effects

To observe the relationship between hippocampus and dopamine, I performed correlational analyses between subfield volumes and L-DOPA mediated changes in memory and sleep. Contrary to my hypotheses, I did not find any relationships between hippocampal volumes and drug-responses, Table 25.

	CA1	CA2	CA3*	DG*	SUB	EC
				r *p*		
Day 1						
Accuracy (D')	.26 *.140*	.31 *.078*	.13 *.470*	.33 *.063*	.31 *.080*	.29 *.110*
Tagging	-.21 *.240*	-.09 *.620*	-.18 *.310*	-.16 *.360*	-.22 *.220*	.17 *.350*
Sleep measures						
Slow wave duration	.02 *.927*	.09 *.663*	-.18 *.366*	.19 *.346*	-.05 *.785*	-.26 *.184*
Spindle amplitude	-.19 *.322*	.02 *.912*	-.19 *.334*	-.05 *.786*	-.10 *.629*	.08 *.691*

Table 25: MRI: The hippocampus and L-DOPA

Correlation coefficients and *p-values*.
*Spearman's test used

Discussion

I sought to investigate the relationship between medial temporal subregion volumes and integrity in relation to memory and sleep. I found several interesting associations between medial

temporal lobe structures and sleep. Interestingly, the integrity or volume of hippocampal subfield or entorhinal cortex were neither associated with recognition memory nor its subcomponents.

However, majority of these findings did not remain significant after correcting for multiple comparisons. Furthermore, these analyses reported in this chapter are exploratory and the sample size is small. The study overall was powered for behavioural effects, not for hippocampal subfield volume analyses. Therefore, these findings are likely to contain at least some false positives and care and caution should be taken when interpreting these results.

Hippocampus, entorhinal cortex and sleep

Slow wave sleep duration was negatively associated with CA2, dentate gyrus and entorhinal cortex volumes. Slow wave sleep spindle density was also associated with CA2 and subiculum volumes but not with these regions' tissue integrity nor with measures of other subfields. CA2 volume was the best predictor of both slow wave sleep duration and spindle density.

The role of the CA2 region in human cognition and sleep is relatively poorly understood. Its small size make its study difficult and majority of protocols for segmenting hippocampal subfields attach it to the adjacent cornu ammonis regions (Bender et al., 2018; Wisse et al., 2017). While its segmentation is now possible using structural MRI, scanning sequences need to be carefully optimised for sufficient resolution. Not only is this region smaller than other subfields of the hippocampus but it is also relatively resistant to damage (Dudek, Alexander, & Farris, 2016). Indeed, focal CA2 lesions are scarcely reported in the literature. Due to the aforementioned caveats in the resolution of available MRI techniques, focal lesions to the CA2 could also not be reliably identified until recently. The CA2 is, however, intimately involved in memory consolidation (Dudek et al., 2016).

While its role in regulating sleep stage durations is not clear, the CA2 is involved in several memory processes that are also supported by sleep. CA2 is mostly studied in animals, where this region instigates hippocampal ripples and replay during wakefulness and sleep (Kay et al., 2016; Oliva et al., 2016) possibly via modulation from the entorhinal cortex (Oliva et al., 2016). In mice, CA2 is necessary for social recognition memory but does not affect sociability (Hitti & Siegelbaum, 2014), and it is involved in synaptic tagging and capture (Dasgupta et al., 2017). While this replay occurs also during the night, it is not clear that this activity is slow wave sleep dependent. However, it aligns well with our finding that CA2 volume is associated with spindle amplitude increase.

Regression models showed that CA2 volume was the best predictor of both slow wave sleep duration and spindle density, with no other structure volumes or body weight being included in final models.

However, as I used linear regression, and as medial temporal lobe sub-structure volumes are intercorrelated with one another, I cannot conclude that the dentate gyrus, entorhinal cortex and subiculum are not associated with measures of sleep. Instead, it is likely that as CA2 was to be the most strongly correlated subfield, it "hid" the relationships between sleep measures and volumes of other structures. This phenomenon is known as multicollinearity or collinearity: it occurs when two or more independent variables are correlated with one another. As a more intuitive example, when trying to predict individuals' body mass using different measures, jean size and waist circumference may both be strong predictors of body mass. However, as jean size and waist circumference are strongly associated with one another, one may mask the effect of the other. In a model predicting body mass, jean size may not add much value above and beyond waist circumference. Therefore, in the final model, it is unlikely that both jean size and circumference would be included. However, that is not to say that both aren't associated with body mass.

Likewise, while CA2 was the *best* predictor, it does not mean that other subfields aren't also involved or important in predicting sleep architecture. Note that predicting here does not refer to a causal relationship.

Each of the identified correlations between subregion volumes and slow wave sleep duration and spindles are anatomically plausible. For example, in humans, connectivity between the subiculum and cortical regions is increased during spindles (Andrade et al., 2011).

Interestingly, the relationship between slow wave sleep duration and hippocampal subfield volumes was negative. Larger CA2, dentate gyrus and entorhinal cortices were associated with shorter slow wave sleep durations. This is paradoxical as reductions in either hippocampal volumes or slow wave sleep are associated with poorer health outcomes in old age (Ancoli-Israel, 2005; Devanand et al., 2012; M. M. S. Lima, 2013; Shokri-Kojori et al., 2018; Videnovic & Golombek, 2013; Vitiello & Borson, 2001).

However, I did not directly test this hypothesis and therefore, this is speculative. The effects observed in this chapter are small and it is possible that these are false positive findings. To be sure, these findings should be replicated in other samples. It is difficult to explain the negative correlations in light of currently available evidence. However, and while speculative, it is also possible that both subfield volumes and slow wave duration are mediated by some third, unmeasured variable.

What might mediate such a disparate relationship between hippocampal structure and sleep? An alternative explanation is that those with larger CA2s have more efficient slow wave sleep – which aligns well with our finding that they also have higher spindle densities. During a shorter slow wave duration, those with larger CA2s can achieve more restoration. The first half of the night is typically dominated by slow wave sleep. It is possible that after slow wave sleep's homeostatic mission has been fulfilled, other sleep stages take over in healthy individuals. Larger

hippocampal subfield volumes may be indicative of healthier sleep physiology. To this end, increased slow wave sleep may also act as a shield against detrimental effects of early hippocampal volume loss.

CA2 is anatomically located so that it has the potential to influence memory: it receives input from the entorhinal cortex and CA3, and primarily projects to the CA1 (Caruana, Alexander, & Dudek, 2012). The supramammillary nucleus of the hypothalamus also projects to CA2, as well as CA3 and the dentate gyrus (Magloczky, Acsady, & Freund, 1994). Suppressing the supramammillary nucleus reduces theta amplitude, effectively disrupting cortico-hippocampal functional connectivity (Kirk & McNaughton, 1993). In other words, this region modulates encoding-related spike-time coordination between cortical regions and the hippocampus, in a process that likely involves the CA2 (Caruana et al., 2012). In animals the modulation of this pathway is associated with learning (Ito, Moser, & Moser, 2018; Oddie, Bland, Colom, & Vertes, 1994). In humans, theta activity during learning may tag salient information to be preferentially stored during sleep (Heib et al., 2015; Pu & Yu, 2019; Vertes, 2005).

In humans, theta during learning is positively correlated with spindle coherence during slow wave sleep (Heib et al., 2015). The number of spindles during sleep is also increased following learning of salient compared to non-salient information (Wilhelm et al., 2011). In Chapter IV I showed that slow wave sleep characteristics (duration and spindle amplitude) were associated with memory for salient information. In this chapter I found that CA2 volume was associated with reduced slow wave sleep duration and increased spindle density but not memory after correcting for multiple comparisons. The link between spindle density and CA2 volume may reflect CA2's involvement in instigating subsequent sleep memory-associated spindles during wakefulness.

Finally, while medial temporal lobe sub-structures are likely to orchestrate sleep architecture, it is possible that sleep is driving the morphological changes reported here. Using whole brain voxel-based morphology, Taki et al (2012) showed that healthy children with poor sleep had reduced grey matter in the hippocampus, and insomnia is associated with reductions in CA3/dentate gyrus volumes in adults (Neylan et al., 2010). While these results are also correlational, Poor sleep is likely to affect brain morphology.

I propose that medial temporal lobe sub-structures might drive the effects observed here on sleep. First, the relationship between slow wave sleep duration and hippocampus is to the opposite direction of what would be expected if poor sleep was reducing hippocampal volume. Second, our sample consisted of healthy elderly adults who reported being good sleepers. This was defined by asking them whether they had self-reported sleep problems and by excluding anyone who slept under 5.5h per night. It is unlikely that in this sample poor sleep would have caused morphological changes to brain structure.

However, it is possible that participants in our sample have had poor sleep previously in their lifetimes. It is not clear if this, or other lifestyle factors not accounted for in here could have affected hippocampal volumetry. In addition, these findings were correlational in nature: it is not clear if hippocampal volumetry has an adverse or positive effect on sleep, or if poor sleep leads to changes in hippocampal volume.

Long-term large cohort studies assessing the relationship between hippocampal volume and integrity against sleep architecture may shed light to the directionality of this relationship. These studies may also be powerful in addressing the transiency of this relationship: if hippocampal volumes are indeed associated with sleep architecture, are these changes adaptive and transient or do they persist in the long term?

The hippocampus, entorhinal cortex, sleep and memory

I sought to investigate the relationship between medial temporal region substructures and recognition memory. Contrary to what was expected, there were no associations between volumes or relaxometry and recognition memory performance after correcting for false discovery rate. Nor did I find any evidence to support a relationship between volumetry and L-DOPA-mediated changes in memory or sleep characteristics. To our awareness, no other studies have investigated hippocampal morphology and tissue integrity in relation to recognition memory over time courses spanning multiple days, or in relation to drug responses. It is important to note that I powered our study on the effects of dopamine on memory and therefore, these imaging correlations should be viewed as exploratory, rather than confirmatory.

I also assessed if hippocampal subfield CA2 volume mediated the relationship between slow wave sleep duration and memory for tagged items using a mediation analysis. CA2 volume was associated with both slow wave sleep duration and memory for tagged items (although not after correcting for false discovery rate), and slow wave sleep duration and memory for tagged items were also associated with one another. While I found that the relationship between slow wave sleep and memory disappeared when controlling for CA2 volume, the effect of this mediation was not significant. Fogel et al (2017) found that age-related changes in hippocampal grey matter volume were associated with both sleep spindles and offline skill enhancement in a procedural motor learning task (S. Fogel et al., 2017). While they did not perform a mediation analysis, they suggested that hippocampal grey matter was modulating motor learning via spindle enhancement. It is noteworthy that a mediation analysis is correlational in nature and does not make any assumptions about causality.

Sleep spindle density is associated with memory persistence. While subiculum volume associated with spindle density, it was not associated with memory in this study. Others have found that subiculum and pre-subiculum volumes are associated poor executive function and with an increased risk of dementia (Evans et al., 2018). It is possible that spindle density mediates the subiculum's effects on other cognitive functions.

Both memory recollection and familiarity have been associated with medial temporal structures, with the former being primarily hippocampal (Brown & Aggleton, 2001; Diana et al., 2007; Duzel et al., 2003; Manns, Hopkins, Reed, Kitchener, & Squire, 2003; Merkow, Burke, & Kahana, 2015; Rugg & Yonelinas, 2003; Suzuki, Miller, & Desimone, 1997; Yonelinas et al., 1998). Contrary to our hypotheses, I found no relationship between either recollected or familiar items and medial temporal lobe structures. This is in contrast with several others who have found recognition memory or memory persistence to be associated with subfield volumes (Bender, Daugherty, & Raz, 2013; Bennett, Stark, & Stark, 2018; Hartopp et al., 2018; Shing et al., 2011; Yassa et al., 2010). Differences in segmentation procedure and sample characteristics may explain the discrepancies. For example, many of the other studies have had larger samples, wider age-ranges for subjects and they have used larger subfield mask (e.g. combining CA3 and dentate gyrus and not segmenting CA2) and lower resolution MRIs.

In the present study, I also explored the effects of dopamine-mediated changes in memory and sleep against hippocampal subfield volumetry. Dopamine within the hippocampus mediates memory persistence (J. Lisman et al., 2011), possibly by guiding memory selection. In Chapters IV and V, I described how overnight L-DOPA enhanced memory selection in this sample. While I did not observe associations between drug-mediated effects on memory and medial temporal lobe sub-structure volumes or integrity, I found several associations between brain morphology and tissue state that were associated with aspects of sleep architecture known to underlie memory. Sleep architecture and memory were together modulated by L-DOPA. The lack of a

relationship between volumetry and relaxometry measures does not mean that hippocampus is not driving these processes. As I did not record hippocampal activity directly using techniques that allow localising signal to the hippocampus, I cannot ascertain whether L-DOPAs modulatory effects are driven by this region.

Limitations

It is possible that this chapter contains false positive findings. While care was taken to correct for multiple comparisons, due to the moderate sample size and number of statistical tests, correcting for all analyses performed in this chapter would have inflated false negative findings. Instead, I only corrected for false discovery rate for all subfields. While, the relationships between subfield volumes and slow wave duration and spindles are anatomically plausible, caution should be taken when interpreting these findings.

Sample characteristics may have introduced further bias. First, I only tested 35 participants, some of whom were not included in all analyses and our study may have been underpowered. For example, it is possible that CA2 volume is associated with memory for salient information via a pathway that is mediated by slow wave sleep characteristics, but a larger sample is needed to determine this. Second, I had strict inclusion and exclusion criteria due to the study involving MRI, overnight stays and pharmacological agents. This led to a sampling bias toward very healthy elderly. Our sample may therefore have less age-related pathology, and therefore less variability in brain tissue integrity.

There are several limitations with using MRI to study brain structure. Perhaps most importantly, structural correlations between behaviour do not allow segmentation of task characteristics to study individual processes or stages of cognitive function. It is possible that MRI volume and relaxometry predict memory encoding and consolidation but when all memory processes are

studied together the added noise of other cognitive processes involved masks this effect out. Functional magnetic resonance may be better suited to answer questions about different stages of memory. Importantly, correlational neuroimaging studies do not offer information about the *necessity* of a region to perform a function. While I found associations between hippocampal morphology and sleep, and structure integrity and memory, I cannot know to what magnitude these regions are involved in these processes.

The resolution of our scans was within the sub-millimetre range, which is high in the MRI context but low in the context of the hippocampus. Your hippocampus is likely smaller than your ring finger, and when divided into subfields measurement errors in the range of a couple of voxels can bias volumetry and relaxometry outcomes. In pathology, hippocampal subfield segmentation is guided by anatomical boundaries that are not seen in MRI (Wisse et al., 2017). The measurement of medial temporal lobe structures by magnetic resonance is coarse in comparison to histopathology.

While volumetry is a widely adapted method for quantifying brain morphology, pooling results across studies on medial temporal subregion volumetry is challenging due to heterogeneity in study designs and analysis approaches. Different research groups use different tools for segmenting the hippocampus and its subfields. For example, the role of the CA2 in humans is not well understood and majority of available hippocampal subfield segmentation procedures do not separate the CA2 from the adjacent CA3 and dentate gyrus (Wisse et al., 2017). The FreeSurfer and ASHS pipelines used here is likely the most widely adopted (Wisse et al., 2017; Worker et al., 2018)

Conventions over normalising subfield volumes to total brain volume or intracranial volume also vary. Head-sizes vary between individuals and this variation is positively associated with differences in intracranial, total brain and regional brain volumes (Jack, Petersen, Obrien, &

Tangalos, 1992; Synek & Reuben, 1976; Wolf et al., 2003). People with larger intracranial cavities are likely to have larger hippocampal subregions. Correcting for total intracranial volume in structural brain imaging studies reduces individual variability in total brain volume (Whitwell, Crum, Watt, & Fox, 2001). The caveat of correcting for intracranial volume or total brain volume is that it introduces an additional source of noise in the data. This is unlikely to be a problem with larger sample sizes but as I was interested in small subfields and I had a moderate sample size. Furthermore, due to movement during scans in one participant, and poor scan alignment in another, two participants' intracranial or total brain volume could not be determined. In a small sample size not including two participants would have largely reduced the statistical power in this study.

For these two reasons, I decided to not correct for intracranial volume. While the correlations observed in this study remained after correcting for body weight, it is possible that controlling for intracranial volume and brain volume would produce disparate results. Therefore, correcting for intracranial volume or total brain volume may have been a more robust approach.

White matter structures such as corpus callosum volume and hippocampal-fornix connections have also been associated with sleep and memory (Buchmann et al., 2011; Hartopp et al., 2018). I only explored the relationships between medial temporal structures and memory and sleep. A natural next step is to extend the scope outside of the medial temporal lobe and assess the relationship between diffusion tensor imaging data, cortical thickness (Dube et al., 2015), long term memory persistence and sleep.

Perhaps the largest limitation of this chapter is that the study was not powered for hippocampal subfield correlations. Therefore, the risk of false positives is increased and the findings should be taken with caution and replicated in independent samples.

Conclusions

Mnemonic functions of the medial temporal lobe are an outcome of the interactions and actions of its component subregions. Here, I showed that larger CA2, dentate gyrus and entorhinal cortex volumes were associated with reduced overall slow wave sleep duration. Surprisingly, I did not find any relationships between subfield volumes or tissue integrity and memory or L-DOPA mediated changes in memory and sleep.

Chapter VII: General discussion

Human brains selectively store knowledge about the world to optimise future behaviour. We automatically rehearse and contextualise or discard information to create a robust collection of facts and events. To achieve this, the memory system, which involves several medial temporal lobe regions, biases memory persistence in favour of important information during sleep. The two double-blind randomised controlled trials reported in this thesis were designed to carefully target different stages of memory evolution and sleep with dopamine in old age. I aimed to examine the relationships between dopamine, memory, sleep and the hippocampus.

Broadly, I found that L-DOPA modulates processes involved in selecting information to-be-kept overnight – L-DOPA accelerated the rate of forgetting newly learnt information, but memories tagged as salient were shielded from this effect. Strikingly, both these effects were also associated with changes in slow wave sleep architecture.

In contrast, L-DOPA did not affect encoding or retrieval when memory is tested a day later. Several medial temporal lobe substructures were also associated with differences in sleep

architecture. However, these findings were exploratory, and the effects were small and weak. Furthermore, and unlike hypothesised, hippocampal subfield volumes or T2 relaxation times, were not associated with memory or the effectiveness of L-DOPA.

These results have been discussed in relation to one another through earlier chapters. In this chapter, I will draw further overarching conclusions about these findings and discuss their implications.

Behavioural findings

Perhaps my most striking finding is that L-DOPA nocturnally selects salient information for storage at the cost of accelerating clearance of unimportant information. L-DOPA after learning accelerated forgetting, but not if information was tagged as salient. Specifically, I found that nocturnal L-DOPA increased the *difference* between remembering re-exposed, or "tagged", and not re-exposed items. The effects of L-DOPA were seen one – but not three or five – days after learning.

These findings therefore suggest that L-DOPA *accelerates* forgetting of information that would be lost later anyway. In other words, information that would be remembered over a longer delay anyway was not affected by L-DOPA, as evidenced by a lack of effect on days 3 and 5. Instead, weakly encoded or stored information that would be forgotten over longer delays, was forgotten quicker, as evidenced by lower memory performance (D') on Day 1. Furthermore, I found that L-DOPA did not increase forgetting of tagged information that was more strongly encoded by presenting the information twice.

I suggest that these effects are explained by dopamine enhancing memory selection both during tagging and during subsequent sleep.

There were two important limitations to this interpretation. First, dopamine's effects on biasing memory could equally be due to dopamine enhancing encoding. I interpreted my findings as dopamine biasing the *tagging* of salient information as opposed to enhancing all encoding equally. Memories were tagged as salient by a re-test. It is therefore possible that rather than affecting tagging, dopamine affected re-encoding.

The second limitation to our interpretation was that dopamine may have biased memory during retrieval. Residual amounts of L-DOPA could still have been present centrally at the 12-hour test, affecting performance then rather than during tagging or nocturnal sleep. I did not measure dopamine levels in plasma, so I cannot exclude this possibility.

However, in Chapter III I show findings from another placebo-controlled randomised trial that indirectly refute these alternative explanations.

Specifically, in the DARet study (Chapter III), I showed that L-DOPA does *not* bias encoding or retrieval. This was based on the observation that when I administered L-DOPA to target encoding (and early, wakeful, consolidation), and retrieval separately, it did not change memory when performance was tested a day after learning. The evidence *against* an effect 1 day after learning was moderate, as indicated by the Bayes Factors (Table 5, page 110). Specifically, these analyses showed that the data collected 1 day from learning, regardless of whether L-DOPA was active at encoding or retrieval, were 4.6 times more likely to have been collected from a null than the alternative distribution.

My findings therefore robustly show that L-DOPA does not affect encoding or retrieval, when information is tested 24 hours after learning. However, there was a small trend-level effect 3 days after learning. I suggest that this finding was a false positive as the effect was small and did not

survive multiple comparison correction, but further replication is needed to establish this with certainly. Considering the findings from the DOPAMIND study (Chapters IV and V) in light of the DARet study (Chapter III), I suggest that dopamine was influencing tagging, sleep-related consolidation or both, and that the observed effects cannot be explained by dopamine's influence on encoding or retrieval.

My findings also align well with drosophila models that have robustly shown that dopamine overnight increases forgetting (Berry et al., 2015; Berry et al., 2012; Berry et al., 2018), and with several studies in humans showing that dopamine enhances memory when learnt information are either associated with rewards (Feld et al., 2014), or when information is repeatedly learnt (Knecht et al., 2004; Shellshear et al., 2015). Others have interpreted their findings as dopamine having an overall effect on encoding or consolidation. I offer an alternative explanation, that dopamine *biases* memory for salient information by enhancing tagging processes either during learning or shortly after.

The concept of increased forgetting is still emerging, and it is not yet clear whether this is due to direct degradation of engram or additional noise or reduced precision of storage and recall.

Limitations of the tagging manipulation

A further important limitation in this thesis is interpreting repeat exposure as behavioural tagging. To reiterate: the purpose of the evening test of a portion of the items (List A) was to "tag" them as salient by repeat exposure. Participants knew they would be tested on these items again, and this manipulation was to act to strengthen the pre-existing memory trace for these items (List A). However, as participants had formed memories of the other lists earlier, it is possible that a repeat exposure of some of the words encouraged participants to actively rehearse the non-tagged words as they knew they would be tested later. Therefore, it is possible that the manipulation here did not increase saliency of the tagged information.

Another caveat is that between learning and re-test participants may have forgotten items. If items were either forgotten or not encoded in the first place, the tagging manipulation would have just acted as initial encoding. Although I attempted to out rule this explanation by showing in DARet (Chapter III) that L-DOPA does *not* affect encoding, this is a real concern and further complicates interpreting the findings from the DOPAMIND study.

The tagged word list (List A) may therefore include both items that were genuinely tagged as important, items that were just repeat exposed (i.e. re-learnt but no saliency was attached to them) as well as items that were only really encoded once due to either forgetting between testing and learning, or due to not encoding them at all during learning.

Considering these limitations, these data become more difficult to interpret. While in the control study (DARet, Chapter III) I did not find any effect of L-DOPA during encoding but it is possible that this was due to other differences in the study, such as fatigue, time of the day, or other factors to do with the study design. However, due to the lack of any effects in DARet on encoding when memory was tested 1 day later it is unlikely that encoding effects alone explain L-DOPA's effects on memory.

A plausible alternative explanation is that re-exposure, rather than salience tagging, explains the observed effects. It is possible that merely seeing the same information alters a disparate route for encoding that can be modulated by dopamine. Indeed, others have shown that medications that increase dopamine levels in the brain enhance learning of items over several learning trials and occurrences (Knecht et al., 2004; Shellshear et al., 2015).

However, in the current studies I did not account for these important limitations. An alternative study design that would have allowed for me to better disentangle these effects could have been more powerful. The current design could have been improved by having a different manipulation for tagging, such as a reward element. This should be considered for future studies.

Furthermore, a condition in which no items are repeat tested would have allowed for me to disentangle if the accelerated forgetting observed for list B was triggered by the List A test, or if this was unrelated.

Dopamine, sleep and memory

Nocturnal dopamine increased duration of slow wave sleep as well as slow wave spindle amplitude, as reported in Chapter V. Importantly, both effects were associated with dopamine's effects on memory. Slow wave sleep duration was associated with memory performance for salient information, but not when L-DOPA active in the system. L-DOPA also was associated with the tagging effect – i.e. the relative memory benefit of salient compared to non-salient information – nocturnally, and this association was also correlated with slow wave spindle amplitude. However, the relationship between sleep physiology and the behavioural measures was correlational and therefore I cannot ascertain if this association is causal.

Slow wave spindles are indirectly associated with hippocampal replay, which likely is an anatomical substrate for, at least some types of, systems consolidation (Molle et al., 2006; M. Y. Yang et al., 2019). It may not be possible or worthwhile to replay *all* memories during sleep. Instead, sleep selectively consolidates some information over others based on behavioural salience (Blaskovich et al., 2017; Himmer et al., 2019; Lipinska, Bolinger, Thomas, Baldwin, & Stuart, 2019; Schapiro et al., 2017). Different spindle characteristics, such as amplitude, are also associated with enhanced memory persistence (Mednick et al., 2013). Therefore, my findings are consistent with dopamine biasing memory selection during sleep.

Spindle amplitude reflects enhanced communication and coherence between cortical nodes during spindles. When memories are successfully stored in the long term, they become more integrated in cortical nodes and less reliant on the hippocampus. As memories are consolidated, the underlying neural activity required for their retrieval becomes more reliant on cortical connectivity and requires less hippocampal recruitment (Brodt et al., 2016). This is important because increased spindle amplitude reflects more coherent cortical activation during spindles. Therefore, spindle amplitude is likely to be a measure of the kind of processes that support information migration from hippocampal to cortical networks.

This type of consolidation can also happen rapidly during learning in some circumstances, for example when information is congruent with previous experiences or rehearsed (Coutanche & Thompson-Schill, 2014; Himmer et al., 2019; McClelland, 2013). Crucially, sleep is required to make this kind of rapid consolidation last (Himmer et al., 2019). Therefore, the tagging effect is likely to be facilitated by fast online consolidation that is subsequently stabilised overnight. In Chapter V I showed that slow wave sleep duration facilitated this kind of learning, but L-DOPA wiped this effect off. It is possible that this was due to L-DOPA enhancing rapid consolidation during tagging, however I did not directly test this hypothesis. Perhaps once memories have been fast-tracked to the cortex during wakeful consolidation, they do not further benefit from increased slow wave sleep, but instead rely on spindle characteristics.

Dopamine's dual effect

Considering the findings across these chapters together, I suggest that L-DOPA modulates memory through a dual process, where it enhances memory tagging by fast mapping online consolidation, and later stabilises these effects during sleep, see Figure 44.

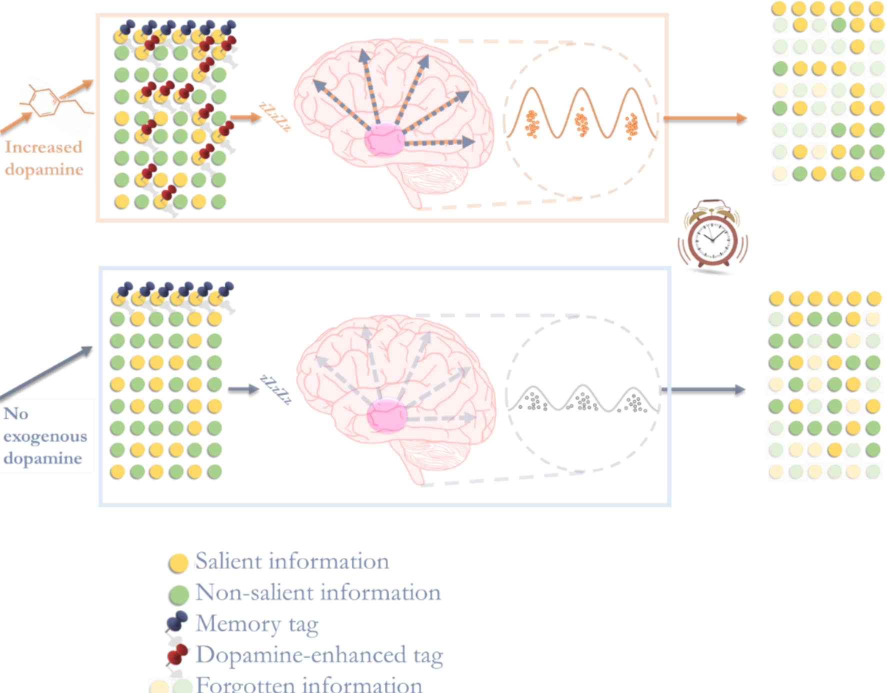


Figure 44: Dopamine has a dual role in memory selection

Some salient information is earmarked as important by a neural tag. Some of this happens during initial encounter and dopamine enhances this effect. During sleep, dopamine modulates selective memory processes that are biased toward tagged information. This leads to enhanced synchronisation in cortical firing patterns during spindles. Together these two processes affect subsequent memory. Salient information is now much more likely to be remembered, and this effect is increased by dopamine.

Implications and future directions

My findings raise several questions about the neurobiological effects of dopamine. First, if dopamine does enhance the tagging process, this should be detectable during tagging as enhanced theta activity. Theta during and after learning is associated with processes that increase memory for salient information (Jarovi et al., 2018; Pu & Yu, 2019). Crucially, the relative increase in theta coherence is also associated with subsequent sleep spindle amplitude increases and memory success (Heib et al., 2015). The model I have suggested (Figure 44) holds that L-DOPA enhances saliency-tagging together with subsequent memory processes. If correct, L-DOPA should enhance theta coherence during tagging. Crucially, I did not record EEG during learning, only during subsequent sleep. Future research is needed to address this question.

To determine whether the dual process hypothesis holds true, L-DOPA needs to be timed to target tagging and consolidation separately, and scale EEG needs to ideally be recorded during tagging.

In addition to ascertaining L-DOPA's effects on tagging and sleep separately, the functions of different dopamine receptors remain unknown. Most studies examining the relationship between dopamine, memory and the hippocampus have focussed on D1 neurons, which are the most abundant and behaviourally salient in the hippocampus (Hansen & Manahan-Vaughan, 2014; Roggenhofer et al., 2010). D2-like neurons seem to also be associated with memory-related behaviours (Feld et al., 2014; Franca et al., 2015; Nyberg et al., 2016; Salami et al., 2019), it is possible that different receptors modulate behaviour in different ways. In this thesis, I used medication, L-DOPA, that excites both D1 and D2 receptor types.

Clinical significance

I explored the possibility that memory and sleep could be modulated by a readily available medication, L-DOPA, in a positive way, that might bring clinical benefits in ageing. This may be a potential future direction for this research: to observe the efficacy of long-term L-DOPA use in reducing age-related pathology. In this section I suggest directions for future research. The current data alone is not sufficient in guiding clinical practice.

L-DOPA's effect on slow wave sleep has several potential implications as a therapeutic route to target age-related cognitive decline. L-DOPA increased slow wave sleep duration by 11% in this sample of elderly adults. Slow wave sleep is known to support several restorative functions in humans, ranging from stabilizing mood, anxiety and satiety to clearing brain amyloid and regulating insulin (Li et al., 2018; Reutrakul & Van Cauter, 2018; Shokri-Kojori et al., 2018). Ageing is associated with reduced sleep, increased incidence of sleep disorders and enhanced risk of developing cognitive problems including dementia. Age is the largest risk factor for dementias. An imbalance in amyloid homeostasis, leading to increased amyloid accumulation in the brain, increases dementia risk further.

The long-term benefits of L-DOPA in modulating age-related risk factors for poor health are not presently known. Our findings together with studies that have shown that L-DOPA accelerates learning over five consecutive days (Knecht et al., 2004; Shellshear et al., 2015) suggest that L-DOPA may have clinical utility. Therefore, future trials targeting slow wave sleep with L-DOPA in ageing may have disease modifying implications and be of clinical benefit.

I also found that L-DOPA enhanced processes involved in memory selection. Future studies are needed to ascertain if these effects may have potential clinical benefit to people living with mild memory problems.

Any medication that can cross the blood brain barrier can potentially also alter sleep architecture. Commonly prescribed pharmacological sleeping aids are often not the most suited for elderly adults and may not be suited as a long-term option for disease modification. First, patients tend to build tolerance over time, and for this reason treatment should be restricted to 4-5 weeks to target poor sleep (Wortelboer, Cohrs, Rodenbeck, & Ruther, 2002) – with the exception of melatonin (Cardinali, 2018). Second, most common sleeping pills actually reduce slow wave activity as well as time spent in slow wave sleep and in REM (Achermann & Borbely, 1987; Borbely, Mattmann, Loepfe, Strauch, & Lehmann, 1985). Third, several common sleeping pills, such as benzodiazepines and diazepam, have been associated with increased falls in elderly (Cumming, 1998; Ryynanen, Kivela, Honkanen, Laippala, & Saano, 1993), increasing fracture risk in the elderly (Cumming & Klineberg, 1993; Cumming et al., 1991). While there is little evidence that L-DOPA reduced falls in healthy ageing, it may moderately reduce them in Parkinson's disease (Abraham Lieberman et al., 2019; F. C. Chang et al., 2015). L-DOPA does not seem to increase risk of falling by promoting somnolence.

L-DOPA is a readily available, safe, and widely used medication which has the potential to impact on sleep efficacy. It may be safer to use than commonly prescribed sleeping pills, especially in the elderly population.

Further research is required to determine whether L-DOPA has clinical utility in targeting memory and sleep.

Sleep and the medial temporal lobe

I also explored the relationship between medial temporal lobe substructures, memory and sleep in Chapter VI. The findings reported in in this chapter were exploratory in nature and all observed effects were small.

The function of the medial temporal lobe is an outcome of the interactions and actions of its component subregions. Hippocampal subfield volume and integrity change with age, and these changes are associated with memory. In Chapter VI, preliminary evidence showed that shorter slow wave sleep durations may be associated with *larger* CA2, dentate gyrus and entorhinal cortex volumes. Slow wave sleep spindle density was also associated with CA2 and subiculum volumes. In contrast to what I expected, I did not see any relationships between medial temporal lobe substructure integrity and sleep, nor did I find any associations between substructures and memory. Although the relationship between hippocampal subfield volumes and sleep were associated with the same sleep characteristics modulated by dopamine, I found no association between subregion volumes or integrity and the dopamine-mediated effects reported in Chapters IV and V. Note that all the MRI structural analyses had low power and findings from this chapter should be taken with a pinch of salt.

Therefore, the hippocampus and the entorhinal cortex are associated with sleep architecture. However, it is not clear whether subregion volumes are affecting sleep stage durations and spindles, or if differences in sleep efficiency can, over time, shield the hippocampus against atrophy.

Conclusion

In this thesis I have shown evidence to support that L-DOPA enhances routine forgetting but selectively stores salient information. These effects were associated with increased slow wave sleep and slow wave spindle amplitude. Together, these findings suggest that saliency increases the likelihood that an item is remembered, and that dopamine further increases this effect. During subsequent sleep, dopamine increases the coherence of thalamocortical communication and causes more synchronous cortical firing during spindles. I propose that this increase in coherence is associated with more selective hippocampal replay. As a result, nocturnal dopamine biases later retention for salient items. These findings have potential clinical impact.

References

Abraham Lieberman, A. D., Olson, M. C., Hussain, V. S., Frames, C. W., McCauley, M., & Lockhart, T. E. (2019). Falls when standing, falls when walking: different mechanisms, different outcomes in Parkinson disease. . *Cureus, 11*(8).

Accili, D., Fishburn, C. S., Drago, J., Steiner, H., Lachowicz, J. E., Park, B. H., . . . Fuchs, S. (1996). A targeted mutation of the D-3 dopamine receptor gene is associated with hyperactivity in mice. *Proceedings of the National Academy of Sciences of the United States of America, 93*(5), 1945-1949. doi:DOI 10.1073/pnas.93.5.1945

Achermann, P., & Borbely, A. A. (1987). Dynamics of EEG slow wave activity during physiological sleep and after administration of benzodiazepine hypnotics. *Hum Neurobiol, 6*(3), 203-210. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2896653

Ancoli-Israel, S. (2005). Sleep and aging: Prevalence of disturbed sleep and treatment considerations in older adults. *Journal of Clinical Psychiatry, 66*, 24-30. Retrieved from <Go to ISI>://WOS:000234110800005

Anderson, M. C., Bjork, E. L., & Bjork, R. A. (2000). Retrieval-induced forgetting: Evidence for a recall-specific mechanism. *Psychonomic Bulletin & Review, 7*(3), 522-530. doi:Doi 10.3758/Bf03214366

References

Anderson, M. C., Bjork, R. A., & Bjork, E. L. (1994). Remembering Can Cause Forgetting - Retrieval Dynamics in Long-Term-Memory. *Journal of Experimental Psychology-Learning Memory and Cognition, 20*(5), 1063-1087. doi:Doi 10.1037//0278-7393.20.5.1063

Andrade, K. C., Spoormaker, V. I., Dresler, M., Wehrle, R., Holsboer, F., Samann, P. G., & Czisch, M. (2011). Sleep spindles and hippocampal functional connectivity in human NREM sleep. *J Neurosci, 31*(28), 10331-10339. doi:10.1523/JNEUROSCI.5660-10.2011

Andretic, R., van Swinderen, B., & Greenspan, R. J. (2005). Dopaminergic modulation of arousal in Drosophila. *Current Biology, 15*(13), 1165-1175. doi:10.1016/j.cub.2005.05.025

Annese, J., Schenker-Ahmed, N. M., Bartsch, H., Maechler, P., Sheh, C., Thomas, N., . . . Corkin, S. (2014). Postmortem examination of patient HM's brain based on histological sectioning and digital 3D reconstruction. *Nature Communications, 5*. doi:Artn 3122

Doi 10.1038/Ncomms4122

Antony, J. W., Ferreira, C. S., Norman, K. A., & Wimber, M. (2017). Retrieval as a Fast Route to Memory Consolidation. *Trends in Cognitive Sciences, 21*(8), 573-576. doi:10.1016/j.tics.2017.05.001

Atkinson, R. C., & Shiffrin, R. M. (1968). Human memory: A proposed system and its control processes. In K. W. Spence & J. T. Spence (Eds.), *The psychology of learning and motivation* (Vol. 2, pp. 89 – 195). New York: Academic Press.

Atucha, E., Karew, A., Kitsukawa, T., & Sauvage, M. M. (2017). Recognition memory: Cellular evidence of a massive contribution of the LEC to familiarity and a lack of involvement of the hippocampal subfields CA1 and CA3. *Hippocampus, 27*(10), 1083-1092. doi:10.1002/hipo.22754

Axmacher, N., Cohen, M. X., Fell, J., Haupt, S., Dumpelmann, M., Elger, C. E., . . . Ranganath, C. (2010). Intracranial EEG Correlates of Expectancy and Memory Formation in the

Human Hippocampus and Nucleus Accumbens. *Neuron, 65*(4), 541-549. doi:10.1016/j.neuron.2010.02.006

Axmacher, N., Elger, C. E., & Fell, J. (2008). Ripples in the medial temporal lobe are relevant for human memory consolidation. *Brain, 131*, 1806-1817. doi:10.1093/brain/awn103

Ballarini, F., Moncada, D., Martinez, M. C., Alen, N., & Viola, H. (2009). Behavioral tagging is a general mechanism of long-term memory formation. *Proceedings of the National Academy of Sciences of the United States of America, 106*(34), 14599-14604. doi:10.1073/pnas.0907078106

Bao, S. W., Chan, W. T., & Merzenich, M. M. (2001). Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature, 412*(6842), 79-83. doi:Doi 10.1038/35083586

Barnes, C. A., Rao, G., & Houston, F. P. (2000). LTP induction threshold change in old rats at the perforant path-granule cell synapse. *Neurobiology of Aging, 21*(5), 613-620. doi:Doi 10.1016/S0197-4580(00)00163-9

Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language, 68*(3), 255-278. doi:10.1016/j.jml.2012.11.001

Barton´, K. (2018). Package 'MuMIn' (Version 1.42.1). CRAN.

Baruzzi, A., Contin, M., Riva, R., Procaccianti, G., Albani, F., Tonello, C., . . . Martinelli, P. (1987). Influence of Meal Ingestion Time on Pharmacokinetics of Orally-Administered Levodopa in Parkinsonian-Patients. *Clinical Neuropharmacology, 10*(6), 527-537. doi:Doi 10.1097/00002826-198712000-00004

Bateman, R. J., Wen, G., Morris, J. C., & Holtzman, D. M. (2007). Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. *Neurology, 68*(9), 666-669. doi:10.1212/01.wnl.0000256043.50901.e3

Bates, D. (2018). Parsimonious Mixed Models. *arXiv.*

References

Battaglia, F. P., Sutherland, G. R., & McNaughton, B. L. (2004). Hippocampal sharp wave bursts coincide with neocortical "up-state" transitions. *Learning & Memory, 11*(6), 697-704. doi:10.1101/lm.73504

Bear, M. F., & Abraham, W. C. (1996). Long-term depression in hippocampus. *Annual Review of Neuroscience, 19*, 437-462. doi:DOI 10.1146/annurev.ne.19.030196.002253

Beaulieu, J. M., & Gainetdinov, R. R. (2014). The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Molecular and Cellular Biology, 34*(3), 182-217. doi:DOI 10.1124/pr.110.002642

Bender, A. R., Daugherty, A. M., & Raz, N. (2013). Vascular Risk Moderates Associations between Hippocampal Subfield Volumes and Memory. *Journal of Cognitive Neuroscience, 25*(11), 1851-1862. doi:10.1162/jocn_a_00435

Bender, A. R., Keresztes, A., Bodammer, N. C., Shing, Y. L., Werkle-Bergner, M., Daugherty, A. M., . . . Raz, N. (2018). Optimization and validation of automated hippocampal subfield segmentation across the lifespan. *Human brain mapping, 39*(2), 916-931. doi:10.1002/hbm.23891

Bendor, D., & Wilson, M. A. (2012). Biasing the content of hippocampal replay during sleep. *Nature Neuroscience, 15*(10), 1439-1444. doi:10.1038/nn.3203

Benilova, I., Karran, E., & De Strooper, B. (2012). The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. *Nature Neuroscience, 15*(3), 349-357. doi:10.1038/nn.3028

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Statistical Methodology, 57*(1), 289-300. Retrieved from <Go to ISI>://WOS:A1995QE45300017

References

Bennett, I. J., Stark, S. M., & Stark, C. E. L. (2018). Recognition Memory Dysfunction Relates to Hippocampal Subfield Volume: A Study of Cognitively Normal and Mildly Impaired Older Adults. *J Gerontol B Psychol Sci Soc Sci*. doi:10.1093/geronb/gbx181

Berry, J. A., Cervantes-Sandoval, I., Chakraborty, M., & Davis, R. L. (2015). Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting. *Cell, 161*(7), 1656-1667. doi:10.1016/j.cell.2015.05.027

Berry, J. A., Cervantes-Sandoval, I., Nicholas, E. P., & Davis, R. L. (2012). Dopamine Is Required for Learning and Forgetting in Drosophila. *Neuron, 74*(3), 530-542. doi:10.1016/j.neuron.2012.04.007

Berry, J. A., Phan, A., & Davis, R. L. (2018). Dopamine Neurons Mediate Learning and Forgetting through Bidirectional Modulation of a Memory Trace. *Cell Reports, 25*(3), 651-+. doi:10.1016/j.celrep.2018.09.051

Bertolucci, P. H., Andrade, L. A., Lima, J. G., & Carlini, E. A. (1987). Total sleep deprivation and Parkinson disease. *Arq Neuropsiquiatr, 45*(3), 224-230. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3439904

Bethus, I., Tse, D., & Morris, R. G. M. (2010). Dopamine and Memory: Modulation of the Persistence of Memory for Novel Hippocampal NMDA Receptor-Dependent Paired Associates. *Journal of Neuroscience, 30*(5), 1610-1618. doi:Doi 10.1523/Jneurosci.2721-09.2010

Billard, J. M., & Freret, T. (2018). Asc-1 transporter activation: an alternative to rescue age-related alterations in functional plasticity at rat hippocampal CA3/CA1 synapses. *Journal of Neurochemistry, 147*(4), 514-525. doi:10.1111/jnc.14586

Bird, C. M. (2017). The role of the hippocampus in recognition memory. *Cortex, 93*, 155-165. doi:10.1016/j.cortex.2017.05.016

References

Birdsall, T. G., & Roberts, R. A. (1965). On the Theory of Signal Detectability - an Optimum Nonsequential Observation-Decision Procedure. *Ieee Transactions on Information Theory, 11*(2), 195-204. doi:Doi 10.1109/Tit.1965.1053740

Blaskovich, B., Szollosi, A., Gombos, F., Racsmany, M., & Simor, P. (2017). The Benefit of Directed Forgetting Persists After a Daytime Nap: The Role of Spindles and Rapid Eye Movement Sleep in the Consolidation of Relevant Memories. *Sleep, 40*(3). doi:10.1093/sleep/zsw076

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356. doi:10.1113/jphysiol.1973.sp010273

Bliss, T. V. P., & Collingridge, G. L. (1993). A Synaptic Model of Memory - Long-Term Potentiation in the Hippocampus. *Nature, 361*(6407), 31-39. doi:DOI 10.1038/361031a0

Bonstrup, M., Iturrate, I., Thompson, R., Cruciani, G., Censor, N., & Cohen, L. G. (2019). A Rapid Form of Offline Consolidation in Skill Learning. *Current Biology, 29*(8), 1346-1351 e1344. doi:10.1016/j.cub.2019.02.049

Borbely, A. A., Mattmann, P., Loepfe, M., Strauch, I., & Lehmann, D. (1985). Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. *Hum Neurobiol, 4*(3), 189-194. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2866173

Boulamery, A., Simon, N., Vidal, J., & Bruguerolle, B. (2010). Effects of L-Dopa on Circadian Rhythms of 6-Ohda Striatal Lesioned Rats: A Radiotelemetric Study. *Chronobiology International, 27*(2), 251-264. doi:10.3109/07420521003664213

Bowles, B., Duke, D., Rosenbaum, R. S., McRae, K., & Kohler, S. (2016). Impaired assessment of cumulative lifetime familiarity for object concepts after left anterior temporal-lobe resection that includes perirhinal cortex but spares the hippocampus. *Neuropsychologia, 90*, 170-179. doi:10.1016/j.neuropsychologia.2016.06.035

References

Boyle, G. J. (1985). Self-report measures of depression: some psychometric considerations. *Br J Clin Psychol, 24 (Pt 1)*, 45-59. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3971068

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A. I., Steur, E. N. H. J., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging, 24*(2), 197-211. doi:Pii S0197-4580(02)00065-9

Doi 10.1016/S0197-4580(02)00065-9

Braun, E. K., Wimmer, G. E., & Shohamy, D. (2018). Retroactive and graded prioritization of memory by reward. *Nature Communications, 9*. doi:ARTN 4886

10.1038/s41467-018-07280-0

Breitenstein, C., & Knecht, S. (2002). Development and validation of a language learning model for behavioral and functional-imaging studies. *J Neurosci Methods, 114*(2), 173-179. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11856568

Brezis, N., Bronfman, Z. Z., Yovel, G., & Goshen-Gottstein, Y. (2017). The Electrophysiological Signature of Remember-Know Is Confounded with Memory Strength and Cannot Be Interpreted as Evidence for Dual-process Theory of Recognition. *J Cogn Neurosci, 29*(2), 322-336. doi:10.1162/jocn_a_01053

Brodt, S., Pohlchen, D., Flanagin, V. L., Glasauer, S., Gais, S., & Schonauer, M. (2016). Rapid and independent memory formation in the parietal cortex. *Proceedings of the National Academy of Sciences of the United States of America, 113*(46), 13251-13256. doi:10.1073/pnas.1605719113

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron, 68*(5), 815-834. doi:DOI 10.1016/j.neuron.2010.11.022

References

Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience, 2*(1), 51-61. doi:Doi 10.1038/35049064

Buchmann, A., Kurth, S., Ringli, M., Geiger, A., Jenni, O. G., & Huber, R. (2011). Anatomical markers of sleep slow wave activity derived from structural magnetic resonance images. *Journal of Sleep Research, 20*(4), 506-513. doi:10.1111/j.1365-2869.2011.00916.x

Bunzeck, N., & Duzel, E. (2006). Absolute coding of stimulus novelty in the human substantia nigra/VTA. *Neuron, 51*(3), 369-379. doi:DOI 10.1016/j.neuron.2006.06.021

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafo, M. R. (2013). Power failure: why small sample size undermines the reliability of neuroscience (vol 14, pg 365-376, 2013). *Nature Reviews Neuroscience, 14*(6), 444-444. doi:10.1038/nrn3502

Buzsaki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Res, 398*(2), 242-252. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3026567

Buzsaki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus, 25*(10), 1073-1188. doi:10.1002/hipo.22488

Buzsaki, G., Horvath, Z., Urioste, R., Hetke, J., & Wise, K. (1992). High-Frequency Network Oscillation in the Hippocampus. *Science, 256*(5059), 1025-1027. doi:DOI 10.1126/science.1589772

Buzsaki, G., Leung, L. W., & Vanderwolf, C. H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res, 287*(2), 139-171. doi:10.1016/0165-0173(83)90037-1

Buzsaki, G., Logothetis, N., & Singer, W. (2013). Scaling Brain Size, Keeping Timing: Evolutionary Preservation of Brain Rhythms. *Neuron, 80*(3), 751-764. doi:10.1016/j.neuron.2013.10.002

References

Cairney, S. A., Guttesen, A. A. V., El Marj, N., & Staresina, B. P. (2018). Memory Consolidation Is Linked to Spindle-Mediated Information Processing during Sleep. *Current Biology, 28*(6), 948-+. doi:10.1016/j.cub.2018.01.087

Callaghan, M. F., Freund, P., Draganski, B., Anderson, E., Cappelletti, M., Chowdhury, R., . . . Weiskopf, N. (2014). Widespread age-related differences in the human brain microstructure revealed by quantitative magnetic resonance imaging. *Neurobiol Aging, 35*(8), 1862-1872. doi:10.1016/j.neurobiolaging.2014.02.008

Canolty, R. T., & Knight, R. T. (2010). The functional role of cross-frequency coupling. *Trends in Cognitive Sciences, 14*(11), 506-515. doi:10.1016/j.tics.2010.09.001

Cardinali, D. P. (2018). Preservation of slow wave sleep as a neuroprotective strategy in aging. *Approaches to Aging Control, 22*.

Carlsson, A. (1993). Thirty years of dopamine research. *Adv Neurol, 60*, 1-10. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8093570

Carr, M. F., Jadhav, S. P., & Frank, L. M. (2011). Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nature Neuroscience, 14*(2), 147-153. doi:10.1038/nn.2732

Carson, N., Leach, L., & Murphy, K. J. (2018). A re-examination of Montreal Cognitive Assessment (MoCA) cutoff scores. *International Journal of Geriatric Psychiatry, 33*(2), 379-388. doi:10.1002/gps.4756

Carter, J. H., Nutt, J. G., & Woodward, W. R. (1992). The Effect of Exercise on Levodopa Absorption. *Neurology, 42*(10), 2042-2045. doi:Doi 10.1212/Wnl.42.10.2042

Caruana, D. A., Alexander, G. M., & Dudek, S. M. (2012). New insights into the regulation of synaptic plasticity from an unexpected place: Hippocampal area CA2. *Learning & Memory, 19*(9), 391-400. doi:10.1101/lm.025304.111

References

Castillo Diaz, F., Hernandez, M. A., Capella, T., & Medina, J. H. (2019). Dopamine Neurotransmission in the Ventral Tegmental Area Promotes Active Forgetting of Cocaine-Associated Memory. *Mol Neurobiol*. doi:10.1007/s12035-019-1516-3

Chang, F. C., Tsui, D. S., Mahant, N., Wolfe, N., Kim, S. D., Ha, A. D., . . . Fung, V. S. (2015). 24 h Levodopa-carbidopa intestinal gel may reduce falls and "unresponsive" freezing of gait in Parkinson's disease. *Parkinsonism Relat Disord, 21*(3), 317-320. doi:10.1016/j.parkreldis.2014.12.019

Chang, H. C., & Guarente, L. (2013). SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. *Cell, 153*(7), 1448-1460. doi:10.1016/j.cell.2013.05.027

Chowdhury, R., Guitart-Masip, M., Bunzeck, N., Dolan, R. J., & Duzel, E. (2012). Dopamine Modulates Episodic Memory Persistence in Old Age. *Journal of Neuroscience, 32*(41), 14193-14204. doi:10.1523/jneurosci.1278-12.2012

Chowdhury, R., Guitart-Masip, M., Lambert, C., Dayan, P., Huys, Q., Duzel, E., & Dolan, R. J. (2013). Dopamine restores reward prediction errors in old age. *Nature Neuroscience, 16*(5), 648-653. doi:10.1038/nn.3364

Chugh, Y., Saha, N., Sankaranarayanan, A., & Sharma, P. L. (1991). Possible Mechanism of Haloperidol-Induced Enhancement of Memory Retrieval. *Methods and Findings in Experimental and Clinical Pharmacology, 13*(3), 161-164. Retrieved from <Go to ISI>://WOS:A1991FJ14000002

Civelli, O., Bunzow, J. R., & Grandy, D. K. (1993). Molecular Diversity of the Dopamine-Receptors. *Annual Review of Pharmacology and Toxicology, 33*, 281-307. doi:DOI 10.1146/annurev.pa.33.040193.001433

Clark, R. E., West, A. N., Zola, S. M., & Squire, L. R. (2001). Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. *Hippocampus, 11*(2), 176-186. doi:DOI 10.1002/hipo.1035

References

Clemens, Z., Molle, M., Eross, L., Barsi, P., Halasz, P., & Born, J. (2007). Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain, 130*, 2868-2878. doi:10.1093/brain/awm146

Clos, M., Bunzeck, N., & Sommer, T. (2019). Dopamine is a double-edged sword: dopaminergic modulation enhances memory retrieval performance but impairs metacognition. *Neuropsychopharmacology, 44*(3), 555-563. doi:10.1038/s41386-018-0246-y

Clos, M., Schwarze, U., Gluth, S., Bunzeck, N., & Sommer, T. (2015). Goal- and retrieval-dependent activity in the striatum during memory recognition. *Neuropsychologia, 72*, 1-11. doi:10.1016/j.neuropsychologia.2015.04.011

Commins, S., Gigg, J., Anderson, M., & O'Mara, S. M. (1998). The projection from hippocampal area CA1 to the subiculum sustains long-term potentiation. *Neuroreport, 9*(5), 847-850. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9579677

Contin, M., Provini, F., Martinelli, P., Riva, R., Albani, F., Vetrugno, R., . . . Baruzzi, A. (2003). Excessive daytime sleepiness and levodopa in Parkinson's disease: polygraphic, placebo-controlled monitoring. *Clin Neuropharmacol, 26*(3), 115-118. doi:10.1097/00002826-200305000-00003

Contreras, D., Destexhe, A., Sejnowski, T. J., & Steriade, M. (1997). Spatiotemporal patterns of spindle oscillations in cortex and thalamus. *J Neurosci, 17*(3), 1179-1196. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8994070

Cools, R., Barker, R. A., Sahakian, B. J., & Robbins, T. W. (2001). Enhanced or impaired cognitive function in Parkinson's disease as a function of dopaminergic medication and task demands. *Cerebral Cortex, 11*(12), 1136-1143. doi:DOI 10.1093/cercor/11.12.1136

Cools, R., & D'Esposito, M. (2011). Inverted-U-Shaped Dopamine Actions on Human Working Memory and Cognitive Control. *Biological Psychiatry, 69*(12), E113-E125. doi:10.1016/j.biopsych.2011.03.028

References

Coulthard, E. J., Bogacz, R., Javed, S., Mooney, L. K., Murphy, G., Keeley, S., & Whone, A. L. (2012). Distinct roles of dopamine and subthalamic nucleus in learning and probabilistic decision making. *Brain, 135*, 3721-3734. doi:10.1093/brain/aws273

Coutanche, M. N., & Thompson-Schill, S. L. (2014). Fast Mapping Rapidly Integrates Information Into Existing Memory Networks. *Journal of Experimental Psychology-General, 143*(6), 2296-2303. doi:10.1037/xge0000020

Cox, R., Schapiro, A. C., Manoach, D. S., & Stickgold, R. (2017). Individual Differences in Frequency and Topography of Slow and Fast Sleep Spindles. *Frontiers in Human Neuroscience, 11*, 433. doi:10.3389/fnhum.2017.00433

Craik, F. I. M., & Tulving, E. (1975). Depth of Processing and Retention of Words in Episodic Memory. *Journal of Experimental Psychology-General, 104*(3), 268-294. doi:Doi 10.1037//0096-3445.104.3.268

Cromwell, H. C., Berridge, K. C., Drago, J., & Levine, M. S. (1998). Action sequencing is impaired in D1A-deficient mutant mice. *European Journal of Neuroscience, 10*(7), 2426-2432. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9749770

Crowley, K., Trinder, J., Kim, Y., Carrington, M., & Colrain, I. M. (2002). The effects of normal aging on sleep spindle and K-complex production. *Clin Neurophysiol, 113*(10), 1615-1622. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12350438

Csicsvari, J., Hirase, H., Czurko, A., Mamiya, A., & Buzsaki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *Journal of Neuroscience, 19*(1), 274-287. Retrieved from <Go to ISI>://WOS:000077839400030

Cumming, R. G. (1998). Epidemiology of medication-related falls and fractures in the elderly. *Drugs Aging, 12*(1), 43-53. doi:10.2165/00002512-199812010-00005

Cumming, R. G., & Klineberg, R. J. (1993). Psychotropics, thiazide diuretics and hip fractures in the elderly. *Med J Aust, 158*(6), 414-417. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8479356

References

Cumming, R. G., Miller, J. P., Kelsey, J. L., Davis, P., Arfken, C. L., Birge, S. J., & Peck, W. A. (1991). Medications and multiple falls in elderly people: the St Louis OASIS study. *Age Ageing, 20*(6), 455-461. doi:10.1093/ageing/20.6.455

da Silva, A. A., de Mello, R. G., Schaan, C. W., Fuchs, F. D., Redline, S., & Fuchs, S. C. (2016). Sleep duration and mortality in the elderly: a systematic review with meta-analysis. *BMJ Open, 6*(2), e008119. doi:10.1136/bmjopen-2015-008119

Dahan, L., Astier, B., Vautrelle, N., Urbain, N., Kocsis, B., & Chouvet, G. (2007). Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. *Neuropsychopharmacology, 32*(6), 1232-1241. doi:10.1038/sj.npp.1301251

Dalton, M. A., McCormick, C., & Maguire, E. A. (2019). Differences in functional connectivity along the anterior-posterior axis of human hippocampal subfields. *Neuroimage, 192*, 38-51. doi:10.1016/j.neuroimage.2019.02.066

Danker-Hopfe, H., Anderer, P., Zeitlhofer, J., Boeck, M., Dorn, H., Gruber, G., . . . Dorffner, G. (2009). Interrater reliability for sleep scoring according to the Rechtschaffen & Kales and the new AASM standard. *Journal of Sleep Research, 18*(1), 74-84. doi:10.1111/j.1365-2869.2008.00700.x

Dasgupta, A., Baby, N., Krishna, K., Hakim, M., Wong, Y. P., Behnisch, T., . . . Sajikumar, S. (2017). Substance P induces plasticity and synaptic tagging/capture in rat hippocampal area CA2. *Proceedings of the National Academy of Sciences of the United States of America, 114*(41), E8741-E8749. doi:10.1073/pnas.1711267114

Davis, R. L., & Zhong, Y. (2017). The Biology of Forgetting-A Perspective. *Neuron, 95*(3), 490-503. doi:10.1016/j.neuron.2017.05.039

Dayan, L., & Finberg, J. P. M. (2003). L-DOPA increases noradrenaline turnover in central and peripheral nervous systems. *Neuropharmacology, 45*(4), 524-533. doi:Doi 10.1016/S0028-3908(03)00190-4

References

Dayan, P., & Willshaw, D. J. (1991). Optimizing Synaptic Learning Rules in Linear Associative Memories. *Biological Cybernetics, 65*(4), 253-265. doi:Doi 10.1007/Bf00206223

De Cock, V. C., Vidailhet, M., Leu, S., Texeira, A., Apartis, E., Elbaz, A., . . . Arnulf, I. (2007). Restoration of normal motor control in Parkinson's disease during REM sleep. *Brain, 130*(Pt 2), 450-456. doi:10.1093/brain/awl363

de Frias, C. M., Annerbrink, K., Westberg, L., Eriksson, E., Adolfsson, R., & Nilsson, L. G. (2004). COMT gene polymorphism is associated with declarative memory in adulthood and old age. *Behavior Genetics, 34*(5), 533-539. doi:10.1023/B:BEGE.0000038491.06972.8c

De Gennaro, L., Ferrara, M., Vecchio, F., Curcio, G., & Bertini, M. (2005). An electroencephalographic fingerprint of human sleep. *Neuroimage, 26*(1), 114-122. doi:10.1016/j.neuroimage.2005.01.020

de Wael, R. V., Lariviere, S., Caldairou, B., Hong, S. J., Margulies, D. S., Jefferies, E., . . . Bernhardt, B. C. (2018). Anatomical and microstructural determinants of hippocampal subfield functional connectome embedding. *Proceedings of the National Academy of Sciences of the United States of America, 115*(40), 10154-10159. doi:10.1073/pnas.1803667115

Dehnavi, F., Moghimi, S., Sadrabadi Haghighi, S., Safaie, M., & Ghorbani, M. (2019). Opposite Effect of Motivated Forgetting on Sleep Spindles during Stage 2 and Slow Wave Sleep. *Sleep*. doi:10.1093/sleep/zsz085

Devanand, D. P., Bansal, R., Liu, J., Hao, X. J., Pradhaban, G., & Peterson, B. S. (2012). MRI hippocampal and entorhinal cortex mapping in predicting conversion to Alzheimer's disease. *Neuroimage, 60*(3), 1622-1629. doi:10.1016/j.neuroimage.2012.01.075

Dew, M. A., Hoch, C. C., Buysse, D. J., Monk, T. H., Begley, A. E., Houck, P. R., . . . Reynolds, C. F. (2003). Healthy older adults' sleep predicts all-cause mortality at 4 to 19 years of follow-up. *Psychosomatic Medicine, 65*(1), 63-73. doi:10.1097/01.Psy.0000039756.23250.7c

References

Diana, R. A., Yonelinas, A. P., & Ranganath, C. (2007). Imaging recollection and familiarity in the medial temporal lobe: a three-component model. *Trends in Cognitive Sciences, 11*(9), 379-386. doi:DOI 10.1016/j.tics.2007.08.001

Diederich, N. J., Paolini, V., & Vaillant, M. (2009). Slow wave sleep and dopaminergic treatment in Parkinson's disease: a polysomnographic study. *Acta Neurol Scand, 120*(5), 308-313. doi:10.1111/j.1600-0404.2009.01167.x

Diekelmann, S., Wilhelm, I., Wagner, U., & Born, J. (2013). Sleep to Implement an Intention. *Sleep, 36*(1), 149-153. doi:10.5665/sleep.2322

Dillon, S. E., Tsivos, D., Knight, M., McCann, B., Pennington, C., Shiel, A. I., . . . Coulthard, E. J. (2017). The impact of ageing reveals distinct roles for human dentate gyrus and CA3 in pattern separation and object recognition memory. *Sci Rep, 7*(1), 14069. doi:10.1038/s41598-017-13853-8

Dimsdale-Zucker, H. R., Ritchey, M., Ekstrom, A. D., Yonelinas, A. P., & Ranganath, C. (2018). CA1 and CA3 differentially support spontaneous retrieval of episodic contexts within human hippocampal subfields. *Nature Communications, 9*. doi:ARTN 294

10.1038/s41467-017-02752-1

Du, H. Y., Deng, W., Aimone, J. B., Ge, M. Y., Parylak, S., Walch, K., . . . Mu, Y. L. (2016). Dopaminergic inputs in the dentate gyrus direct the choice of memory encoding. *Proceedings of the National Academy of Sciences of the United States of America, 113*(37), E5501-E5510. doi:10.1073/pnas.1606951113

Dube, J., Lafortune, M., Bedetti, C., Bouchard, M., Gagnon, J. F., Doyon, J., . . . Carrier, J. (2015). Cortical Thinning Explains Changes in Sleep Slow Waves during Adulthood. *Journal of Neuroscience, 35*(20), 7795-7807. doi:10.1523/Jneurosci.3956-14.2015

Dubois, B., & Pillon, B. (1997). Cognitive deficits in Parkinson's disease. *Journal of Neurology, 244*(1), 2-8. Retrieved from <Go to ISI>://WOS:A1997VX03900002

References

Dudek, S. M., Alexander, G. M., & Farris, S. (2016). Rediscovering area CA2: unique properties and functions. *Nature Reviews Neuroscience, 17*(2), 89-102. doi:10.1038/nrn.2015.22

Dudek, S. M., & Bear, M. F. (1992). Homosynaptic Long-Term Depression in Area Ca1 of Hippocampus and Effects of N-Methyl-D-Aspartate Receptor Blockade. *Proceedings of the National Academy of Sciences of the United States of America, 89*(10), 4363-4367. doi:DOI 10.1073/pnas.89.10.4363

Dupret, D., O'Neill, J., Pleydell-Bouverie, B., & Csicsvari, J. (2010). The reorganization and reactivation of hippocampal maps predict spatial memory performance. *Nature Neuroscience, 13*(8), 995-1002. doi:10.1038/nn.2599

Duszkiewicz, A. J., McNamara, C. G., Takeuchi, T., & Genzel, L. (2019). Novelty and Dopaminergic Modulation of Memory Persistence: A Tale of Two Systems. *Trends in Neurosciences, 42*(2), 102-114. doi:10.1016/j.tins.2018.10.002

Duvernoy, H. M., Cattin, F., & Risold, P.-Y. (2013). *The Human Hippocampus; Functional Anatomy, Vascularization and Serial Sections with MRI* (4 ed.). Verlag Berlin Heidelberg: Springer.

Duzel, E., Habib, R., Rotte, M., Guderian, S., Tulving, E., & Heinze, H. J. (2003). Human hippocampal and parahippocampal activity during visual associative recognition memory for spatial and nonspatial stimulus configurations. *J Neurosci, 23*(28), 9439-9444. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14561873

Eban-Rothschild, A., Rothschild, G., Giardino, W. J., Jones, J. R., & de Lecea, L. (2016). VTA dopaminergic neurons regulate ethologically relevant sleep-wake behaviors. *Nature Neuroscience, 19*(10), 1356-1366. doi:10.1038/nn.4377

Ebbinghaus, H. (1885). *Memoy: A contribution to experimental psychology*. New York: Dover.

Eckart, C., Fuentemilla, L., Bauch, E. M., & Bunzeck, N. (2014). Dopaminergic stimulation facilitates working memory and differentially affects prefrontal low theta oscillations. *Neuroimage, 94*, 185-192. doi:10.1016/j.neuroimage.2014.03.011

Edelmann, E., & Lessmann, V. (2013). Dopamine regulates intrinsic excitability thereby gating successful induction of spike timing-dependent plasticity in CA1 of the hippocampus. *Frontiers in neuroscience, 7*, 25. doi:10.3389/fnins.2013.00025

Ego-Stengel, V., & Wilson, M. A. (2010). Disruption of Ripple-Associated Hippocampal Activity During Rest Impairs Spatial Learning in the Rat. *Hippocampus, 20*(1), 1-10. doi:10.1002/hipo.20707

Elger, C. E., Grunwald, T., Lehnertz, K., Kutas, M., Helmstaedter, C., Brockhaus, A., . . . Heinze, H. J. (1997). Human temporal lobe potentials in verbal learning and memory processes. *Neuropsychologia, 35*(5), 657-667. doi:Doi 10.1016/S0028-3932(96)00110-8

Ellis, B. W., Johns, M. W., Lancaster, R., Raptopoulos, P., Angelopoulos, N., & Priest, R. G. (1981). The St. Mary's Hospital sleep questionnaire: a study of reliability. *Sleep, 4*(1), 93-97. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7232974

Evans, T. E., Adams, H. H. H., Licher, S., Wolters, F. J., van der Lugt, A., Ikram, M. K., . . . Ikram, M. A. (2018). Subregional volumes of the hippocampus in relation to cognitive function and risk of dementia. *Neuroimage, 178*, 129-135. doi:10.1016/j.neuroimage.2018.05.041

Ezzyat, Y., Inhoff, M. C., & Davachi, L. (2018). Differentiation of Human Medial Prefrontal Cortex Activity Underlies Long-Term Resistance to Forgetting in Memory. *J Neurosci, 38*(48), 10244-10254. doi:10.1523/JNEUROSCI.2290-17.2018

Feld, G. B., Besedovsky, L., Kaida, K., Munte, T. F., & Born, J. (2014). Dopamine D2-like Receptor Activation Wipes Out Preferential Consolidation of High over Low Reward Memories during Human Sleep. *Journal of Cognitive Neuroscience, 26*(10), 2310-2320. doi:10.1162/jocn_a_00629

Feld, G. B., & Born, J. (2017). Sculpting memory during sleep: concurrent consolidation and forgetting. *Current Opinion in Neurobiology, 44*, 20-27. doi:10.1016/j.conb.2017.02.012

References

Feld, G. B., Weis, P. P., & Born, J. (2016). The Limited Capacity of Sleep-Dependent Memory Consolidation. *Front Psychol, 7*. doi:ARTN 1368

10.3389/fpsyg.2016.01368

Ferreira, J. J., Galitzky, M., Thalamas, C., Tiberge, M., Montastruc, J. L., Sampaio, C., & Rascol, O. (2002). Effect of ropinirole on sleep onset: a randomized, placebo-controlled study in healthy volunteers. *Neurology, 58*(3), 460-462. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11839850

Fischer, S., & Born, J. (2009). Anticipated reward enhances offline learning during sleep. *J Exp Psychol Learn Mem Cogn, 35*(6), 1586-1593. doi:10.1037/a0017256

Floresco, S. B., & Phillips, A. G. (2001). Delay-dependent modulation of memory retrieval by infusion of a dopamine D-1 agonist into the rat medial prefrontal cortex. *Behavioral Neuroscience, 115*(4), 934-939. doi:Doi 10.1037//0735-7044.115.4.934

Fogel, S., Vien, C., Karni, A., Benali, H., Carrier, J., & Doyon, J. (2017). Sleep spindles: a physiological marker of age-related changes in gray matter in brain regions supporting motor skill memory consolidation. *Neurobiology of Aging, 49*, 154-164. doi:10.1016/j.neurobiolaging.2016.10.009

Fogel, S. M., & Smith, C. T. (2006). Learning-dependent changes in sleep spindles and Stage 2 sleep. *Journal of Sleep Research, 15*(3), 250-255. doi:DOI 10.1111/j.1365-2869.2006.00522.x

Fogel, S. M., Smith, C. T., & Beninger, R. J. (2009). Evidence for 2-stage models of sleep and memory: learning-dependent changes in spindles and theta in rats. *Brain Research Bulletin, 79*(6), 445-451. doi:10.1016/j.brainresbull.2009.03.002

Foster, D. J., & Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature, 440*(7084), 680-683. doi:10.1038/nature04587

References

Franca, A. S., Lobao-Soares, B., Muratori, L., Nascimento, G., Winne, J., Pereira, C. M., . . . Ribeiro, S. (2015). D2 dopamine receptor regulation of learning, sleep and plasticity. *Eur Neuropsychopharmacol, 25*(4), 493-504. doi:10.1016/j.euroneuro.2015.01.011

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nat Rev Neurosci, 6*(2), 119-130. doi:10.1038/nrn1607

Fraser, M. A., Shaw, M. E., & Cherbuin, N. (2015). A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing. *Neuroimage, 112*, 364-374. doi:10.1016/j.neuroimage.2015.03.035

Frey, U., & Morris, R. G. M. (1997a). Synaptic tagging and long-term potentiation. *Nature, 385*(6616), 533-536. doi:10.1038/385533a0

Frey, U., & Morris, R. G. M. (1997b). Synaptic tagging: Synapse specificity during protein synthesis dependent long-term potentiation. *Journal of Neurochemistry, 69*, S130-S130. Retrieved from <Go to ISI>://WOS:A1997XM28500508

Frey, U., Schroeder, H., & Matthies, H. (1990). Dopaminergic Antagonists Prevent Long-Term Maintenance of Posttetanic Ltp in the Ca1 Region of Rat Hippocampal Slices. *Brain Research, 522*(1), 69-75. doi:Doi 10.1016/0006-8993(90)91578-5

Gagnon, J. F., Bedard, M. A., Fantini, M. L., Petit, D., Panisset, M., Rompre, S., . . . Montplaisir, J. (2002). REM sleep behavior disorder and REM sleep without atonia in Parkinson's disease. *Neurology, 59*(4), 585-589. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12196654

Gais, S., & Born, J. (2004). Declarative memory consolidation: Mechanisms acting during human sleep. *Learning & Memory, 11*(6), 679-685. doi:10.1101/lm.80504

Gasbarri, A., Packard, M. G., Campana, E., & Pacitti, C. (1994). Anterograde and Retrograde Tracing of Projections from the Ventral Tegmental Area to the Hippocampal-Formation in the Rat. *Brain Research Bulletin, 33*(4), 445-452. doi:Doi 10.1016/0361-9230(94)90288-7

References

Gasbarri, A., Sulli, A., & Packard, M. G. (1997). The dopaminergic mesencephalic projections to the hippocampal formation in the rat. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 21*(1), 1-22. doi:Doi 10.1016/S0278-5846(96)00157-1

Gelman, A. (2005). Analysis of Variance - Why it is More Important Than Ever. *The Annals of Statistics, 33*.

Genzel, L., Rossato, J. I., Jacobse, J., Grieves, R. M., Spooner, P. A., Battaglia, F. P., . . . Morris, R. G. M. (2017). The Yin and Yang of Memory Consolidation: Hippocampal and Neocortical. *Plos Biology, 15*(1). doi:ARTN e200531

10.1371/journal.pbio.2000531

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsaki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nature Neuroscience, 12*(10), 1222-1223. doi:10.1038/nn.2384

Gjedde, A., Kumakura, Y., Cumming, P., Linnet, J., & Moller, A. (2010). Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proceedings of the National Academy of Sciences of the United States of America, 107*(8), 3870-3875. doi:DOI 10.1073/pnas.0912319107

Gotham, A. M., Brown, R. G., & Marsden, C. D. (1988). 'Frontal' cognitive function in patients with Parkinson's disease 'on' and 'off' levodopa. *Brain, 111 (Pt 2)*, 299-321. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3378138

Gould, E., Reeves, A. J., Fallah, M., Tanapat, P., Gross, C. G., & Fuchs, E. (1999). Hippocampal neurogenesis in adult Old World primates. *Proceedings of the National Academy of Sciences of the United States of America, 96*(9), 5263-5267. doi:DOI 10.1073/pnas.96.9.5263

Granado, N., Ortiz, O., Suarez, L. M., Martin, E. D., Cena, V., Solis, J. M., & Moratalla, R. (2008). D-1 but not D-5 dopamine receptors are critical for LTP, spatial learning, and

LTP-Induced arc and zif268 expression in the hippocampus. *Cerebral Cortex, 18*(1), 1-12. doi:DOI 10.1093/cercor/bhm026

Greenland, S., Senn, S. J., Rothman, K. J., Carlin, J. B., Poole, C., Goodman, S. N., & Altman, D. G. (2016). Statistical tests, P values, confidence intervals, and power: a guide to misinterpretations. *Eur J Epidemiol, 31*(4), 337-350. doi:10.1007/s10654-016-0149-3

Grogan, J. P., Bogacz, R., Tsivos, D., Whone, A., & Coulthard, E. J. (2015). Dopamine and Consolidation of Episodic Memory: Timing is Everything. *J Cogn Neurosci, 27*(10), 2035-2050. doi:10.1162/jocn_a_00840

Grogan, J. P., Isotalus, H. K., Howat, A., Irigoras Izagirre, N., Knight, L. E., & Coulthard, E. J. (2019). Levodopa does not affect expression of reinforcement learning in older adults. *Scientific Reports, 9*(6349).

Grogan, J. P., Knight, L. E., Smith, L., Irigoras Izagirre, N., Howat, A., Knight, B. E., . . . Coulthard, E. J. (2018). Effects of Parkinson's disease and dopamine on digit span measures of working memory. *Psychopharmacology (Berl), 235*(12), 3443-3450. doi:10.1007/s00213-018-5058-6

Gruber, M. J., Ritchey, M., Wang, S. F., Doss, M. K., & Ranganath, C. (2016). Post-learning Hippocampal Dynamics Promote Preferential Retention of Rewarding Events. *Neuron, 89*(5), 1110-1120. doi:10.1016/j.neuron.2016.01.017

Hamilton, T. J., Wheatley, B. M., Sinclair, D. B., Bachmann, M., Larkum, M. E., & Colmers, W. F. (2010). Dopamine modulates synaptic plasticity in dendrites of rat and human dentate granule cells. *Proceedings of the National Academy of Sciences of the United States of America, 107*(42), 18185-18190. doi:10.1073/pnas.1011558107

Han, S., Huettel, S. A., Raposo, A., Adcock, R. A., & Dobbins, I. G. (2010). Functional Significance of Striatal Responses during Episodic Decisions: Recovery or Goal Attainment? *Journal of Neuroscience, 30*(13), 4767-4775. doi:10.1523/Jneurosci.3077-09.2010

References

Hansen, N., & Manahan-Vaughan, D. (2014). Dopamine D1/D5 Receptors Mediate Informational Saliency that Promotes Persistent Hippocampal Long-Term Plasticity. *Cerebral Cortex, 24*(4), 845-858. doi:10.1093/cercor/bhs362

Hardt, O., Nader, K., & Nadel, L. (2013). Decay happens: the role of active forgetting in memory. *Trends in Cognitive Sciences, 17*(3), 111-120. doi:10.1016/j.tics.2013.01.001

Harrington, M. O., Johnson, J. M., Croom, H. E., Pennington, K., & Durrant, S. J. (2018). The influence of REM sleep and SWS on emotional memory consolidation in participants reporting depressive symptoms. *Cortex, 99*, 281-295. doi:10.1016/j.cortex.2017.12.004

Hartopp, N., Wright, P., Ray, N. J., Evans, T. E., Metzler-Baddeley, C., Aggleton, J. P., & O'Sullivan, M. J. (2018). A Key Role for Subiculum-Fornix Connectivity in Recollection in Older Age. *Front Syst Neurosci, 12*, 70. doi:10.3389/fnsys.2018.00070

Hasselmo, M. E. (2005). What is the function of hippocampal theta Rhythm? Linking behavioral data to phasic properties of field potential and unit recording data. *Hippocampus, 15*(7), 936-949. doi:10.1002/hipo.20116

Hasselmo, M. E., & Wyble, B. P. (1997). Free recall and recognition in a network model of the hippocampus: simulating effects of scopolamine on human memory function. *Behavioural Brain Research, 89*(1-2), 1-34. doi:Doi 10.1016/S0166-4328(97)00048-X

Hayakawa, N., Kato, H., & Araki, T. (2007). Age-related changes of astorocytes, oligodendrocytes and microglia in the mouse hippocampal CA1 sector. *Mechanisms of Ageing and Development, 128*(4), 311-316. doi:10.1016/j.mad.2007.01.005

Hayes, S. M., Hayes, J. P., Williams, V. J., Liu, H. T., & Verfaellie, M. (2017). FMRI activity during associative encoding is correlated with cardiorespiratory fitness and source memory performance in older adults. *Cortex, 91*, 208-220. doi:10.1016/j.cortex.2017.01.002

Hebb, D. (1949). *The Organization of Behavior: A Neuropsychological Theory*. New York: John Wiley and Sons.

References

Heib, D. P. J., Hoedlmoser, K., Anderer, P., Gruber, G., Zeitlhofer, J., & Schabus, M. (2015). Oscillatory Theta Activity during Memory Formation and Its Impact on Overnight Consolidation: A Missing Link? *Journal of Cognitive Neuroscience, 27*(8), 1648-1658. doi:10.1162/jocn_a_00804

Heine, V. M., Maslam, S., Joels, M., & Lucassen, P. J. (2004). Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation. *Neurobiol Aging, 25*(3), 361-375. doi:10.1016/S0197-4580(03)00090-3

Himmer, L., Schönauer, M., Heib, D. P. J., Schabus, M., & Gais, S. (2019). Rehearsal initiates systems memory consolidation, sleep makes it last. *Science Advances, 5*(4).

Hitti, F. L., & Siegelbaum, S. A. (2014). The hippocampal CA2 region is essential for social memory. *Nature, 508*(7494), 88-+. doi:10.1038/nature13028

Homann, C. N., Wenzel, K., Suppan, K., Ivanic, G., Kriechbaum, N., Crevenna, R., & Ott, E. (2002). Sleep attacks in patients taking dopamine agonists: review. *BMJ, 324*(7352), 1483-1487. doi:10.1136/bmj.324.7352.1483

Hornykiewicz, O. (1966). Dopamine (3-Hydroxytyramine) and Brain Function. *Pharmacological Reviews, 18*(2), 925-+. Retrieved from <Go to ISI>://WOS:A19667972500001

Hu, P., Stylos-Allan, M., & Walker, M. P. (2006). Sleep facilitates consolidation of emotional declarative memory. *Psychological Science, 17*(10), 891-898. doi:DOI 10.1111/j.1467-9280.2006.01799.x

Huang, Y. F., Potter, R., Sigurdson, W., Santacruz, A., Shih, S., Ju, Y. E., . . . Bateman, R. J. (2012). Effects of Age and Amyloid Deposition on A beta Dynamics in the Human Central Nervous System. *Arch Neurol, 69*(1), 51-58. doi:10.1001/archneurol.2011.235

Huang, Y. Y., & Kandel, E. R. (1995). D1/D5 Receptor Agonists Induce a Protein Synthesis-Dependent Late Potentiation in the Ca1 Region of the Hippocampus. *Proceedings of the*

National Academy of Sciences of the United States of America, 92(7), 2446-2450. doi:DOI 10.1073/pnas.92.7.2446

Hyacinthe, C., Barraud, Q., Tison, F., Bezard, E., & Ghorayeb, I. (2014). D1 receptor agonist improves sleep-wake parameters in experimental parkinsonism. *Neurobiol Dis, 63*, 20-24. doi:10.1016/j.nbd.2013.10.029

Hyman, B. T., Van Hoesen, G. W., Kromer, L. J., & Damasio, A. R. (1986). Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann Neurol, 20*(4), 472-481. doi:10.1002/ana.410200406

Igloi, K., Gaggioni, G., Sterpenich, V., & Schwartz, S. (2015). A nap to recap or how reward regulates hippocampal-prefrontal memory networks during daytime sleep in humans. *Elife, 4*. doi:ARTN e07903

10.7554/eLife.07903

Introinicollison, I. B., & Mcgaugh, J. L. (1989). Cocaine Enhances Memory Storage in Mice. *Psychopharmacology, 99*(4), 537-541. doi:Doi 10.1007/Bf00589905

Isaac, S. O., & Berridge, C. W. (2003). Wake-promoting actions of dopamine D1 and D2 receptor stimulation. *J Pharmacol Exp Ther, 307*(1), 386-394. doi:10.1124/jpet.103.053918

Ito, H. T., Moser, E. I., & Moser, M. B. (2018). Supramammillary Nucleus Modulates Spike-Time Coordination in the Prefrontal-Thalamo-Hippocampal Circuit during Navigation. *Neuron, 99*(3), 576-+. doi:10.1016/j.neuron.2018.07.021

Jack, C. R., Petersen, R. C., Obrien, P. C., & Tangalos, E. G. (1992). Mr-Based Hippocampal Volumetry in the Diagnosis of Alzheimers-Disease. *Neurology, 42*(1), 183-188. doi:Doi 10.1212/Wnl.42.1.183

Jadhav, S. P., Kemere, C., German, P. W., & Frank, L. M. (2012). Awake Hippocampal Sharp-Wave Ripples Support Spatial Memory. *Science, 336*(6087), 1454-1458. doi:10.1126/science.1217230

References

Janak, P. H., Keppel, G., & Martinez, J. L. (1992). Cocaine Enhances Retention of Avoidance-Conditioning in Rats. *Psychopharmacology, 106*(3), 383-387. doi:Doi 10.1007/Bf02245422

Jankovic, J. (2008). Parkinson's disease: clinical features and diagnosis. *Journal of Neurology Neurosurgery and Psychiatry, 79*(4), 368-376. doi:10.1136/jnnp.2007.131045

Janssen, D. P. (2012). Twice random, once mixed: applying mixed models to simultaneously analyze random effects of language and participants. *Behavioural Research Methods, 1*(44), 232-247.

Jarovi, J., Volle, J., Yu, X. T., Guan, L. S., & Takehara-Nishiuchi, K. (2018). Prefrontal Theta Oscillations Promote Selective Encoding of Behaviorally Relevant Events. *Eneuro, 5*(6). doi:UNSP e0407-18.2018

10.1523/ENEURO.0407-18.2018

JASP. (2018). JASP (Version Version 0.9).

Jeffreys, H. (1961). *Theory of Probability* (3 ed.). Oxford: Clarendon Press.

Jenkins, J. G., & Dallenbach, K. M. (1924). Obliviscence during sleep and waking. *American Journal of Psychology, 35*, 605-612. doi:Doi 10.2307/1414040

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). Fsl. *Neuroimage, 62*(2), 782-790. doi:10.1016/j.neuroimage.2011.09.015

Jinno, S. (2016). Aging affects new cell production in the adult hippocampus: A quantitative anatomic review. *Journal of Chemical Neuroanatomy, 76*, 64-72. doi:10.1016/j.jchemneu.2015.10.009

Johnson, A., & Redish, A. D. (2007). Neural ensembles in CA3 transiently encode paths forward of the animal at a decision point. *Journal of Neuroscience, 27*(45), 12176-12189. doi:10.1523/Jneurosci.3761-07.2007

Jones, B. E., Bobillier, P., Pin, C., & Jouvet, M. (1973). The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral

waking in the cat. *Brain Res, 58*(1), 157-177. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4581335

Joo, H. R., & Frank, L. M. (2018). The hippocampal sharp wave-ripple in memory retrieval for immediate use and consolidation. *Nature Reviews Neuroscience, 19*(12), 744-757. doi:10.1038/s41583-018-0077-1

Ju, Y.-E. S., Ooms, S. J., Sutphen, C., Macauley, S. L., Zangrilli, M. A., Jerome, G., . . . Holtzman, D. M. (2017). Slow wave sleep disruption increases cerebrospinal fluid amyloid-β levels. *Brain, 140*(8), 2104-2111. doi:10.1093/brain/awx148

Ju, Y. E., Ooms, S. J., Sutphen, C., Macauley, S. L., Zangrilli, M. A., Jerome, G., . . . Holtzman, D. M. (2017). Slow wave sleep disruption increases cerebrospinal fluid amyloid-beta levels. *Brain, 140*, 2104-2111. doi:10.1093/brain/awx148

Kales, A., Ansel, R. D., Markham, C. H., Scharf, M. B., & Tan, T. L. (1971). Sleep in patients with Parkinson's disease and normal subjects prior to and following levodopa administration. *Clin Pharmacol Ther, 12*(2), 397-406. doi:10.1002/cpt1971122part2397

Kaminski, J., Mamelak, A. N., Birch, K., Mosher, C. P., Tagliati, M., & Rutishauser, U. (2018). Novelty-Sensitive Dopaminergic Neurons in the Human Substantia Nigra Predict Success of Declarative Memory Formation. *Current Biology, 28*(9), 1333-1343 e1334. doi:10.1016/j.cub.2018.03.024

Kamp, S. M., Bader, R., & Mecklinger, A. (2017). ERP Subsequent Memory Effects Differ between Inter-Item and Unitization Encoding Tasks. *Frontiers in Human Neuroscience, 11*. doi:ARTN 30

10.3389/fnhum.2017.00030

Karpicke, J. D., & Roediger, H. L. (2008). The critical importance of retrieval for learning. *Science, 319*(5865), 966-968. doi:10.1126/science.1152408

References

Kay, K., Sosa, M., Chung, J. E., Karlsson, M. P., Larkin, M. C., & Frank, L. M. (2016). A hippocampal network for spatial coding during immobility and sleep. *Nature, 531*(7593), 185-190. doi:10.1038/nature17144

Kelleher, R. J., Govindarajan, A., Jung, H. Y., Kang, H. J., & Tonegawa, S. (2004). Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell, 116*(3), 467-479. doi:10.1016/s0092-8674(04)00115-1

Kelly, M. A., Rubinstein, M., Phillips, T. J., Lessov, C. N., Burkhart-Kasch, S., Zhang, G., . . . Low, M. J. (1998). Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. *J Neurosci, 18*(9), 3470-3479. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9547254

Kemp, A., & Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proc Natl Acad Sci U S A, 101*(21), 8192-8197. doi:10.1073/pnas.0402650101

Kemp, A., & Manahan-Vaughan, D. (2007). Hippocampal long-term depression: master or minion in declarative memory processes? *Trends Neurosci, 30*(3), 111-118. doi:10.1016/j.tins.2007.01.002

Kensinger, E. A., Clarke, R. J., & Corkin, S. (2003). What neural correlates underlie successful encoding and retrieval? A functional magnetic resonance imaging study using a divided attention paradigm. *J Neurosci, 23*(6), 2407-2415. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12657700

Kim, H. (2013). Differential neural activity in the recognition of old versus new events: An Activation Likelihood Estimation Meta-Analysis. *Human brain mapping, 34*(4), 814-836. doi:10.1002/hbm.21474

References

Kim, H. (2019). Neural correlates of explicit and implicit memory at encoding and retrieval: A unified framework and meta-analysis of functional neuroimaging studies. *Biol Psychol, 145*, 96-111. doi:10.1016/j.biopsycho.2019.04.006

Kirk, I. J., & McNaughton, N. (1993). Mapping the differential effects of procaine on frequency and amplitude of reticularly elicited hippocampal rhythmical slow activity. *Hippocampus, 3*(4), 517-525. doi:10.1002/hipo.450030411

Kish, S. J., Shannak, K., Rajput, A., Deck, J. H. N., & Hornykiewicz, O. (1992). Aging Produces a Specific Pattern of Striatal Dopamine Loss - Implications for the Etiology of Idiopathic Parkinsons-Disease. *Journal of Neurochemistry, 58*(2), 642-648. doi:DOI 10.1111/j.1471-4159.1992.tb09766.x

Kleiner, M., Brainard, D., & Pelli, D. (2007). What's new in Psychtoolbox-3? *Perception, 36*, 14-14. Retrieved from <Go to ISI>://WOS:000250594600049

Knecht, S., Breitenstein, C., Bushuven, S., Wailke, S., Kamping, S., Floel, A., . . . Ringelstein, E. B. (2004). Levodopa: Faster and better word learning in normal humans. *Annals of Neurology, 56*(1), 20-26. doi:Doi 10.1002/Ana.20125

Knickmeyer, R. C., Wang, J. P., Zhu, H. T., Geng, X. J., Woolson, S., Hamer, R. M., . . . Gilmore, J. H. (2014). Common Variants in Psychiatric Risk Genes Predict Brain Structure at Birth. *Cerebral Cortex, 24*(5), 1230-1246. doi:10.1093/cercor/bhs401

Knight, M. J., Wearn, A., Coulthard, E., & Kauppinen, R. A. (2019). T2 Relaxometry and Diffusion Tensor Indices of the Hippocampus and Entorhinal Cortex Improve Sensitivity and Specificity of MRI to Detect Amnestic Mild Cognitive Impairment and Alzheimer's Disease Dementia. *J Magn Reson Imaging, 49*(2), 445-455. doi:10.1002/jmri.26195

Kobayashi, K., & Yasoshima, Y. (2001). The central noradrenaline system and memory consolidation. *Neuroscientist, 7*(5), 371-376. doi:Doi 10.1177/107385840100700506

Kuhl, B. A., Dudukovic, N. M., Kahn, I., & Wagner, A. D. (2007). Decreased demands on cognitive control reveal the neural processing benefits of forgetting. *Nature Neuroscience, 10*(7), 908-914. doi:10.1038/nn1918

Kuhn, H. G., Toda, T., & Gage, F. H. (2018). Adult Hippocampal Neurogenesis: A Coming-of-Age Story. *Journal of Neuroscience, 38*(49), 10401-10410. doi:10.1523/Jneurosci.2144-18.2018

Kume, K., Kume, S., Park, S. K., Hirsh, J., & Jackson, F. R. (2005). Dopamine is a regulator of arousal in the fruit fly. *Journal of Neuroscience, 25*(32), 7377-7384. doi:10.1523/Jneurosci.2048-05.2005

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 1-26. Retrieved from <Go to ISI>://WOS:000417711600001

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2019). lmerTest: Tests in Linear Mixed Effects Models (Version 3.1-0).

Lansink, C. S., Goltstein, P. M., Lankelma, J. V., McNaughton, B. L., & Pennartz, C. M. A. (2009). Hippocampus Leads Ventral Striatum in Replay of Place-Reward Information. *Plos Biology, 7*(8). doi:ARTN e1000173

10.1371/journal.pbio.1000173

Lazenka, M. F., Legakis, L. P., & Negus, S. S. (2016). Opposing Effects of Dopamine D1-and D2-Like Agonists on Intracranial Self-Stimulation in Male Rats. *Experimental and Clinical Psychopharmacology, 24*(3), 193-205. doi:10.1037/pha0000067

Leal, S. L., & Yassa, M. A. (2015). Neurocognitive Aging and the Hippocampus across Species. *Trends in Neurosciences, 38*(12), 800-812. doi:10.1016/j.tins.2015.10.003

References

Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F., & Huganir, R. L. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature, 405*(6789), 955-959. Retrieved from <Go to ISI>://WOS:000087732700052

Lee, J. L. C. (2010). Memory reconsolidation mediates the updating of hippocampal memory content. *Frontiers in Behavioral Neuroscience, 4*. doi:ARTN 168

10.3389/fnbeh.2010.00168

Lee, M. D., Wagenmakers, E-J., . (2013). *Bayesian Cognitive Modeling: A Practical Course*. New York, NY, USA: Cambridge University Press.

Lemon, N., & Manahan-Vaughan, D. (2006). Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *Journal of Neuroscience, 26*(29), 7723-7729. doi:10.1523/Jneurosci.1454-06.2006

Lena, I., Parrot, S., Deschaux, O., Muffat-Joly, S., Sauvinet, V., Renaud, B., . . . Gottesmann, C. (2005). Variations in extracellular levels of dopamine, noradrenaline, glutamate, and aspartate across the sleep-wake cycle in the medial prefrontal cortex and nucleus accumbens of freely moving rats. *Journal of Neuroscience Research, 81*(6), 891-899. doi:Doi 10.1002/Jnr.20602

Lewandowski, A., Rosipal, R., & Dorffner, G. (2013). On the Individuality of Sleep EEG Spectra. *Journal of Psychophysiology, 27*(3), 105-112. doi:10.1027/0269-8803/a000092

Li, M. J., Kechter, A., Olmstead, R. E., Irwin, M. R., & Black, D. S. (2018). Sleep and mood in older adults: coinciding changes in insomnia and depression symptoms. *Int Psychogeriatr, 30*(3), 431-435. doi:10.1017/S1041610217001454

Lima, M. M., Andersen, M. L., Reksidler, A. B., Silva, A., Zager, A., Zanata, S. M., . . . Tufik, S. (2008). Blockage of dopaminergic D(2) receptors produces decrease of REM but not of slow wave sleep in rats after REM sleep deprivation. *Behavioural Brain Research, 188*(2), 406-411. doi:10.1016/j.bbr.2007.11.025

References

Lima, M. M., Reksidler, A. B., & Vital, M. (2008). The dopaminergic dilema: Sleep or wake? Implications in Parkinson's disease. *Bioscience Hypotheses, 1*(1), 9–13.

Lima, M. M. S. (2013). Sleep disturbances in Parkinson's disease: The contribution of dopamine in REM sleep regulation. *Sleep Medicine Reviews, 17*(5), 367-375. doi:10.1016/j.smrv.2012.10.006

Lipinska, G., Bolinger, E. M., Thomas, K. G., Baldwin, D. S., & Stuart, B. (2019). Preferential Consolidation of Emotional Memory during Sleep: A meta-analysis. *Front Psychol.* doi:10.3389/fpsyg.2019.01014

Lisman, J., Grace, A. A., & Duzel, E. (2011). A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP. *Trends in Neurosciences, 34*(10), 536-547. doi:10.1016/j.tins.2011.07.006

Lisman, J., Schulman, H., & Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat Rev Neurosci, 3*(3), 175-190. doi:10.1038/nrn753

Lisman, J. E. (1999). Relating hippocampal circuitry to function: Recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron, 22*(2), 233-242. doi:Doi 10.1016/S0896-6273(00)81085-5

Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron, 46*(5), 703-713. doi:DOI 10.1016/j.neuron.2005.05.002

Liu, L. D., Wong, T. P., Pozza, M. F., Lingenhoehl, K., Wang, Y. S., Sheng, M., . . . Wang, Y. T. (2004). Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science, 304*(5673), 1021-1024. doi:DOI 10.1126/science.1096615

LME4-Authors. (2019). Package ‘lme4’ (Version 1.1-21).

Logothetis, N. K., Eschenko, O., Murayama, Y., Augath, M., Steudel, T., Evrard, H. C., . . . Oeltermann, A. (2012). Hippocampal-cortical interaction during periods of subcortical silence. *Nature, 491*(7425), 547-553. doi:10.1038/nature11618

References

Lomo, T. (2003). The discovery of long-term potentiation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 358*(1432), 617-620. doi:10.1098/rstb.2002.1226

Long, N. M., Burke, J. F., & Kahana, M. J. (2014). Subsequent memory effect in intracranial and scalp EEG. *Neuroimage, 84*, 488-494. doi:10.1016/j.neuroimage.2013.08.052

Louie, K., & Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron, 29*(1), 145-156. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11182087

Lu, Y., Christian, K., & Lu, B. (2008). BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiology of Learning and Memory, 89*(3), 312-323. doi:10.1016/j.nlm.2007.08.018

Lucey, B. P., & Bateman, R. J. (2014). Amyloid-beta diurnal pattern: possible role of sleep in Alzheimer's disease pathogenesis. *Neurobiology of Aging, 35*, S29-S34. doi:10.1016/j.neurobiolaging.2014.03.035

Magezi, D. A. (2015). Linear mixed-effects models for within-participant psychology experiments: an introductory tutorial and free, graphical user interface (LMMgui). *Front Psychol.*

Magloczky, Z., Acsady, L., & Freund, T. F. (1994). Principal Cells Are the Postsynaptic Targets of Supramammillary Afferents in the Hippocampus of the Rat. *Hippocampus, 4*(3), 322-334. doi:DOI 10.1002/hipo.450040316

Maloney, K. J., Mainville, L., & Jones, B. E. (2002). c-Fos expression in dopaminergic and GABAergic neurons of the ventral mesencephalic tegmentum after paradoxical sleep deprivation and recovery. *European Journal of Neuroscience, 15*(4), 774-778. doi:DOI 10.1046/j.1460-9568.2002.01907.x

Manahan-Vaughan, D. (1997). Group 1 and 2 metabotropic glutamate receptors play differential roles in hippocampal long-term depression and long-term potentiation in freely moving

rats. *J Neurosci, 17*(9), 3303-3311. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9096163

Manahan-Vaughan, D., & Braunewell, K. H. (1999). Novelty acquisition is associated with induction of hippocampal long-term depression. *Proceedings of the National Academy of Sciences of the United States of America, 96*(15), 8739-8744. doi:DOI 10.1073/pnas.96.15.8739

Manahan-Vaughan, D., Kulla, A., & Frey, J. (2001). Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *Journal of Neuroscience, 15*(20).

Mander, B. A., Marks, S. M., Vogel, J. W., Rao, V., Lu, B., Saletin, J. M., . . . Walker, M. P. (2015). beta-amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nature Neuroscience, 18*(7), 1051-1057. doi:10.1038/nn.4035

Manns, J. R., Hopkins, R. O., Reed, J. M., Kitchener, E. G., & Squire, L. R. (2003). Recognition memory and the human hippocampus. *Neuron, 37*(1), 171-180. doi:Doi 10.1016/S0896-6273(02)01147-9

Marsden, C. A. (2006). Dopamine: the rewarding years. *British Journal of Pharmacology, 147*, S136-S144. doi:10.1038/sj.bjp.0706473

Marsman, M., & Wagenmakers, E. J. (2017). Bayesian benefits with JASP. *European Journal of Developmental Psychology, 14*(5), 545-555. doi:10.1080/17405629.2016.1259614

Matsumae, M., Kurita, D., Atsumi, H., Haida, M., Sato, O., & Tsugane, R. (2001). Sequential changes in MR water proton relaxation time detect the process of rat brain myelination during maturation. *Mechanisms of Ageing and Development, 122*(12), 1281-1291. doi:Doi 10.1016/S0047-6374(01)00265-2

Matthies, H., Schroder, H., Hollt, V., & Krug, M. (1997). Dopamine D-1-deficient mutant mice do not show the maintenance of hippocampal LTP. *Naunyn-Schmiedebergs Archives of Pharmacology, 356*(4), 60-60. Retrieved from <Go to ISI>://WOS:A1997XY87200060

References

Mayer, M. L., Westbrook, G. L., & Guthrie, P. B. (1984). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. *Nature, 309*(5965), 261-263. doi:10.1038/309261a0

Mayes, A. R., Montaldi, D., Roper, A., Migo, E. M., Gholipour, T., & Kafkas, A. (2019). Amount, not strength of recollection, drives hippocampal activity: A problem for apparent word familiarity-related hippocampal activation. *Hippocampus, 29*(1), 46-59. doi:10.1002/hipo.23031

McClelland, J. L. (2013). Incorporating Rapid Neocortical Learning of New Schema-Consistent Information Into Complementary Learning Systems Theory. *Journal of Experimental Psychology-General, 142*(4), 1190-1210. doi:10.1037/a0033812

Mcclelland, J. L., Mcnaughton, B. L., & Oreilly, R. C. (1995). Why There Are Complementary Learning-Systems in the Hippocampus and Neocortex - Insights from the Successes and Failures of Connectionist Models of Learning and Memory. *Psychological Review, 102*(3), 419-457. doi:Doi 10.1037//0033-295x.102.3.419

McNamara, C. G., Tejero-Cantero, A., Trouche, S., Campo-Urriza, N., & Dupret, D. (2014). Dopaminergic neurons promote hippocampal reactivation and spatial memory persistence. *Nature Neuroscience, 17*(12), 1658-1660. doi:Doi 10.1038/Nn.3843

Mednick, S. C., McDevitt, E. A., Walsh, J. K., Wamsley, E., Paulus, M., Kanady, J. C., & Drummond, S. P. A. (2013). The Critical Role of Sleep Spindles in Hippocampal-Dependent Memory: A Pharmacology Study. *Journal of Neuroscience, 33*(10), 4494-4504. doi:10.1523/Jneurosci.3127-12.2013

Meiboom, S., & Gill, D. (1958). Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Review of Scientific Instruments, 29*(8), 688-691. doi:Doi 10.1063/1.1716296

Merkow, M. B., Burke, J. F., & Kahana, M. J. (2015). The human hippocampus contributes to both the recollection and familiarity components of recognition memory. *Proceedings of the*

National Academy of Sciences of the United States of America, 112(46), 14378-14383. doi:10.1073/pnas.1513145112

Micallef, J., Rey, M., Eusebio, A., Audebert, C., Rouby, F., Jouve, E., . . . Blin, O. (2009). Antiparkinsonian drug-induced sleepiness: a double-blind placebo-controlled study of L-dopa, bromocriptine and pramipexole in healthy subjects. *British Journal of Clinical Pharmacology, 67*(3), 333-340. doi:10.1111/j.1365-2125.2008.03310.x

Michon, F., Sun, J. J., Kim, C. Y., Ciliberti, D., & Kloosterman, F. (2019). Post-learning Hippocampal Replay Selectively Reinforces Spatial Memory for Highly Rewarded Locations. *Current Biology*. doi:10.1016/j.cub.2019.03.048

Migo, E. M., Mayes, A. R., & Montaldi, D. (2012). Measuring recollection and familiarity: Improving the remember/know procedure. *Consciousness and Cognition, 21*(3), 1435-1455. doi:10.1016/j.concog.2012.04.014

Molle, M., Yeshenko, O., Marshall, L., Sara, S. J., & Born, J. (2006). Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *Journal of Neurophysiology, 96*(1), 62-70. doi:10.1152/jn.00014.2006

Montastruc, J. L., Brefel-Courbon, C., Senard, J. M., Bagheri, H., Ferreira, J., Rascol, O., & Lapeyre-Mestre, M. (2001). Sleep attacks and antiparkinsonian drugs: a pilot prospective pharmacoepidemiologic study. *Clin Neuropharmacol, 24*(3), 181-183. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11391132

Monti, J. M., Hawkins, M., Jantos, H., D'Angelo, L., & Fernandez, M. (1988). Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. *Psychopharmacology (Berl), 95*(3), 395-400. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3137628

Monti, J. M., Hawkins, M., Jantos, H., Dangelo, L., & Fernandez, M. (1988). Biphasic Effects of Dopamine D-2 Receptor Agonists on Sleep and Wakefulness in the Rat. *Psychopharmacology, 95*(3), 395-400. Retrieved from <Go to ISI>://WOS:A1988N918900020

References

Monti, J. M., Jantos, H., & Fernandez, M. (1989). Effects of the Selective Dopamine D-2 Receptor Agonist, Quinpirole on Sleep and Wakefulness in the Rat. *European Journal of Pharmacology, 169*(1), 61-66. doi:Doi 10.1016/0014-2999(89)90817-0

Monti, J. M., & Monti, D. (2007). The involvement of dopamine in the modulation of sleep and waking. *Sleep Med Rev, 11*(2), 113-133. doi:10.1016/j.smrv.2006.08.003

Morcom, A. M., Bullmore, E. T., Huppert, F. A., Lennox, B., Praseedom, A., Linnington, H., & Fletcher, P. C. (2010). Memory Encoding and Dopamine in the Aging Brain: A Psychopharmacological Neuroimaging Study. *Cerebral Cortex, 20*(3), 743-757. doi:10.1093/cercor/bhp139

Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*(6056), 774-776. doi:10.1038/319774a0

Morris, R. G. M. (2006). Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *European Journal of Neuroscience, 23*(11), 2829-2846. doi:10.1111/j.1460-9568.2006.04888.x

Murdock, B. B. (1976). Item and Order Information in Short-Term Serial Memory. *Journal of Experimental Psychology-General, 105*(2), 191-216. doi:Doi 10.1037//0096-3445.105.2.191

Murphy, M. P., & LeVine, H., 3rd. (2010). Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis, 19*(1), 311-323. doi:10.3233/JAD-2010-1221

Nader, K. (2015). Reconsolidation and the Dynamic Nature of Memory. *Cold Spring Harbor Perspectives in Biology, 7*(10). doi:ARTN a021782

10.1101/cshperspect.a021782

Nader, K., & Hardt, O. (2009). A single standard for memory: the case for reconsolidation. *Nature Reviews Neuroscience, 10*(3), 224-234. doi:10.1038/nrn2590

Nasreddine, Z. S., Phillips, N., & Chertkow, H. (2012). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology, 78*(10), 765-766; author reply 766. doi:10.1212/01.wnl.0000413072.54070.a3

Nausieda, P. A., Weiner, W. J., Kaplan, L. R., Weber, S., & Klawans, H. L. (1982). Sleep disruption in the course of chronic levodopa therapy: an early feature of the levodopa psychosis. *Clin Neuropharmacol, 5*(2), 183-194. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7139632

Neylan, T. C., Mueller, S. G., Wang, Z., Metzler, T. J., Lenoci, M., Truran, D., . . . Schuff, N. (2010). Insomnia Severity Is Associated with a Decreased Volume of the CA3/Dentate Gyrus Hippocampal Subfield. *Biological Psychiatry, 68*(5), 494-496. doi:10.1016/j.biopsych.2010.04.035

Nicolas, A., Petit, D., Rompre, S., & Montplaisir, J. (2001). Sleep spindle characteristics in healthy subjects of different age groups. *Clin Neurophysiol, 112*(3), 521-527. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11222974

Nir, Y., Staba, R. J., Andrillon, T., Vyazovskiy, V. V., Cirelli, C., Fried, I., & Tononi, G. (2011). Regional slow waves and spindles in human sleep. *Neuron, 70*(1), 153-169. doi:10.1016/j.neuron.2011.02.043

Nofzinger, E. A., Buysse, D. J., Miewald, J. M., Meltzer, C. C., Price, J. C., Sembrat, R. C., . . . Moore, R. Y. (2002). Human regional cerebral glucose metabolism during non-rapid eye movement sleep in relation to waking. *Brain, 125*, 1105-1115. doi:DOI 10.1093/brain/awf103

Norimoto, H., Matsumoto, N., Miyawaki, T., Matsuki, N., & Ikegaya, Y. (2013). Subicular activation preceding hippocampal ripples in vitro. *Sci Rep, 3*, 2696. doi:10.1038/srep02696

References

Nyberg, L., Habib, R., McIntosh, A. R., & Tulving, E. (2000). Reactivation of encoding-related brain activity during memory retrieval. *Proceedings of the National Academy of Sciences of the United States of America, 97*(20), 11120-11124. doi:DOI 10.1073/pnas.97.20.11120

Nyberg, L., Karalija, N., Salami, A., Andersson, M., Wahlin, A., Kaboovand, N., . . . Backman, L. (2016). Dopamine D2 receptor availability is linked to hippocampal-caudate functional connectivity and episodic memory. *Proceedings of the National Academy of Sciences of the United States of America, 113*(28), 7918-7923. doi:10.1073/pnas.1606309113

Nygard, M., Hill, R. H., Wikstrom, M. A., & Kristensson, K. (2005). Age-related changes in electrophysiological properties of the mouse suprachiasmatic nucleus in vitro. *Brain Research Bulletin, 65*(2), 149-154. doi:10.1016/j.brainresbull.2004.12.006

Nyhus, E., & Curran, T. (2010). Functional role of gamma and theta oscillations in episodic memory. *Neuroscience and Biobehavioral Reviews, 34*(7), 1023-1035. doi:10.1016/j.neubiorev.2009.12.014

O'Carroll, C. M., Martin, S. J., Sandin, J., Frenguelli, B., & Morris, R. G. M. (2006). Dopaminergic modulation of the persistence of one-trial hippocampus-dependent memory. *Learning & Memory, 13*(6), 760-769. doi:Doi 10.1101/Lm.321006

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Experimental Neurology, 51*(1), 78-109. doi:10.1016/0014-4886(76)90055-8

Oddie, S. D., Bland, B. H., Colom, L. V., & Vertes, R. P. (1994). The midline posterior hypothalamic region comprises a critical part of the ascending brainstem hippocampal synchronizing pathway. *Hippocampus, 4*(4), 454-473. doi:10.1002/hipo.450040408

Oliva, A., Fernandez-Ruiz, A., Buzsaki, G., & Berenyi, A. (2016). Role of Hippocampal CA2 Region in Triggering Sharp-Wave Ripples. *Neuron, 91*(6), 1342-1355. doi:10.1016/j.neuron.2016.08.008

Otmakhova, N. A., & Lisman, J. E. (1996). D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *Journal of*

Neuroscience, 16(23), 7478-7486. Retrieved from <Go to ISI>://WOS:A1996VY43300008

Otmakhova, N. A., & Lisman, J. E. (1998). D1/D5 dopamine receptors inhibit depotentiation at CA1 synapses via cAMP-dependent mechanism. *Journal of Neuroscience, 18*(4), 1270-1279. Retrieved from <Go to ISI>://WOS:000071825800009

Oudiette, D., Antony, J. W., Creery, J. D., & Paller, K. A. (2013). The Role of Memory Reactivation during Wakefulness and Sleep in Determining Which Memories Endure. *Journal of Neuroscience, 33*(15), 6672-U6758. doi:10.1523/Jneurosci.5497-12.2013

Oudiette, D., Constantinescu, I., Leclair-Visonneau, L., Vidailhet, M., Schwartz, S., & Arnulf, I. (2011). Evidence for the Re-Enactment of a Recently Learned Behavior during Sleepwalking. *Plos One, 6*(3). doi:ARTN e18056

10.1371/journal.pone.0018056

Pacini, R., & Epstein, S. (1999). The relation of rational and experiential information processing styles to personality, basic beliefs, and the ratio-bias phenomenon. *Journal of Personality and Social Psychology, 76*(6), 972-987. doi:Doi 10.1037/0022-3514.76.6.972

Pal, S., Bhattacharya, K. F., Agapito, C., & Chaudhuri, K. R. (2001). A study of excessive daytime sleepiness and its clinical significance in three groups of Parkinson's disease patients taking pramipexole, cabergoline and levodopa mono and combination therapy. *J Neural Transm (Vienna), 108*(1), 71-77. doi:10.1007/s007020170098

Papadaki, E., Kavroulakis, E., Kalaitzakis, G., Karageorgou, D., Makrakis, D., Maris, T. G., & Simos, P. G. (2019). Age-related deep white matter changes in myelin and water content: A T2 relaxometry study. *J Magn Reson Imaging*. doi:10.1002/jmri.26707

Papaleonidopoulos, V., Kouvaros, S., & Papatheodoropoulos, C. (2018). Effects of endogenous and exogenous D1/D5 dopamine receptor activation on LTP in ventral and dorsal CA1 hippocampal synapses. *Synapse, 72*(8). doi:ARTN e22033

10.1002/syn.22033

Parrott, A. C., & Hindmarch, I. (1980). The Leeds Sleep Evaluation Questionnaire in psychopharmacological investigations - a review. *Psychopharmacology (Berl), 71*(2), 173-179. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6777817

Patil, A., Murty, V. P., Dunsmoor, J. E., Phelps, E. A., & Davachi, L. (2017). Reward retroactively enhances memory consolidation for related items. *Learning & Memory, 24*(1), 65-69. doi:10.1101/lm.042978

Paus, T., Collins, D. L., Evans, A. C., Leonard, G., Pike, B., & Zijdenbos, A. (2001). Maturation of white matter in the human brain: A review of magnetic resonance studies. *Brain Research Bulletin, 54*(3), 255-266. doi:Doi 10.1016/S0361-9230(00)00434-2

Pearson, H. A., & Peers, C. (2006). Physiological roles for amyloid beta peptides. *Journal of Physiology-London, 575*(1), 5-10. doi:10.1113/jphysiol.2006.111203

Pearson, K., & Filon, L. (1898). Mathematical Contributions to Theory of Evolution: IV. On the Probable Errors of Frequency Constants and on the Influence of Random Selection and Correlation. *Philosophical Transactions of the Royal Society of London. Series A, Containing Papers of a Mathematical or Physical Character, 191*, 229-311.

Peitikainen, P. (2007). *Alchemists of Human Nature : Psychological Utopianism in Gross, Jung, Reich and Fromm*. eBook: Routledge.

Pennartz, C. M. A., Lee, E., Verheul, J., Lipa, P., Barnes, C. A., & McNaughton, B. L. (2004). The ventral striatum in off-line processing: Ensemble reactivation during sleep and modulation by hippocampal ripples. *Journal of Neuroscience, 24*(29), 6446-6456. doi:10.1523/Jneurosci.0575-04.2004

Penner, M. R., & Mizumori, S. J. Y. (2012). Age-associated changes in the hippocampal-ventral striatum-ventral tegmental loop that impact learning, prediction, and context discrimination. *Frontiers in Aging Neuroscience, 4*. doi:ARTN 22

10.3389/fnagi.2012.00022

Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuroscience, 12*(7), 919-U143. doi:10.1038/nn.2337

Poschel, B., & Manahan-Vaughan, D. (2007). Persistent (> 24 h) long-term depression in the dentate gyrus of freely moving rats is not dependent on activation of NMDA receptors, L-type voltage-gated calcium channels or protein synthesis. *Neuropharmacology, 52*(1), 46-54. doi:10.1016/j.neuropharm.2006.07.019

Proenca, M. B., Dombrowski, P. A., Da Cunha, C., Fischer, L., Ferraz, A. C., & Lima, M. M. (2014). Dopaminergic D2 receptor is a key player in the substantia nigra pars compacta neuronal activation mediated by REM sleep deprivation. *Neuropharmacology, 76 Pt A*, 118-126. doi:10.1016/j.neuropharm.2013.08.024

Pu, M., & Yu, R. J. (2019). Post-encoding frontal theta activity predicts incidental memory in the reward context. *Neurobiology of Learning and Memory, 158*, 14-23. doi:10.1016/j.nlm.2019.01.008

Purcell, S. M., Manoach, D. S., Demanuele, C., Cade, B. E., Mariani, S., Cox, R., . . . Stickgold, R. (2017). Characterizing sleep spindles in 11,630 individuals from the National Sleep Research Resource. *Nature Communications, 8*, 15930. doi:10.1038/ncomms15930

Quintana, D. S., & Williams, D. R. (2018). Bayesian alternatives for common null-hypothesis significance tests in psychiatry: a non-technical guide using JASP. *Bmc Psychiatry, 18*. doi:ARTN 178

10.1186/s12888-018-1761-4

Ramadan, W., Eschenko, O., & Sara, S. J. (2009). Hippocampal Sharp Wave/Ripples during Sleep for Consolidation of Associative Memory. *Plos One, 4*(8). doi:ARTN e6697

10.1371/journal.pone.0006697

References

Rasch, B., & Born, J. (2013). About Sleep's Role in Memory. *Physiological Reviews, 93*(2), 681-766. doi:10.1152/physrev.00032.2012

Rasch, B., Buechel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science, 315*(5817), 1426-1429. doi:10.1126/science.1138581

Rauchs, G., Desgranges, B., Foret, J., & Eustache, F. (2005). The relationships between memory systems and sleep stages. *Journal of Sleep Research, 14*(2), 123-140. doi:DOI 10.1111/j.1365-2869.2005.00450.x

Rauchs, G., Feyers, D., Landeau, B., Bastin, C., Luxen, A., Maquet, P., & Collette, F. (2011). Sleep Contributes to the Strengthening of Some Memories Over Others, Depending on Hippocampal Activity at Learning. *Journal of Neuroscience, 31*(7), 2563-2568. doi:10.1523/Jneurosci.3972-10.2011

Ravassard, P., Pachoud, B., Comte, J. C., Mejia-Perez, C., Scote-Blachon, C., Gay, N., . . . Salin, P. A. (2009). Paradoxical (REM) sleep deprivation causes a large and rapidly reversible decrease in long-term potentiation, synaptic transmission, glutamate receptor protein levels, and ERK/MAPK activation in the dorsal hippocampus. *Sleep, 32*(2), 227-240. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19238810

RCoreTeam. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Retrieved from http://www.R-project.org/

Redline, S., Kirchner, L., Quan, S. F., Gottlieb, D. J., Kapur, V., & Newman, A. (2004). The effects of age, sex, ethnicity, and sleep-disordered breathing on sleep architecture. *Archives of Internal Medicine, 164*(4), 406-418. doi:DOI 10.1001/archinte.164.4.406

Redondo, R. L., & Morris, R. G. (2011). Making memories last: the synaptic tagging and capture hypothesis. *Nat Rev Neurosci, 12*(1), 17-30. doi:10.1038/nrn2963

References

Reinhard, M. A., Regen, W., Baglioni, C., Nissen, C., Feige, B., Hennig, J., . . . Spiegelhalder, K. (2014). The Relationship between Brain Morphology and Polysomnography in Healthy Good Sleepers. *Plos One, 9*(10).

Reist, C., Sokolski, K. N., Chen, C. C., Coskinas, E., & Demet, E. M. (1995). The effect of sleep deprivation on motor impairment and retinal adaptation in Parkinson's disease. *Prog Neuropsychopharmacol Biol Psychiatry, 19*(3), 445-454. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7624495

Rempel-Clower, N. L., Zola, S. M., Squire, L. R., & Amaral, D. G. (1996). Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. *J Neurosci, 16*(16), 5233-5255. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8756452

Reutrakul, S., & Van Cauter, E. (2018). Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. *Metabolism, 84*, 56-66. doi:10.1016/j.metabol.2018.02.010

Roberts, D. E., Killiany, R. J., & Rosene, D. L. (2012). Neuron numbers in the hypothalamus of the normal aging rhesus monkey: Stability across the adult lifespan and between the sexes. *Journal of Comparative Neurology, 520*(6), 1181-1197. doi:10.1002/cne.22761

Robertson, D. R. C., Wood, N. D., Everest, H., Monks, K., Waller, D. G., Renwick, A. G., & George, C. F. (1989). The Effect of Age on the Pharmacokinetics of Levodopa Administered Alone and in the Presence of Carbidopa. *British Journal of Clinical Pharmacology, 28*(1), 61-69. doi:DOI 10.1111/j.1365-2125.1989.tb03506.x

Roggenhofer, E., Fidzinski, P., Bartsch, J., Kurz, F., Shor, O., & Behr, J. (2010). Activation of dopamine D1/D5 receptors facilitates the induction of presynaptic long-term potentiation at hippocampal output synapses. *European Journal of Neuroscience, 32*(4), 598-605. doi:10.1111/j.1460-9568.2010.07312.x

Rosburg, T., Johansson, M., Weigl, M., & Mecklinger, A. (2015). How does testing affect retrieval-related processes? An event-related potential (ERP) study on the short-term

effects of repeated retrieval. *Cognitive Affective & Behavioral Neuroscience, 15*(1), 195-210. doi:10.3758/s13415-014-0310-y

Rossato, J. I., Bevilaqua, L. R. M., Izquierdo, I., Medina, J. H., & Cammarota, M. (2009). Dopamine Controls Persistence of Long-Term Memory Storage. *Science, 325*(5943), 1017-1020. doi:DOI 10.1126/science.1172545

Rudoy, J. D., Voss, J. L., Westerberg, C. E., & Paller, K. A. (2009). Strengthening Individual Memories by Reactivating Them During Sleep. *Science, 326*(5956), 1079-1079. doi:10.1126/science.1179013

Rugg, M. D., & Vilberg, K. L. (2013). Brain networks underlying episodic memory retrieval. *Current Opinion in Neurobiology, 23*(2), 255-260. doi:10.1016/j.conb.2012.11.005

Rugg, M. D., & Yonelinas, A. P. (2003). Human recognition memory: a cognitive neuroscience perspective. *Trends in Cognitive Sciences, 7*(7), 313-319. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12860190

Rutishauser, U., Mamelak, A. N., & Schuman, E. M. (2006). Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. *Neuron, 49*(6), 805-813. doi:10.1016/j.neuron.2006.02.015

Rye, D. B. (2004). The two faces of Eve - Dopamine's modulation of wakefulness and sleep. *Neurology, 63*(8), S2-S7. Retrieved from <Go to ISI>://WOS:000224796900002

http://graphics.tx.ovid.com/ovftpdfs/FPDDNCJCDEGKNB00/fs047/ovft/live/gv031/00006114/00006114-200410263-00002.pdf

Ryynanen, O. P., Kivela, S. L., Honkanen, R., Laippala, P., & Saano, V. (1993). Medications and chronic diseases as risk factors for falling injuries in the elderly. *Scand J Soc Med, 21*(4), 264-271. doi:10.1177/140349489302100406

Sadowski, J. H., Jones, M. W., & Mellor, J. R. (2016). Sharp-Wave Ripples Orchestrate the Induction of Synaptic Plasticity during Reactivation of Place Cell Firing Patterns in the Hippocampus. *Cell Reports, 14*(8), 1916-1929. doi:10.1016/j.celrep.2016.01.061

Salami, A., Eriksson, J., & Nyberg, L. (2012). Opposing Effects of Aging on Large-Scale Brain Systems for Memory Encoding and Cognitive Control. *Journal of Neuroscience, 32*(31), 10749-10757. doi:10.1523/Jneurosci.0278-12.2012

Salami, A., Garrett, D. D., Wahlin, A., Rieckmann, A., Papenberg, G., Karalija, N., . . . Nyberg, L. (2019). Dopamine D-2/3 Binding Potential Modulates Neural Signatures of Working Memory in a Load-Dependent Fashion. *Journal of Neuroscience, 39*(3), 537-547. doi:10.1523/Jneurosci.1493-18.2018

Saletin, J. M., Goldstein-Piekarski, A. N., Greer, S. M., Stark, S., Stark, C. E., & Walker, M. P. (2016). Human Hippocampal Structure: A Novel Biomarker Predicting Mnemonic Vulnerability to, and Recovery from, Sleep Deprivation. *J Neurosci, 36*(8), 2355-2363. doi:10.1523/JNEUROSCI.3466-15.2016

Saletin, J. M., Goldstein, A. N., & Walker, M. P. (2011). The Role of Sleep in Directed Forgetting and Remembering of Human Memories. *Cerebral Cortex, 21*(11), 2534-2541. doi:10.1093/cercor/bhr034

Saletin, J. M., van der Helm, E., & Walker, M. P. (2013). Structural brain correlates of human sleep oscillations. *Neuroimage, 83*, 658-668. doi:10.1016/j.neuroimage.2013.06.021

Saletu, M., Anderer, P., Saletu-Zyhlarz, G., Hauer, C., & Saletu, B. (2002). Acute placebo-controlled sleep laboratory studies and clinical follow-up with pramipexole in restless legs syndrome. *Eur Arch Psychiatry Clin Neurosci, 252*(4), 185-194. doi:10.1007/s00406-002-0380-7

Saletu, M., Anderer, P., Saletu-Zyhlarz, G., Prause, W., Semler, B., Zoghlami, A., . . . Saletu, B. (2001). Restless legs syndrome (RLS) and periodic limb movement disorder (PLMD): acute placebo-controlled sleep laboratory studies with clonazepam. *Eur*

Neuropsychopharmacol, 11(2), 153-161. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11313161

Sanchez-Cubillo, I., Perianez, J. A., Adrover-Roig, D., Rodriguez-Sanchez, J. M., Rios-Lago, M., Tirapu, J., & Barcelo, F. (2009). Construct validity of the Trail Making Test: Role of task-switching, working memory, inhibition/interference control, and visuomotor abilities. *Journal of the International Neuropsychological Society, 15*(3), 438-450. doi:10.1017/S1355617709090626

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature, 437*(7063), 1257-1263. doi:10.1038/nature04284

Sara, S. J. (1986). Haloperidol Facilitates Memory Retrieval in the Rat. *Psychopharmacology, 89*(3), 307-310. Retrieved from <Go to ISI>://WOS:A1986C640000011

Satoh, A., Imai, S., & Guarente, L. (2017). The brain, sirtuins, and ageing. *Nature Reviews Neuroscience, 18*(6), 362-374. doi:10.1038/nrn.2017.42

Sauvage, M. M., Beer, Z., & Eichenbaum, H. (2010). Recognition memory: adding a response deadline eliminates recollection but spares familiarity. *Learn Mem, 17*(2), 104-108. doi:10.1101/lm.1647710

Schacter, D. L., & Wagner, A. D. (1999). Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus, 9*(1), 7-24. doi:Doi 10.1002/(Sici)1098-1063(1999)9:1<7::Aid-Hipo2>3.0.Co;2-K

Schapiro, A. C., McDevitt, E. A., Chen, L., Norman, K. A., Mednick, S. C., & Rogers, T. T. (2017). Sleep Benefits Memory for Semantic Category Structure While Preserving Exemplar-Specific Information. *Scientific Reports, 7*. doi:ARTN 14869

10.1038/s41598-017-12884-5

Schoemaker, D., Mascret, C., Collins, D. L., Yu, E., Gauthier, S., & Pruessner, J. C. (2017). Recollection and familiarity in aging individuals: Gaining insight into relationships with

medial temporal lobe structural integrity. *Hippocampus, 27*(6), 692-701. doi:10.1002/hipo.22725

Schultz, W. (1998). Predictive reward signal of dopamine neurons. *Journal of Neurophysiology, 80*(1), 1-27. Retrieved from <Go to ISI>://WOS:000074750800003

http://jn.physiology.org/content/jn/80/1/1.full.pdf

Schwarze, U., Bingel, U., Badre, D., & Sommer, T. (2013). Ventral Striatal Activity Correlates with Memory Confidence for Old- and New-Responses in a Difficult Recognition Test. *Plos One, 8*(3). doi:ARTN e54324

10.1371/journal.pone.0054324

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry, 20*(1), 11-21. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/13406589

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med, 8*(6), 595-608. doi:10.15252/emmm.201606210

Semon, R. (1909). *Die nmemischen Empfindungen*. Leipzig: Willhelm Engelmann.

Seugnet, L., Galvin, J. E., Suzuki, Y., Gottschalk, L., & Shaw, P. J. (2009). Persistent short-term memory defects following sleep deprivation in a drosophila model of Parkinson disease. *Sleep, 32*(8), 984-992. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19725249

Shapiro, C. M., Auch, C., Reimer, M., Kayumov, L., Heslegrave, R., Huterer, N., . . . Devins, G. M. (2006). A new approach to the construct of alertness. *Journal of Psychosomatic Research, 60*(6), 595-603. doi:10.1016/j.jpsychores.2006.04.012

Shellshear, L., MacDonald, A. D., Mahoney, J., Finch, E., McMahon, K., Silburn, P., . . . Copland, D. A. (2015). Levodopa enhances explicit new-word learning in healthy adults: a preliminary study. *Hum Psychopharmacol*. doi:10.1002/hup.2480

References

Shimizu, R. E., Connolly, P. M., Cellini, N., Armstrong, D. M., Hernandez, L. T., Estrada, R., . . . Simons, S. B. (2018). Closed-Loop Targeted Memory Reactivation during Sleep Improves Spatial Navigation. *Frontiers in Human Neuroscience, 12*. doi:ARTN 28

10.3389/fnhum.2018.00028

Shing, Y. L., Rodrigue, K. M., Kennedy, K. M., Fandakova, Y., Bodammer, N., Werkle-Bergner, M., . . . Raz, N. (2011). Hippocampal subfield volumes: age, vascular risk, and correlation with associative memory. *Frontiers in Aging Neuroscience, 3*, 2. doi:10.3389/fnagi.2011.00002

Shohamy, D., & Adcock, R. A. (2010). Dopamine and adaptive memory. *Trends in Cognitive Sciences, 14*(10), 464-472. doi:10.1016/j.tics.2010.08.002

Shohamy, D., Myers, C. E., Geghman, K. D., Sage, J., & Gluck, M. A. (2006). L-dopa impairs learning, but spares generalization, in Parkinson's disease. *Neuropsychologia, 44*(5), 774-784. doi:10.1016/j.neuropsychologia.2005.07.013

Shokri-Kojori, E., Wang, G. J., Wiers, C. E., Demiral, S. B., Guo, M., Kim, S. W., . . . Volkow, N. D. (2018). beta-Amyloid accumulation in the human brain after one night of sleep deprivation. *Proceedings of the National Academy of Sciences of the United States of America, 115*(17), 4483-4488. doi:10.1073/pnas.1721694115

Siapas, A. G., & Wilson, M. A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron, 21*(5), 1123-1128. doi:Doi 10.1016/S0896-6273(00)80629-7

Siegel, J. M. (2001). The REM sleep - Memory consolidation hypothesis. *Science, 294*(5544), 1058-1063. doi:DOI 10.1126/science.1063049

Singer, A. C., & Frank, L. M. (2009). Rewarded Outcomes Enhance Reactivation of Experience in the Hippocampus. *Neuron, 64*(6), 910-921. doi:10.1016/j.neuron.2009.11.016

Sirota, A., Csicsvari, J., Buhl, D., & Buzsaki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America, 100*(4), 2065-2069. doi:10.1073/pnas.0437938100

Skaggs, W. E., & McNaughton, B. L. (1996). Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science, 271*(5257), 1870-1873. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8596957

Smith, C. N., Jeneson, A., Frascino, J. C., Kirwan, C. B., Hopkins, R. O., & Squire, L. R. (2014). When recognition memory is independent of hippocampal function. *Proceedings of the National Academy of Sciences of the United States of America, 111*(27), 9935-9940. doi:10.1073/pnas.1409878111

Smith, C. N., Wixted, J. T., & Squire, L. R. (2011). The Hippocampus Supports Both Recollection and Familiarity When Memories Are Strong. *Journal of Neuroscience, 31*(44), 15693-15702. doi:10.1523/Jneurosci.3438-11.2011

Smith, S. M. (2002). Fast robust automated brain extraction. *Human brain mapping, 17*(3), 143-155. doi:10.1002/hbm.10062

Smith, W. B., Starck, S. R., Roberts, R. W., & Schuman, E. M. (2005). Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron, 45*(5), 765-779. doi:10.1016/j.neuron.2005.01.015

Sockeel, P., Dujardin, K., Devos, D., Deneve, C., Destee, A., & Defebvre, L. (2006). The Lille apathy rating scale (LARS), a new instrument for detecting and quantifying apathy: validation in Parkinson's disease. *J Neurol Neurosurg Psychiatry, 77*(5), 579-584. doi:10.1136/jnnp.2005.075929

Spaniol, J., Davidson, P. S. R., Kim, A. S. N., Han, H., Moscovitch, M., & Grady, C. L. (2009). Event-related fMRI studies of episodic encoding and retrieval: Meta-analyses using

activation likelihood estimation. *Neuropsychologia, 47*(8-9), 1765-1779. doi:10.1016/j.neuropsychologia.2009.02.028

Sprecher, K. E., Bendlin, B. B., Racine, A. M., Okonkwo, O. C., Christian, B. T., Koscik, R. L., . . . Benca, R. M. (2015). Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiology of Aging, 36*(9), 2568-2576. doi:10.1016/j.neurobiolaging.2015.05.004

Sprecher, K. E., Koscik, R. L., Carlsson, C. M., Zetterberg, H., Blennow, K., Okonkwo, O. C., . . . Bendlin, B. B. (2017). Poor sleep is associated with CSF biomarkers of amyloid pathology in cognitively normal adults. *Neurology, 89*(5), 445-453. doi:10.1212/Wnl.0000000000004171

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev, 99*(2), 195-231. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1594723

Squire, L. R. (2009). The Legacy of Patient HM for Neuroscience. *Neuron, 61*(1), 6-9. doi:DOI 10.1016/j.neuron.2008.12.023

Squire, L. R., Genzel, L., Wixted, J. T., & Morris, R. G. (2015). Memory Consolidation. *Cold Spring Harbor Perspectives in Biology, 7*(8). doi:ARTN a021766

10.1101/cshperspect.a021766

Squire, L. R., Stark, C. E. L., & Clark, R. E. (2004). The medial temporal lobe. *Annual Review of Neuroscience, 27*, 279-306. doi:10.1146/annurev.neuro.27.070203.144130

Stanton, P. K. (1996). LTD, LTP, and the sliding threshold for long-term synaptic plasticity. *Hippocampus, 6*(1), 35-42. doi:Doi 10.1002/(Sici)1098-1063(1996)6:1<35::Aid-Hipo7>3.0.Co;2-6

References

Steiger, T. K., & Bunzeck, N. (2017). Reward Dependent Invigoration Relates to Theta Oscillations and Is Predicted by Dopaminergic Midbrain Integrity in Healthy Elderly. *Frontiers in Aging Neuroscience, 9*. doi:ARTN 1

10.3389/fnagi.2017.00001

Sterpenich, V., Albouy, G., Darsaud, A., Schmidt, C., Vandewalle, G., Vu, T. T. D., . . . Maquet, P. (2009). Sleep Promotes the Neural Reorganization of Remote Emotional Memory. *Journal of Neuroscience, 29*(16), 5143-5152. doi:10.1523/Jneurosci.0561-09.2009

Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature, 437*(7063), 1272-1278. doi:Doi 10.1038/Nature04286

Storm, B. C., & Levy, B. J. (2012). A progress report on the inhibitory account of retrieval-induced forgetting. *Memory & Cognition, 40*(6), 827-843. doi:10.3758/s13421-012-0211-7

Suchecki, D., Tiba, P. A., & Machado, R. B. (2012). REM Sleep Rebound as an Adaptive Response to Stressful Situations. *Front Neurol, 3*, 41. doi:10.3389/fneur.2012.00041

Suzuki, W. A., Miller, E. K., & Desimone, R. (1997). Object and place memory in the macaque entorhinal cortex. *Journal of Neurophysiology, 78*(2), 1062-1081. Retrieved from <Go to ISI>://WOS:A1997XT27300041

Swanson-Park, J. L., Coussens, C. M., Mason-Parker, S. E., Raymond, C. R., Hargreaves, E. L., Dragunow, M., . . . Abraham, W. C. (1999). A double dissociation within the hippocampus of dopamine D-1/D-5 receptor and beta-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience, 92*(2), 485-497. doi:Doi 10.1016/S0306-4522(99)00010-X

Swick, T. J. (2012). Parkinson's disease and sleep/wake disturbances. *Parkinsons Dis, 2012*, 205471. doi:10.1155/2012/205471

References

Synek, V., & Reuben, J. R. (1976). Ventricular-Brain Ratio Using Planimetric Measurement of Emi Scans. *British Journal of Radiology, 49*(579), 233-237. doi:Doi 10.1259/0007-1285-49-579-233

Szucs, D., & Ioannidis, J. P. A. (2017). Empirical assessment of published effect sizes and power in the recent cognitive neuroscience and psychology literature. *Plos Biology, 15*(3). doi:ARTN e2000797

10.1371/journal.pbio.2000797

Takashima, A., Nieuwenhuis, I. L. C., Jensen, O., Talamini, L. M., Rijpkema, M., & Fernandez, G. (2009). Shift from Hippocampal to Neocortical Centered Retrieval Network with Consolidation. *Journal of Neuroscience, 29*(32), 10087-10093. doi:10.1523/Jneurosci.0799-09.2009

Taki, Y., Hashizume, H., Thyreau, B., Sassa, Y., Takeuchi, H., Wu, K., . . . Kawashima, R. (2012). Sleep duration during weekdays affects hippocampal gray matter volume in healthy children. *Neuroimage, 60*(1), 471-475. doi:10.1016/j.neuroimage.2011.11.072

Tamminen, J., Lambon Ralph, M. A., & Lewis, P. A. (2013). The role of sleep spindles and slow-wave activity in integrating new information in semantic memory. *J Neurosci, 33*(39), 15376-15381. doi:10.1523/JNEUROSCI.5093-12.2013

Tan, E. K., Lum, S. Y., Fook-Chong, S. M., Teoh, M. L., Yih, Y., Tan, L., . . . Wong, M. C. (2002). Evaluation of somnolence in Parkinson's disease: comparison with age- and sex-matched controls. *Neurology, 58*(3), 465-468. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11839852

Tang, X., Cai, F., Ding, D. X., Zhang, L. L., Cai, X. Y., & Fang, Q. (2018). Magnetic resonance imaging relaxation time in Alzheimer's disease. *Brain Research Bulletin, 140*, 176-189. doi:10.1016/j.brainresbull.2018.05.004

References

Tasali, E., Leproul, R., Ehrmann, D. A., & Van Cauter, E. (2008). Slow-wave sleep and the risk of type 2 diabetes in humans. *Proceedings of the National Academy of Sciences of the United States of America, 105*(3), 1044-1049. doi:10.1073/pnas.0706446105

Tonegawa, S., Liu, X., Ramirez, S., & Redondo, R. (2015). Memory Engram Cells Have Come of Age. *Neuron, 87*(5), 918-931. doi:10.1016/j.neuron.2015.08.002

Tonegawa, S., Pignatelli, M., Roy, D. S., & Ryan, T. J. (2015). Memory engram storage and retrieval. *Current Opinion in Neurobiology, 35*, 101-109. doi:10.1016/j.conb.2015.07.009

Travis, S. G., Huang, Y., Fujiwara, E., Radomski, A., Olsen, F., Carter, R., . . . Malykhin, N. V. (2014). High field structural MRI reveals specific episodic memory correlates in the subfields of the hippocampus. *Neuropsychologia, 53*, 233-245. doi:10.1016/j.neuropsychologia.2013.11.016

Treisman, M. (1964). The Effect of One Stimulus on the Threshold for Another - an Application of Signal Detectability Theory. *British Journal of Statistical Psychology, 17*(1), 15-35. Retrieved from <Go to ISI>://WOS:A1964CCL7400002

Tsien, J. Z., Huerta, P. T., & Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell, 87*(7), 1327-1338. doi:Doi 10.1016/S0092-8674(00)81827-9

Tsumoto, T. (1993). Long-Term Depression in Cerebral-Cortex - a Possible Substrate of Forgetting That Should Not Be Forgotten. *Neuroscience Research, 16*(4), 263-270. doi:Doi 10.1016/0168-0102(93)90036-P

Tucker, A. M., Dinges, D. F., & Van Dongen, H. P. A. (2007). Trait interindividual differences in the sleep physiology of healthy young adults. *Journal of Sleep Research, 16*(2), 170-180. doi:DOI 10.1111/j.1365-2869.2007.00594.x

Tulving, E. (1985). Memory and Consciousness. *Canadian Psychology-Psychologie Canadienne, 26*(1), 1-12. doi:DOI 10.1037/h0080017

Tulving, E. (1987). Multiple memory systems and consciousness. *Hum Neurobiol, 6*(2), 67-80. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3305441

Uribe, C., Segura, B., Baggio, H. C., Campabadal, A., Abos, A., Compta, Y., . . . Junque, C. (2018). Differential Progression of Regional Hippocampal Atrophy in Aging and Parkinson's Disease. *Frontiers in Aging Neuroscience, 10*, 325. doi:10.3389/fnagi.2018.00325

Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., . . . Girault, J. A. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. *Proceedings of the National Academy of Sciences of the United States of America, 102*(2), 491-496. doi:10.1073/pnas.0408305102

Vallone, D., Pignatelli, M., Grammatikopoulos, G., Ruocco, L., Bozzi, Y., Westphal, H., . . . Sadile, A. G. (2002). Activity, non-selective attention and emotionality in dopamine D2/D3 receptor knock-out mice. *Behavioural Brain Research, 130*(1-2), 141-148. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11864730

van de Ven, G. M., Trouche, S., McNamara, C. G., Allen, K., & Dupret, D. (2016). Hippocampal Offline Reactivation Consolidates Recently Formed Cell Assembly Patterns during Sharp Wave-Ripples. *Neuron, 92*(5), 968-974. doi:10.1016/j.neuron.2016.10.020

van der Meer, M. A. A., Johnson, A., Schmitzer-Torbert, N. C., & Redish, A. D. (2010). Triple Dissociation of Information Processing in Dorsal Striatum, Ventral Striatum, and Hippocampus on a Learned Spatial Decision Task. *Neuron, 67*(1), 25-32. doi:10.1016/j.neuron.2010.06.023

Van Overschelde, J. P., Rawson, K. A., & Dunlosky, J. (2004). Category norms: An updated and expanded version of the Battig and Montague (1969) norms. *Journal of Memory and Language, 50*(3), 289-335. doi:10.1016/j.jml.2003.10.003

van Rijn, E., Lucignoli, C., Izura, C., & Blagrove, M. T. (2016). Sleep-dependent memory consolidation is related to perceived value of learned material. *Journal of Sleep Research, 25*, 204-205. Retrieved from <Go to ISI>://WOS:000383445200541

References

Vanderwolf, C. H. (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol, 26*(4), 407-418. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4183562

Vertes, R. P. (2005). Hippocampal theta rhythm: A tag for short-term memory. *Hippocampus, 15*(7), 923-935. doi:10.1002/hipo.20118

Videnovic, A., & Golombek, D. (2013). Circadian and sleep disorders in Parkinson's disease. *Experimental Neurology, 243*, 45-56. doi:10.1016/j.expneurol.2012.08.018

Vitiello, M. V., & Borson, S. (2001). Sleep disturbances in patients with Alzheimer's disease - Epidemiology, pathophysiology and treatment. *Cns Drugs, 15*(10), 777-796. doi:Doi 10.2165/00023210-200115100-00004

Vogel, G. W. (1975). A review of REM sleep deprivation. *Arch Gen Psychiatry, 32*(6), 749-761. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/165795

Volkow, N. D., Tomasi, D., Wang, G. J., Telang, F., Fowler, J. S., Logan, J., . . . Ferre, S. (2012). Evidence That Sleep Deprivation Downregulates Dopamine D2R in Ventral Striatum in the Human Brain. *Journal of Neuroscience, 32*(19), 6711-6717. doi:Doi 10.1523/Jneurosci.0045-12.2012

Volkow, N. D., Wang, G. J., Telang, F., Fowler, J. S., Logan, J., Wong, C., . . . Jayne, M. (2008). Sleep deprivation decreases binding of [11C]raclopride to dopamine D2/D3 receptors in the human brain. *J Neurosci, 28*(34), 8454-8461. doi:10.1523/JNEUROSCI.1443-08.2008

Wailke, S., Herzog, J., Witt, K., Deuschl, G., & Volkmann, J. (2011). Effect of controlled-release levodopa on the microstructure of sleep in Parkinson's disease. *European Journal of Neurology, 18*(4), 590-596. doi:10.1111/j.1468-1331.2010.03213.x

Walker, M. P. (2008). Cognitive consequences of sleep and sleep loss. *Sleep Medicine, 9*, S29-S34. doi:Doi 10.1016/S1389-9457(08)70014-5

Walker, M. P. (2009). The Role of Slow Wave Sleep in Memory Processing. *J Clin Sleep Med, 5*(2 Suppl), S20-S26. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2824214/

Walker, M. P., & Stickgold, R. (2004). Sleep-dependent learning and memory consolidation. *Neuron, 44*(1), 121-133. doi:10.1016/j.neuron.2004.08.031

Wallace, M. L., Stone, K., Smagula, S. F., Hall, M. H., Simsek, B., Kado, D. M., . . . Buysse, D. J. (2018). Which Sleep Health Characteristics Predict All-Cause Mortality in Older Men? An Application of Flexible Multivariable Approaches. *Sleep, 41*(1). doi:UNSP zsx189

10.1093/sleep/zsx189

Wang, S. H., & Morris, R. G. (2010). Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. *Annu Rev Psychol, 61*, 49-79, C41-44. doi:10.1146/annurev.psych.093008.100523

Werner, R., Marcus, C., Sheikhbahaei, S., Higuchi, T., Solnes, L., Rowe, S., . . . Javadi, M. (2018). The Impact of Ageing on Dopamine Transporter Imaging. *Journal of Nuclear Medicine, 59*. Retrieved from <Go to ISI>://WOS:000467489902327

Werth, E., Achermann, P., Dijk, D. J., & Borbely, A. A. (1997). Spindle frequency activity in the sleep EEG: individual differences and topographic distribution. *Electroencephalogr Clin Neurophysiol, 103*(5), 535-542. doi:Doi 10.1016/S0013-4694(97)00070-9

Westbrook, A., & Frank, M. (2018). Dopamine and Proximity in Motivation and Cognitive Control. *Curr Opin Behav Sci, 22*, 28-34. doi:10.1016/j.cobeha.2017.12.011

Whitwell, J. L., Crum, W. R., Watt, H. C., & Fox, N. C. (2001). Normalization of cerebral volumes by use of intracranial volume: Implications for longitudinal quantitative MR imaging. *American Journal of Neuroradiology, 22*(8), 1483-1489. Retrieved from <Go to ISI>://WOS:000171119500009

References

Wiesner, C. D., Pulst, J., Krause, F., Elsner, M., Baving, L., Pedersen, A., . . . Goder, R. (2015). The effect of selective REM-sleep deprivation on the consolidation and affective evaluation of emotional memories. *Neurobiology of Learning and Memory, 122*, 131-141. doi:10.1016/j.nlm.2015.02.008

Wigstrom, H., & Gustafsson, B. (1986). Postsynaptic Control of Hippocampal Long-Term Potentiation. *Journal De Physiologie, 81*(4), 228-236. Retrieved from <Go to ISI>://WOS:A1986G306400002

Wilhelm, I., Diekelmann, S., Molzow, I., Ayoub, A., Molle, M., & Born, J. (2011). Sleep Selectively Enhances Memory Expected to Be of Future Relevance. *Journal of Neuroscience, 31*(5), 1563-1569. doi:10.1523/Jneurosci.3575-10.2011

Wilson, I. A., Gallagher, M., Eichenbaum, H., & Tanila, H. (2006). Neurocognitive aging: prior memories hinder new hippocampal encoding. *Trends in Neurosciences, 29*(12), 662-670. doi:10.1016/j.tins.2006.10.002

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science, 265*(5172), 676-679. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8036517

Wimber, M., Alink, A., Charest, I., Kriegeskorte, N., & Anderson, M. C. (2015). Retrieval induces adaptive forgetting of competing memories via cortical pattern suppression. *Nature Neuroscience, 18*(4), 582-+. doi:10.1038/nn.3973

Wing, E. A., Marsh, E. J., & Cabeza, R. (2013). Neural correlates of retrieval-based memory enhancement: An fMRI study of the testing effect. *Neuropsychologia, 51*(12), 2360-2370. doi:10.1016/j.neuropsychologia.2013.04.004

Wisse, L. E. M., Daugherty, A. M., Olsen, R. K., Berron, D., Carr, V. A., Stark, C. E. L., . . . Grp, H. S. (2017). A Harmonized Segmentation Protocol for Hippocampal and Parahippocampal Subregions: Why Do We Need One and What Are the Key Goals? *Hippocampus, 27*(1), 3-11. doi:10.1002/hipo.22671

Wittmann, B. C., Bunzeck, N., Dolan, R. J., & Duzel, E. (2007). Anticipation of novelty recruits reward system and hippocampus while promoting recollection. *Neuroimage, 38*(1), 194-202. doi:10.1016/j.neuroimage.2007.06.038

Wixted, J. T., & Squire, L. R. (2010). The role of the human hippocampus in familiarity-based and recollection-based recognition memory. *Behavioural Brain Research, 215*(2), 197-208. doi:10.1016/j.bbr.2010.04.020

Wolf, H., Kruggel, F., Hensel, A., Wahlund, L. O., Arendt, T., & Gertz, H. J. (2003). The relationship between head size and intracranial volume in elderly subjects. *Brain Research, 973*(1), 74-80. doi:10.1016/S0006-8993(03)02552-6

Worker, A., Dima, D., Combes, A., Crum, W. R., Streffer, J., Einstein, A., . . . O'daly, O. (2018). Test–retest reliability and longitudinal analysis of automated hippocampal subregion volumes in healthy ageing and Alzheimer's disease populations. *Human brain mapping, 39*(4), 1743-1754.

Wortelboer, U., Cohrs, S., Rodenbeck, A., & Ruther, E. (2002). Tolerability of hypnosedatives in older patients. *Drugs Aging, 19*(7), 529-539. doi:10.2165/00002512-200219070-00006

Xie, Kang, H. Y., Xu, Q. W., Chen, M. J., Liao, Y. H., Thiyagarajan, M., . . . Nedergaard, M. (2013). Sleep Drives Metabolite Clearance from the Adult Brain. *Science, 342*(6156), 373-377. doi:10.1126/science.1241224

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., . . . Nedergaard, M. (2013). Sleep Drives Metabolite Clearance from the Adult Brain. *Science, 342*(6156), 373-377. doi:10.1126/science.1241224

Xu, M., Koeltzow, T. E., Santiago, G. T., Moratalla, R., Cooper, D. C., Hu, X. T., . . . Tonegawa, S. (1997). Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. *Neuron, 19*(4), 837-848. doi:Doi 10.1016/S0896-6273(00)80965-4

References

Yang, C., Potts, R., & Shanks, D. R. (2018). Enhancing learning and retrieval of new information: a review of the forward testing effect. *NPJ Sci Learn, 3*, 8. doi:10.1038/s41539-018-0024-y

Yang, M. Y., Logothetis, N. K., & Eschenko, O. (2019). Occurrence of Hippocampal Ripples is Associated with Activity Suppression in the Mediodorsal Thalamic Nucleus. *Journal of Neuroscience, 39*(3), 434-444. doi:10.1523/Jneurosci.2107-18.2018

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *Neuroimage, 51*(3), 1242-1252. doi:10.1016/j.neuroimage.2010.03.040

Yonelinas, A. P., Kroll, N. E., Dobbins, I., Lazzara, M., & Knight, R. T. (1998). Recollection and familiarity deficits in amnesia: convergence of remember-know, process dissociation, and receiver operating characteristic data. *Neuropsychology, 12*(3), 323-339. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9673991

Yoshii, A., & Constantine-Paton, M. (2010). Postsynaptic BDNF-TrkB Signaling in Synapse Maturation, Plasticity, and Disease. *Developmental Neurobiology, 70*(5), 304-322. doi:10.1002/dneu.20765

Yoshinori, A., & Rubin, G. M. (2016). Dopaminergic neurons write and update memories with cell-type-specific rules. *Elife, 6*(e16135).

Yushkevich, P. A., Avants, B. B., Pluta, J., Das, S., Minkoff, D., Mechanic-Hamilton, D., . . . Detre, J. A. (2009). A high-resolution computational atlas of the human hippocampus from postmortem magnetic resonance imaging at 9.4 T. *Neuroimage, 44*(2), 385-398. doi:10.1016/j.neuroimage.2008.08.042

Yushkevich, P. A., Pluta, J. B., Wang, H., Xie, L., Ding, S. L., Gertje, E. C., . . . Wolk, D. A. (2015). Automated volumetry and regional thickness analysis of hippocampal subfields

and medial temporal cortical structures in mild cognitive impairment. *Hum Brain Mapp, 36*(1), 258-287. doi:10.1002/hbm.22627

Zevon, M. A., & Tellegen, A. (1982). The Structure of Mood Change - an Idiographic Nomothetic Analysis. *Journal of Personality and Social Psychology, 43*(1), 111-122. doi:Doi 10.1037//0022-3514.43.1.111

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *J Neurosci, 6*(10), 2950-2967. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3760943

Zweifel, L. S., Parker, J. G., Lobb, C. J., Rainwater, A., Wall, V. Z., Fadok, J. P., . . . Palmiter, R. D. (2009). Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proceedings of the National Academy of Sciences of the United States of America, 106*(18), 7281-7288. doi:DOI 10.1073/pnas.0813415106

Appendices

Appendices

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Overview: Initially, the DOPAMIND study had three study arms: Ropinirole 8mg, co-beneldopa (containing L-DOPA 200mg), and placebo. Five volunteers were dosed with ropinirole and each of them experienced significant nausea and vomiting for up to 24 hours after dosing, despite having received 10mg of domperidone to mitigate nausea first.

After first two participants had experienced nausea, the study protocol was amended to increase the maximum domperidone dose from 10mg to 30mg. Ropinirole continued to cause vomiting and nausea despite the increased domperidone dose on two participants. One of the participants dosed with domperidone experienced a significant serious adverse event temporally associated with the domperidone administration. The participant experienced an asymptomatic prolonged QT interval, observed in routine ECG measures with an onset after 30mg of domperidone had been given. The prolongation of the QT interval continued overnight and was still present the following morning. Cardiac side effects have been reported in participants over the age of 65 taking domperidone routinely at a 30mg or above daily dose.

This resulted in a trial halt during which significant protocol changes were made:

The ropinirole arm of the study was withdrawn

Domperidone maximum dose was reduced to 20mg

Four participants from block 1 completed 3 visits, one participant completed two visits from this block (co-beneldopa and placebo) and one just the ropinirole visit. The participant who only completed the ropinirole visit was recruited again after the study halt and allocated another participant number to complete the study. The data collected from the ropinirole visits was not analysed.

Standard Operating Procedures for Medication Blinding

Version 2: 14th April 2016

DARet1 is a double blind randomised study, in which each volunteer is completes to two conditions; drug (domperidone + Madopar) and placebo. Both drugs are water soluble and therefore blinding will be completed by offering medication mixed with cordial. Each volunteer gets two cups of cordial. The person designated to blinding the pharmaceuticals will be referred to as the blinder in this SOP.

The experimenter will provide the blinder with an envelope that contains information on the condition. The **envelope must be opened out of sight from the researcher and the person completing the blinding must hold onto the envelope until the volunteer leaves the research facility**, so that emergency unblinding can be completed by contacting this person.

The researcher will provide the blinder with the following equipment:

- Envelope addressed with the participant ID and visit number
- Yellow cordial
- Purple cordial
- Plastic cups
- Measuring cup (20ml)
- Oral syringes (10ml)
- Madopar tablets (two jars)
- Domperidone liquid
- Vitamin tablets

The blinder must adhere to following the steps when preparing the medication.

1. Wash hands

2. Mixing the cordial

Using the 20ml measuring cup, measure 40ml of purple cordial into one and 40ml of yellow cordial into the other. Do not dispose of the measuring cup as this will be washed and used again.

3. Opening the envelope

The envelope will state the volunteer participant number and the visit day. The researcher will ensure the blinder receives the correct envelope. The blinder will ensure the researcher will not see contents of this envelope.

- If the envelope indicates the volunteer is in the placebo condition proceed to 4
- If the envelope indicates the volunteer is in the medication conditions, proceed to 5

4. Placebo

The purple mix is done. You do not need to add anything to this.

Get a fraction of a vitamin tablet that equals around ¼ or 1/5 of a tablet, and add this to the yellow mix. If you notice the colour of the mix changing, mix a new cup with less vitamin.

You may need to crush a vitamin tablet to do this. These tablets are easy to crush so this can be achieved by hand.

If there are less than four oral syringes, dispose of one and notify the experimenter of how many are left. If there are more than four, do not dispose any.

As the placebo takes less time to prepare than the drug, please wait at least 30 seconds before you proceed to step 6.

5. Medicine

Using an oral syringe, measure 10ml of Domperidone (the liquid in a glass bottle) and add this to the purple mix.

Add two Madopar tablets to the yellow mix. There are two jars, add one tablet from each.

Wait a moment for the Madopar to dilute. Some residue may be visible on the juice. Do not worry about this.

6. Fill with water

Fill both plastic cups with water (up to roughly the third 'line' from the top).

7. Take juice mixes to the researcher

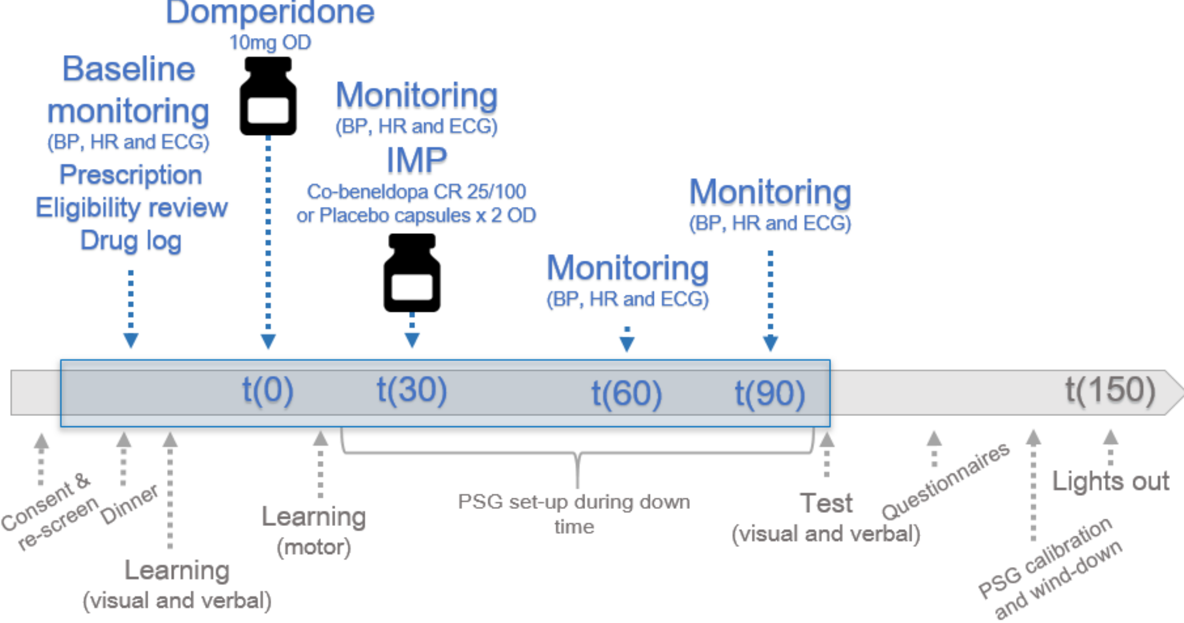
Once you have provided the juice mixes to the researcher, ensure you can be contacted by the researcher at any point when the research volunteer is on site, in case there is need for emergency unblinding. You may leave the Brain Centre but make sure the researcher has your number.

8. Disposing of materials

Envelopes. Ensure you have the envelope with its contents on you until the volunteer has left the study site. After this time you may dispose of the envelope securely. You can do this by placing it in a confidential waste bin or disposing of it somewhere where the researcher cannot see it (for example your home).

Syringes. The oral syringes should be disposed of so that the researcher cannot see them. You can do this by placing them in a lidded bin in any of the clinic rooms or the day ward, as long as this is not the room where the researcher will be conducting the experiment. Do not dispose of these in the Brain Centre office or toilet bins as there is a chance the researcher will see them.

DOPAMIND medical cover information



Blue areas indicate responsibilities allocated to the medical cover (MC) and the blue box shows when the MC is needed on site. All the timings are dependent on the "Lights out" time, which is the time when the volunteer typically goes to bed, typically 10-12PM. The MC is expected to be available to stay on site for 3 hours and will be reimbursed £125 for their time per night. If our volunteers have very incongruent bedtimes we might need cover for a longer period (and we adjust reimbursement accordingly). All testing takes place at CRICBristol (in St Michael's hospital).

The MC is responsible for:

- Drug logs
- Prescriptions
- Eligibility review (based on re-screen)
- Monitoring (ECG, BP, HR)
- Dosing
- Filling and signing relevant pages on the case report form (CRF)

An on-call neurologist (typically the PI, Liz Coulthard) will be available for advice over the phone throughout the evening. Their phone number will be in the CRF. The PI will also be liable in the highly unlikely case anything went wrong.

Each volunteer will have been screened and signed off as eligible by the PI prior to sleep visits. The MC will only review *continued* eligibility. Where the medical cover is a nurse ECGs will be interpreted by the clinical cover remotely and the prescription(s) will be written beforehand.

The most likely side effect we may experience is nausea and vomiting from the co-beneldopa. Other side effects are very unlikely at current doses. We always have an additional 10mg of domperidone we can give to volunteers if they present with nausea, and you should not hesitate to use that.

Before starting, the MC will need to present evidence of **good clinical practice, a signed and dated CV** and **a declaration of ability to perform basic life support** (or an ABLS certificate). They will also need to be **signed off on the delegation log** and they will need to **read and sign the SOP** for monitoring and dosing.

DARet study
(relevant section from testing SOP used by experimenters)

You need to have someone not involved in the testing for this participant perform the drug/placebo preparation. The instructions are in 'Drug Preparation SOPV2.docx'. [APPENDIX B]

Begin Testing

1. Sign consent form
2. Go through continuance criteria

Drug/placebo administration 1

3. Go to day ward and measure blood pressure and heart rate (t0)
4. Administer domperidone/placebo (purple drink)
5. 30 minute delay
6. Can't be unattended, but can be left in day ward if other staff members are around. Check on them occasionally

Drug/placebo administration 2

7. After 30 minutes take BP and HR again (t30 – 30 minutes after baseline)
8. Administer madopar/placebo (orange drink)
9. Can't be unattended, but can be left in day ward if other staff members are around. Check on them occasionally
10. Wait 30 minutes and check BP and HR again (t60)
11. Wait another 30 minutes and check BP and HR again (t90)
12. Begin testing session

NOTE THAT ONLY THE RELEVANT SECTIONS OF SOPs ARE INCLUDED HERE.

Participants learn a list of 100 words and delayed recognition is tested using the Remember-Know paradigm.

Remembering refers to the conscious recollection of information while knowing refers to a sense of familiarity. It is important to note that this is not a measure of certainty. Some have suggested that remember responses are analogous to episodic memories and know responses are analogous to semantic memories. The testing of this task is performed using a vigorous protocol to ensure participants understand the difference between remembering and knowing.

In the learning block, a white fixation cross is shown for 1000ms and then a word is presented in red font on a black background for 2600. The participants are initially shown a practice version with 5 words to learn, and a 5 word test, to get used to the lay out of the task.

The participants are instructed to try to remember the words, along with selecting if they think the word is 'alive' – 'yes' or 'no', via button press. There are 80words (presented in a randomised order).

Stimuli selection

All words were drawn from the MRC psycholinguistic database (Wilson, MD, 1988) and controlled for concreteness (range 100-700, mean 438) and word length (4-8 letter). Only concrete nouns were selected. This yielded a database of 1985 from which 480 words (3x test versions, 100x distractors, 80x test words) were drawn at random. These were then split across test groups at random. A number of t-tests were run to ensure the lists were matched for frequency, imageability, word length and proportion of animate versus non animate (alive or not) items in each list.

Learning – RKNLearnIncidental

In the learning block, a white fixation cross is shown for 1000ms and then a word is presented in red font on a black background for 3600ms. During learning participants are instructed to try to remember the words, along with selecting if they think the word is 'alive' – 'yes' or 'no', via button press. There are 80 target words (presented in a randomised order). The purpose of the question is to get participants to think about the meaning of the word, as this will help them remember the words later. If they press 'yes' or 'no' before 3600ms the word will remain on the screen until 3600ms has elapsed. If they do not respond within 3600ms the word will disappear and the next trial will begin.

Script:

You will see a series of words presented on the computer screen. Your task is to remember them later. We will test your memory by showing you a word on the screen and asking you if the word was on the list you see next, so you don't need to be able to freely recall all the words.

While you try to memorise the words we will ask you if each item is 'alive' or not. For example, a tree would be alive and a rock would not be alive. The reason we ask this is to get you to think about the meaning of the words as we know this will help you remember them later. Not all of the words are easy to categorise, so if you are unsure, make your best guess. We do not mark your performance on the alive/not judgements.

We will test your memory on these words later today, tomorrow morning and then again over the phone 3 and 5 days later. Please read the instructions on the screen and let me know if you have any questions once you have read them.

[Give the volunteer time to read the instructions and answer any questions]

On-screen instructions:

'You will see a series of words displayed in the centre of the screen in red.

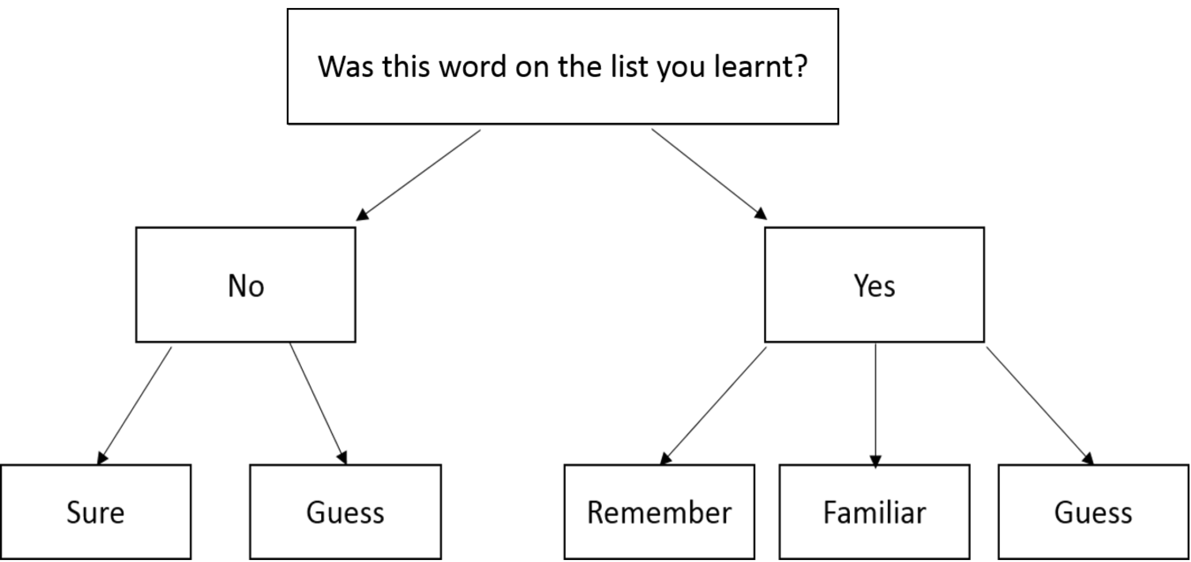
You will decide if each word is alive using left and right buttons.

Try to remember the words, as you will be tested on them later.

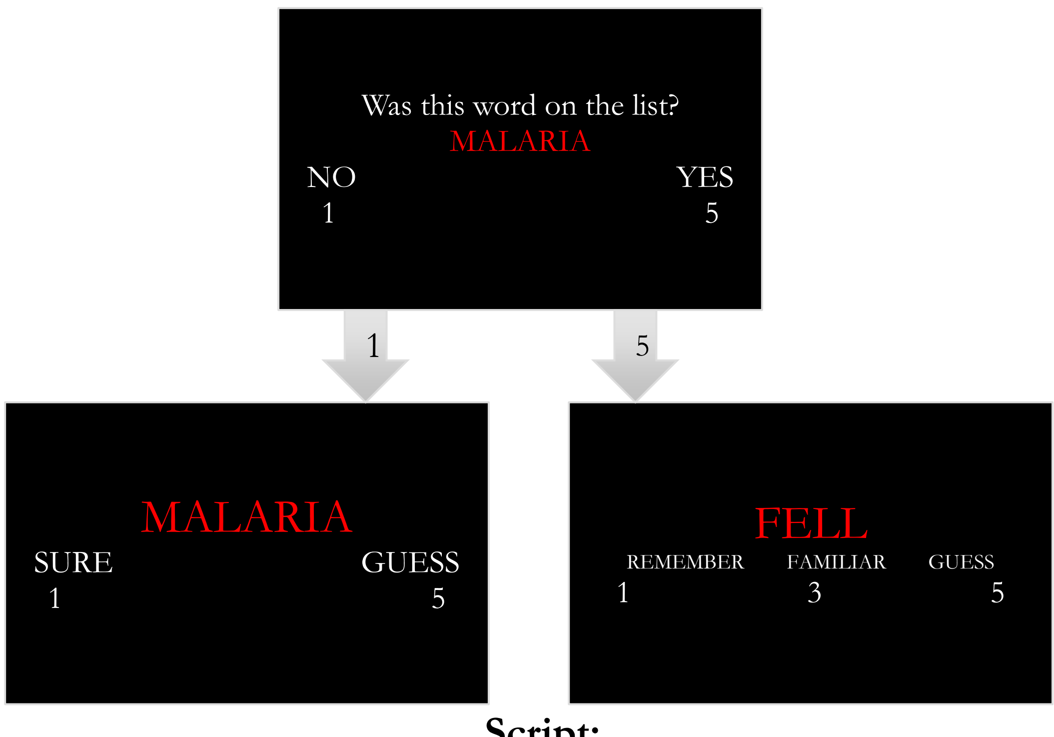
Please press the middle button when you are ready to begin'

Testing – RKNTestIncidental

At test, participants see a word on the screen and have to say if it is on the list they learned. **The experimenter will use the button box to make responses on the volunteers' behalf.** If they respond 'yes' then they must decide whether the word is 'remembered', 'familiar' or 'guessed'. They are instructed to respond 'remember' if they can recollect what they thought of when they saw the word on the screen. They respond 'familiar' if they do not have an actual memory of the word but it seems familiar, and 'guess' if they are purely guessing. If they respond 'no' they must decide if they are 'sure' or if they made a 'guess'. The below diagram summarises the response options. This diagram can be given to the participants.



20 target words are tested (with 20 novel distractors) at each test. In the morning test, to assess reconsolidation, the 20 target words that were tested the previous night are assessed together with 20 novel distractor words. All distractors are novel.



Script:

You will see a series of words presented on the computer screen. For each word your task is to determine if the word was on the list you learnt in the evening. If you say that the word was on the list, we will then ask you if you remember the word, if you are familiar with it, or if you are making a guess.

The difference between remembering and being familiar is not simply how confident you are. These refer to different types of memories. If you

remember a word, you should be able to explain how you remember it. For example, if you saw the word 'giraffe' maybe you remember thinking about the giraffe and the rolling pin from the other task, or thinking about whether the giraffe was alive or not. If you are familiar with a word, you can be just as certain it was on the list, but not have any recollection of when you saw it. So for a familiar response you would not be able to explain how you know it was on the list. And a guess is a guess.

I will be making all the responses for you, and each time you say you remember a word I will ask you how you remember it.

If you say a word was not on the list we will ask you if you are sure or if you made a guess.

Please read the instructions on the screen and let me know if you have any questions once you have read them.

[Most do not have any questions. Sometimes during test volunteers insist on having a memory even when they cannot say how they remember the item. These are 'familiar' responses. This task is difficult for some volunteers to understand.]

Script for the morning:

You will see a series of words presented on the computer screen. For each word your task is to determine if the word was on the list you learnt in the evening. If you say that the word was on the list, we will then ask you if you remember the word, if you are familiar with it, or if you are making a guess. If you say it was not on the list I will ask you if you are sure of if you are making a guess.

If you think a word you see now was on either of the lists you saw last night, then it would have been on the original list. All the distractor words are novel, so there are no tricks to this list and you are not asked to say whether you saw a word in one list but not the other. If you think you have seen a word before, make a 'yes' response.

I will be making all the responses for you, and each time you say you remember a word I will ask you how you remember it, just as last night. Please read the instructions on the screen and let me know if you have any questions once you have read them.

[Complete the test as it was completed the night before]

RKN phone calls

The phone call tests are run similarly to the RKNTest they complete on the computer, except that the words are read aloud by the experimenter, over the phone. Detailed step-by-step instructions on set-up are given in 'DOPAMIND Phone Call RKN Test SOP.docx'.

Each word is read aloud clearly, and the participant must decide if the word was on the list they learned in the evening and answer 'yes' or 'no'. If 'yes', they will then decide if they 'remember', if it is 'familiar' or if it is a 'guess'. Again, 'remember' refers to recalling extra details of the memory, and they must explain what it is they remember. If they say 'no', they should then say whether they are 'sure' or 'guess'.

They should have been given a paper diagram showing these responses, which should also have the times of their phone calls written on it.

You can repeat the word as needed, or spell it out, but **cannot** give a definition or use it in a sentence.

This can be run either on MATLAB (the same as the computer tests), via excel, or on a paper copy.

For matlab, run RKNTestIncidental in the command window, and fill in the participant and version details. Then run as normal.

For excel, open the '\DOPAMIND\Task Development\DOPAMIND\RKNtask\long term test version A.xlsx' (or version B or C - check version number), and go to the appropriate sheet for the version (e.g. sheet A1 or B4). You need to randomise the order of the words, so double click on cell A2 and press enter, then highlight cells A2:C41, right click on column A and select 'sort' by 'smallest to largest'. This will ensure the target and distractor words are presented in a random order. Save this file with the participant ID and version number in the name (e.g. 'RKNTestIncidental_PPID101_SesNo1_VersA1_20183112', replacing the PPID (e.g. 115), Session (1 or 2), Version (e.g. B3), and date in yyyymmdd format (e.g. 4th May 2018 becomes 20180504)). During the test, record their responses by putting a number 1 in the appropriate cell under 'yes' or 'no' then under 'remember','famililar' etc. There MUST be 2 responses per row.

For paper copy, go through the same steps for setting up the excel file, and after saving it, print out the cells A1:J42 – use Print Setup to print the gridlines to make it easier to fill in. After completing the test, you will have to enter the data back into excel, so make sure you have saved the file with the words in the same order as the printed version to make this easier. If you haven't done this, you will have to put the words (and their word numbers) into excel by hand, in the order they were presented.

Script:

Hello, my name is [NAME] *from the DOPAMIND study at CRIC. I'm calling to do the word test. Is now a good time? Do you have the diagram we gave you with the responses on it?*

This test will be like the ones you did in CRIC. I will read a word out, and you will decide if it was on the list you learned the other evening. The words I read out will either have come from that list you learned or they will be brand new words you did not see at all when you came into CRIC.

If you think the word was on the list you read out, please say 'yes' and then whether you 'remember' the word, it is 'familiar' or a 'guess'. 'Remember' responses mean you can recall extra details about the word such as what you thought, or what words came before or after it. If you cannot recall any extra details, but are sure it was on the list, it is 'familiar' and if you are not sure it is a 'guess'.

If you think the word was not on the list you learned, say 'no' and then 'sure' or 'guess'.

I can repeat the word as many times as you need, and spell it out for you, but I cannot give you a definition or use it in a sentence.

Do you have any questions before we begin?

DOPAMIND Phone Call RKN Test SOP

This document contains instructions for running the RKN test over the phone for the DOPAMIND study.

These tests are done on day 3 and day 5 after each sleep visit.

Booking

1. Each call takes 10-15 minutes. Book the phone calls in for a time that suits the participant and yourself (or whichever experimenter will be running the phone calls).
2. Write the times and dates of the phone calls on the back of the 'RKN Diagram' paper and give this to the participant.
3. Create a calendar event for each phone call and invite whoever is doing the phone call, and also invite Hanna Isotalus to it so she has a record of all phone calls.
    a. This calendar event should include the version letter and number for each phone call test, the participant's name and phone number and any extra details e.g. things to confirm for the next visit.

Testing

Preparation

Before the phone call starts you need to setup for the test. You can run the test either on Matlab, Excel or a hard copy (e.g. paper print out of excel).

1. Matlab:
    a. If using a button box, plug this in
    b. Open matlab
    c. Make sure you are in the '\DOPAMIND \Task Development\DOPAMIND\' folder
    d. Double check the participant ID, test number and version number and letter
    e. Run 'RKNTestIncidental'
    f. Enter the details
    g. Leave it on the instruction screen until the phone call starts
    h. When you begin the test, press the button as instructed on the screen, and the appropriate buttons to record the responses.
2. Excel:
    a. Open Excel
    b. Double check the participant ID, version letter and number
    c. Open the appropriate version of the excel file– i.e. if the test is version B3 then open '\DOPAMIND\Task Development\DOPAMIND\RKNtask\long term test version B.xlsx'
    d. Move to the appropriate sheet in the file – i.e. sheet 'B3'.
    e. You now need to randomise the order of the words
        i. Double click on cell A2
        ii. Press enter
        iii. This will randomise the cells A2:A41
        iv. Highlight the cells A2:C41
        v. Right click on column A
        vi. Click 'Sort'
        vii. Click 'Sort Smallest To Largest'
        viii. This will randomise the order of column C – the words.
    f. Use 'File' -> 'Save As' to save this file as 'RKNTestIncidental_PPID101_SesNo1_VersA1_20183112', replacing the PPID (e.g. 115), Session (1 or 2), Version (e.g. B3), and date in yyyymmdd format (e.g. 4th May 2018 becomes 20180504).
    g. Move the cursor to cell D2 to start.

   h. During the test, put a number 1 in the appropriate boxes for 'yes' or 'no', and then the box for 'remember' etc
   i. **EACH LINE MUST HAVE ONLY 2 BOXES FILLED IN!!!!!!!!!!**
3. Hard-copy:
   a. If you will not have access to a computer, you can print out a copy of the Excel sheet and fill this in by hand. You will need to then copy this data back into excel afterwards.
   b. Follow steps 2a-2f to randomise the word order for the appropriate version, and save the file (this will make it easier to enter the data later).
   c. Expand column B so that you can see the 'word number'
   d. Print cells A1:J42 – **MAKE SURE YOU HAVE THE WHOLE LIST OF WORDS!!!!**
      i. When printing, use 'Print Setup' to include the gridlines on the printout
   e. Make a note of the participant's name and phone number if you may not have access to them later.
   f. When doing the word test, tick a box for 'yes' or 'no' and then one more box for secondary response (e.g. 'remember' or 'guess'). There should be 2 ticks per line.
   g. After testing, you will need to enter the data back into an excel file.
      i. If you saved the version you printed, open that one
         1. Ensure the words are in the same order
         2. Enter the responses, putting a number 1 in the boxes
         3. There should be 2 responses per line
         4. **SAVE THIS FILE**
      ii. If you did not save the version you printed, you will need to put the words in the excel sheet into the same order as it was on the printed copy
         1. You can use 'sort' and the column of numbers on the printed sheet to do this if you know how
         2. Otherwise, enter the number (from Column B 'Number') and the word (Column C 'WORD') into the excel sheet
         3. Enter the responses as a number 1 – there should be 2 responses per line.
         4. Repeat for each word
         5. **SAVE THIS FILE – See naming convention in Step 2f**

Test instructions

These are the instructions to give to the participant at the start of each phone call test.

1. Call the participant
2. Introduce yourself and say you are calling to do the memory test
3. Check that they have time to do the test
   a. If they don't, or if they do not pick up, try to call back at a later point on the same day
4. If they are happy to continue, you need to give them instructions mentioning:
   a. You will read out a list of words, some of which were on the list they made the 'alive/not alive' judgements about, and the others of which they won't have seen at all during the testing.
   b. If they think the word was on the list, they should say 'YES' and then 'REMEMBER', 'FAMILIAR', or 'GUESS'
      i. 'REMEMBER' is if they can recall extra details about the word e.g. what they thought when they saw it, what came before or after etc. You must ask them what it is they can remember about each word they say REMEMBER for. If they cannot say what they can recall, then suggest it is FAMILIAR instead.
      ii. 'FAMILIAR' is if they are sure the word was on the list, but cannot recall any extra details (see above).
      iii. 'GUESS' is if they are not sure.
   c. If the think the word was not on the list, they should say 'NO' and then 'SURE' or 'GUESS'

   d. You can repeat the word as many times as necessary, or spell it out for them, in case they do not hear it properly. But, you **cannot** give them any definitions or use it in a sentence. This is because many of the words have multiple meanings and you do not know which meaning they remembered it as.
      i. You may want to spell out certain words even if they don't ask you to – especially for short words, words with homophones (e.g. STARE/STAIR) or if the phone-line is poor quality. You can also spell them out using the phonetic alphabet if needed (e.g. A for alpha, T for tango).
5. Ask if they have any questions, and begin.

Running the test

1. Read the word
2. Spell or repeat if necessary (see above, 4d)
3. Record their responses in matlab/excel
4. If they change their mind after making a response, this is allowed, so you can just edit this in excel/paper copies. If you are running it on matlab and have already pressed something else, just make a note of the word and what their response should be, and email Hanna about it.
5. Do not try to sway their judgements about words if you happen to know what the correct response should be – always say them in the same tone of voice, and keep any prompts the same for targets and distractors.
6. At the end of the test:
   a. Confirm the time/date of their next phone call or visit (if there is one)
   b. Thank them for their time

Remember-know

Participants learned a list of words and delayed recognition was tested. In the learning block, a white fixation cross was shown for 2000ms and then a word was presented in white font on a black background (Figure 5.3) for 2000ms. The participants were instructed to read the word aloud and try to remember it. There were 48 words (presented in a randomised order), drawn from four semantic categories (e.g. trees, countries, weather phenomenon, body parts); 12 words from each.

To test learning a recognition task was used. Participants saw a word on the screen and had to say if it was 'old' or 'new' via button press (Figure 2). If they responded 'old' then they had to decide whether they 'remembered', 'knew' or 'guessed'. They were instructed to respond 'remember' if they could actually remember seeing the word on the screen, or reading it aloud or what they thought when they saw it. They responded 'know' if they did not have an actual memory of the word but it seemed familiar, and 'guess' if they purely guessed. If they had responded 'new' they would have to decide 'sure' or 'guess'.

There were 48 words in the test, 24 of which were targets from the learning block and 24 of which were new distractors (see Appendix Table A.3 for the words used in each version). Of the 24 new words, 12 were from the same 4 categories as the learning words (e.g. trees, countries, weather phenomenon, and body parts), 3 from each, and the other 12 were from unrelated categories.

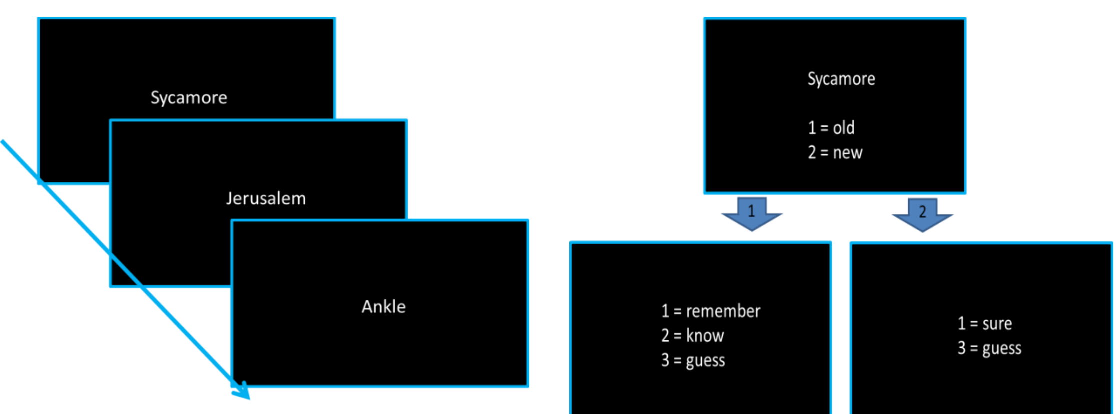


FIGURE 2 DIAGRAM OF THE REMEMBER-KNOW TEST. THE LEARNING TRIALS ARE SHOWN ON THE LEFT, AND THE TEST TRIALS ON THE RIGHT.

The target words and semantically related distractors were drawn from two word lists (Murdock, 1976; Van Overschelde, Rawson, & Dunlosky, 2004), and the unrelated distractors from the Toronto Noun Word Pool (Friendly, Franklin, Hoffman, & Rubin, 1982). The unrelated distractors were chosen so that none fit any of the semantic categories used in the study. The frequencies of the words were compared with a univariate ANOVA (for 36/192 target words and 23/96 related distractor words the frequencies could not be found, this was mainly for cities/countries, animals, boats and kitchen utensils). The difference between frequencies of targets and distractors approached significance ($F(1, 323) = 3.601$, $p = .059$) with targets having lower frequencies. When the semantically related and unrelated distractors were grouped separately, there was a significant (Bonferroni corrected) difference between related and unrelated distractors ($p = .004$), unrelated distractors and targets ($p = .003$) but no difference between related distractors and targets ($p = 1$). This means that the targets and related distractors were equally as frequent words, but that the unrelated distractors were more frequent words than the other two groups. As word frequency affects recognition memory (Brébion,

David, Bressan, & Pilowsky, 2005; Park, Reder, & Dickison, 2005), this is not ideal, and the effects of this will be addressed in the discussion.

Four versions of the test were created with new words in each (no words occurred in more than one version). Participants completed the learning block, and two test blocks, one after 30 minutes and one after 24 hours. The second test had the other 24 words that did not appear on the 30 minute test, and all new distractor words.

Learning

I say:

You're going to see a list of words on the screen. For each word, you will have to read it out loud and memorise the word, and then we'll test your memory for the words in about 30 minutes and again tomorrow.

Testing

I say:

Now we're going to test you on the words you learned earlier. You'll see a word and have to decide if it was on the list you read out loud earlier (i.e. it's an OLD word), or if it wasn't on the list (i.e. it's NEW).

If you decide it's OLD, you'll have to say whether you REMEMBER it, KNOW it or GUESS. The difference between remembering and knowing is whether you can recall anything about it, or if you just have a sense of familiarity about it. For example, if you see someone on the street and you can recall their name, or where you last met, that would be a REMEMBER response. However, if you recognised them, but couldn't recall their name, or where you knew them from or where you last met, that would be a KNOW response. And if it was a pure guess, then press GUESS.

And if you pressed NEW, you'll be asked if you were SURE or GUESSed.

Database searched: MRC psycholinguistic database
Accessed September, 2016

Words searched by:

Concreteness (range 100-700, mean 438) = 400 - 700
Comprehensive syntactic category = NOUN
Word length = 4-8 letters

480 randomly selected from 1985
Randomised using random number generator, and split in to 6 groups:
3 x sets of distractor words (100 each)
3 x sets of test words (80 each)

Variables analysed:

Frequency (KFFRQ) - measure of frequency in language
Concreteness (CNC)
Imageability (IMG)
Word length (NLET)
Inanimate/animate word (Animate)

Results in -t-tests.txt files for each variable

Inanimate = 1, animate = 0. No significant difference in proportion inanimate between groups.

Version 1 targets

Word	CNC	IMG	KFFRQ	NLET	CSYN	RN
FAMILY	525	577	331	6	N	0.7776905
STAR	574	623	25	4	N	0.4195123
COMPANY	424	426	290	7	N	0.6953901
SWEEP	476	513	15	5	N	0.78569521
INCENSE	499	555	2	7	N	0.95797438
NURSE	588	617	17	5	N	0.41077601
EDGE	465	495	78	4	N	0.60144248
ICICLE	569	526	1	6	N	0.43386703
LINKS	454	454	7	5	N	0.65575717
MOUNTAIN	616	629	33	8	N	0.62344649
SELLER	444	427	6	6	N	0.68561063
TWEEZERS	590	619	-	8	N	0.62037132
MOVIE	590	571	29	5	N	0.3753811
DUCK	606	632	9	4	N	0.65798276
ULCER	558	516	5	5	N	0.33834753
RIBBON	600	563	12	6	N	0.87726555
TICKET	590	574	16	6	N	0.12665878
BROTHER	585	589	73	7	N	0.4760468
MARCH	440	497	120	5	N	0.10823534
LIMB	590	580	5	4	N	0.59536936
LETTER	577	595	145	6	N	0.28749628
FOIL	509	495	20	4	N	0.83328444
JUMP	449	506	24	4	N	0.43472038
DYNASTY	406	386	5	7	N	0.04465784
MANURE	644	534	6	6	N	0.30087794
CAGE	593	585	9	4	N	0.66855998
CRYSTAL	587	579	23	7	N	0.69433876
SOPRANO	497	535	6	7	N	0.32827687
WATCH	487	525	81	5	N	0.94160774
TIMBER	578	553	19	6	N	0.25912384
ALGAE	545	424	7	5	N	0.58361547
BOOTH	556	486	7	5	N	0.33495688
TORNADO	644	591	1	7	N	0.03608038
DRUG	555	564	24	4	N	0.57688664
IODINE	576	508	18	6	N	0.02643328
CARAVAN	539	562	8	7	N	0.86206998
HERO	428	483	52	4	N	0.28137359
SETTLER	533	528	3	7	N	0.63816102
VODKA	576	613	-	5	N	0.91974612
BOURBON	570	606	8	7	N	0.55622143
NAIL	598	588	6	4	N	0.37992311
HEEL	579	597	9	4	N	0.6015143
TEST	520	528	119	4	N	0.42087697
SALARY	456	452	43	6	N	0.70321106
WEAPON	560	546	42	6	N	0.54826699
KETTLE	602	594	3	6	N	0.05761774
WIND	552	535	63	4	N	0.78563931
SACK	582	548	8	4	N	0.98262205
HARNESS	563	513	10	7	N	0.39289319
HAWK	623	591	14	4	N	0.56973325
HORN	618	566	31	4	N	0.99187891
DRESS	595	595	67	5	N	0.93575812

ANIMAL	587	575	68	6	N	0.75065276
SILK	538	510	12	4	N	0.60255075
OVEN	593	599	7	4	N	0.00027783
ASPIRIN	574	542	3	7	N	0.0720831
POPE	593	576	40	4	N	0.21815235
COLUMN	520	491	71	6	N	0.53625956
MUCUS	565	570	2	5	N	0.94203231
NERVE	488	486	12	5	N	0.95056153
PLANK	592	598	7	5	N	0.84701924
GASKET	525	487	4	6	N	0.78359895
MAPLE	534	511	7	5	N	0.39921713
GRAVE	535	619	33	5	N	0.53202194
PRIZE	474	517	28	5	N	0.46544407
OPIUM	552	487	16	5	N	0.62664598
VESSEL	571	525	16	6	N	0.6528884
SPEECH	453	447	61	6	N	0.59873327
BLADE	584	568	13	5	N	0.53664393
TEETH	618	611	103	5	N	0.01444932
TUNNEL	555	578	10	6	N	0.64439578
WORLD	532	560	787	5	N	0.59798935
NEST	557	571	20	4	N	0.16795943
SWELL	411	410	7	5	N	0.4296664
GRAVEL	587	569	9	6	N	0.48686868
TASK	409	410	60	4	N	0.53359044
BUSH	585	549	14	4	N	0.707428
PROPERTY	460	466	156	8	N	0.76470776
PAPER	599	590	157	5	N	0.3802828
CENT	544	518	158	4	N	0.09027234

Version 1 distractors

GLACIER	STATUE	EARTH	SLEET
TOMATO	RICE	STRAND	NICKEL
ABDOMEN	SQUEAK	BANNER	PIGEON
CRUCIFIX	CAVE	RACKET	LAKE
HOBBY	SHOE	BACTERIA	LORD
CAVERN	VINEGAR	MAIDEN	NATION
MOUSE	QUAKE	HYMN	SHIVER
MEDICINE	PODIUM	FORT	VEHICLE
LIFT	STOVE	BEAN	ARTIST
WALNUT	JAIL	HOUND	GADFLY
CEREMONY	WAVE	ARROW	CHERRY
SHOVEL	INDIAN	AXLE	BATTLE
BUFFER	STOOL	CLOVER	FROCK
LOOT	WIGWAM	CHILD	RHAPSODY
CHEEK	MOTHER	FLUTE	MIRROR
VEIL	BLISTER	PHONE	FACTORY
LOTION	LEAD	RADIO	CANNON
MULE	CANOE	DOCTOR	OYSTER
STEEPLE	GARBAGE	BIRD	MANKIND
WORK	TOASTER	RAKE	CAMERA
WIRE	WICKET	MILK	IGNITION
COTTON	BEARD	UNIFORM	BIBLE
ONION	FUEL	BANANA	STORM
FLOCK	VIOLET	VIOLIN	BRANCH
WHIP	PARCEL	SCENE	BARON

Version B targets

Word	CNC	IMG	KFFRQ	NLET	CSYN	RN
CITIZEN	455	445	30	7	N	0.62125315
IVORY	571	529	17	5	N	0.42768221
PALETTE	565	437	5	7	N	0.69584421
DISEASE	504	487	53	7	N	0.87741572
CHINA	578	597	69	5	N	0.72552419
CHOIR	567	567	8	5	N	0.04023133
NOVEL	529	547	59	5	N	0.15084077
ARCHERY	470	550	1	7	N	0.88962047
SPECIMEN	481	417	24	8	N	0.77231011
BENCH	614	555	35	5	N	0.49887357
ESTATE	541	474	51	6	N	0.41236174
TEAR	504	550	11	4	N	0.99871589
BORDER	444	453	20	6	N	0.60049009
HARD	425	460	202	4	N	0.26490238
HOTEL	591	597	126	5	N	0.28712155
POLLEN	584	526	11	6	N	0.37446559
CARRIAGE	576	529	11	8	N	0.55233116
COSTUME	544	538	10	7	N	0.63879656
MORNING	515	579	211	7	N	0.84767042
STICK	604	517	39	5	N	0.51170625
SWARM	406	488	3	5	N	0.76444367
WRITE	446	548	106	5	N	0.37961763
CANDY	602	601	16	5	N	0.98908211
HUSBAND	549	537	131	7	N	0.34642469
SPECK	484	503	7	5	N	0.53944705
DUCHESS	568	525	1	7	N	0.41255198
INVADER	485	419	1	7	N	0.73769252
MOON	581	585	60	4	N	0.89344872
FOUNTAIN	593	602	18	8	N	0.2462212
PYRAMID	615	613	2	7	N	0.71154758
WEDDING	509	594	32	7	N	0.90809052
CHALK	634	601	3	5	N	0.25658903
SULTAN	563	541	3	6	N	0.70323256
WALLET	584	617	6	6	N	0.03636808
FAIRY	433	536	4	5	N	0.33214816
COLLAR	622	582	17	6	N	0.86161709
EARL	500	435	12	4	N	0.6500271
WOOL	608	586	10	4	N	0.82810093
BERET	578	517	-	5	N	0.77259066
CARROT	622	577	1	6	N	0.04721404
BRASS	577	524	19	5	N	0.4072141
DOLLAR	575	611	46	6	N	0.42375795
PYTHON	580	559	14	6	N	0.65373504
PEAR	634	590	6	4	N	0.68807519
AUTHOR	502	460	46	6	N	0.0660602
GENTRY	452	462	1	6	N	0.45009962
DAMSEL	544	551	1	6	N	0.77998607
RIDGE	547	543	18	5	N	0.34520657
AVENUE	539	564	46	6	N	0.50076406
RHYME	434	475	3	5	N	0.56586328
LIME	590	563	13	4	N	0.20049344
ROBIN	637	615	2	5	N	0.62496588

STOCKING	551	555	1	8	N	0.18233298
CLAM	564	541	3	4	N	0.78801261
BAYONET	600	548	6	7	N	0.36210065
SCORPION	590	596	-	8	N	0.52943374
HIGHWAY	575	581	40	7	N	0.60985419
FILM	604	562	96	4	N	0.53358287
SOAP	598	600	22	4	N	0.0114836
RECTOR	517	494	33	6	N	0.64511765
WALK	452	505	100	4	N	0.84730126
NEWS	437	484	102	4	N	0.59609322
TOILET	586	603	13	6	N	0.02400484
REFLEX	402	476	4	6	N	0.9136434
TROUT	617	617	4	5	N	0.07823542
DUST	550	549	70	4	N	0.47598977
COOKIE	634	600	1	6	N	0.22901282
MENU	555	613	5	4	N	0.16734743
TRAIL	511	525	31	5	N	0.79104466
MUSCLE	573	553	42	6	N	0.33742786
CAROL	535	499	2	5	N	0.35723784
WALRUS	629	590	1	6	N	0.73824496
OXYGEN	484	430	43	6	N	0.22004256
PLUG	558	583	23	4	N	0.94275856
PORK	585	522	10	4	N	0.39307345
CLOSET	599	525	16	6	N	0.95104557
FOAM	577	600	37	4	N	0.60311278
MILEAGE	421	460	15	7	N	0.71042793
CANAL	598	588	3	5	N	0.94400366
CHLORINE	591	480	33	8	N	0.42585371

Version B distractors

SHAWL	SUNSHINE	WILLOW	LITRE
STADIUM	SPINACH	SMILE	PILL
POSTER	FLEA	SELF	EAGLE
PROFILE	BUBBLE	BEGGAR	FOREST
MARBLE	SPRING	CATTLE	CHURCH
OFFICE	STRIPE	LIAR	TENT
SCHOLAR	TRIPOD	GERM	TROUPE
LIGHTER	HATCHET	TEACHER	CHEESE
LEADER	NEPHEW	TEMPLE	CORN
QUARTER	COIL	VOTER	SOUP
GUARD	PLANET	SCOUT	DUMMY
SOIL	MIXER	PENNY	FACE
NATURE	BASKET	SERVANT	GINGHAM
SNAKE	BLOOM	VISITOR	CIDER
COFFIN	BEACH	HALL	PIER
NECKLACE	CURLER	SKATE	FLOAT
COURT	SWORD	MEAT	DRAIN
MINNOW	DIRT	VAULT	COMPOSER
SCARLET	BRICK	YAWN	HUMAN
FOREHEAD	MAROON	DECK	SATIN
BODY	SEDATIVE	VALLEY	GONDOLA
CRUMB	INCOME	CIRCLE	PESTLE
CONTRACT	WOMB	BOMB	COFFEE
CATFISH	MACARONI	MURAL	SILVER
STEM	LARD	MALARIA	MAGNET

APPENDIX E: II WORD LISTS FOR DARET

Words used in the retrieval phase of the DARet study.

Remember/Know Task version 1.

Target words		Distractor words	
		Related words	Unrelated words
ankle	angelfish	knee	vapor
hip	eel	lung	witness
foot	carp	hand	herald
tooth	trout	ear	traffic
wrist	mackerel	rib	kitten
lip	blowfish	eye	echo
chest	catfish	fir	feather
thigh	shark	cypress	basket
toe	swordfish	birch	butter
head	sturgeon	maple	armour
liver	marlin	spruce	ribbon
arm	goldfish	redwood	blessing
oak	silk	tuna	navy
evergreen	mohair	salmon	Acid
sycamore	velvet	minnow	Water
beech	polyester	cod	Station
cedar	fleece	halibut	Event
balsam	flannel	sardine	Minute
elm	satin	corduroy	Product
willow	suede	linen	Button
walnut	leather	tweed	Helmet
hickory	cashmere	cotton	Moment
teak	wool	spandex	Candle
aspen	lycra	denim	Tiger

Remember/Know Task version 2.

Target words		Distractor words	
		Related words	Unrelated words
ladle	blizzard	rollingpin	Empire
grater	tornado	spatula	District
knife	mist	sieve	Refuge
cup	thunder	blender	Letter
fork	storm	tongs	Journal
dish	sleet	toaster	Captive
spoon	sunshine	cantaloupe	puzzle
bowl	hail	papaya	wagon
stove	wind	honeydew	motor
kettle	rain	cherry	resort
pan	flood	raisin	arrow
colander	lightning	mango	habit
tangerine	afganistan	drizzle	treasure
grape	cuba	clouds	fever
prune	switzerland	snow	spirit
pear	norway	fog	pillow
strawberry	finland	typhoon	dinner
apple	egypt	frost	career
grapefruit	iraq	mexico	gesture
banana	brazil	venezuela	banner
orange	ethiopia	peru	pitcher
plum	australia	canada	acre
watermelon	iran	sweden	college
raspberry	india	portugal	project

Remember/Know Task version 3.

Target words		Distractor words	
		Related words	Unrelated words
california	hall	alabama	sandwich
florida	cellar	tennessee	ticket
texas	ceiling	mississippi	monkey
new jersey	kitchen	kansas	market
arizona	stairway	massachusetts	agent
maine	livingroom	kentucky	errand
alaska	floor	bay	barrel
ohio	office	ocean	tunnel
colorado	room	glacier	total
nevada	chimney	volcano	column
hawaii	closet	cave	prison
michigan	wall	mountain	pontoon
desert	sailboat	window	rattle
lake	cruiseship	hearth	record
river	Yacht	corridor	canvas
stream	speedboat	bedroom	fortune
gully	Canoe	cupboard	heaven
ravine	Rowboat	bathroom	soldier
canyon	Motorboat	raft	level
geyser	Tugboat	barge	device
crater	Submarine	dinghy	blanket
valley	paddle boat	boundary	bubble
tundra	battleship	titanic	contest
waterfall	kayak	catamaran	novel

Remember/Know Task version 4.

Target words		Distractor words	
		Related words	Unrelated words
honda	los angeles	corvette	circle
toyota	chicago	cadillac	fountain
bmw	rome	chrysler	machine
chevrolet	mumbai	nissan	penny
jeep	montreal	mitsubishi	angle
porsche	tokyo	subaru	survey
lexus	moscow	chlorine	model
volkswagen	berlin	calcium	patent
ferrari	stockholm	lithium	credit
audi	jerusalem	copper	cannon
mazda	cairo	neon	marble
volvo	vancouver	aluminium	concert
oxygen	fly	copenhagen	powder
hydrogen	ant	brussels	anchor
carbon	spider	paris	captain
helium	bee	madrid	orchard
nitrogen	mosquito	naples	poem
gold	beetle	venice	temple
iron	ladybird	firefly	couple
silver	grasshopper	dragonfly	carriage
sodium	butterfly	hornet	chapter
potassium	wasp	caterpillar	needle
sulfur	moth	centipede	picture
zinc	cockroach	flea	contract

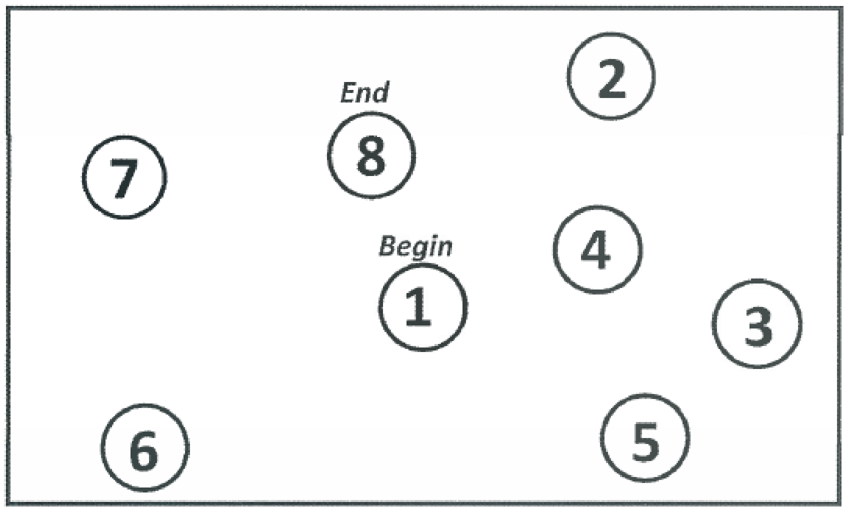
ankle	ladle	california	honda	hip	grater	jersey	toyota
foot	knife	texas	bmw	tooth	cup	maine	chevrolet/chevy
wrist	fork	arizona	jeep	lip	dish	ohio	porsche
chest	spoon	alaska	lexus	thigh	bowl	nevada	volkswagen
toe	stove	colorado	ferrari	head	kettle	michigan	audi
liver	pan	hawaii	mazda	arm	colander	lake	volvo
oak	tangerine	desert	oxygen	evergreen	grape	stream	hydrogen
sycamore	prune	river	carbon	beech	pear	ravine	helium
cedar	strawberry	gully	nitrogen	balsam	apple	geyser	gold
elm	grapefruit	canyon	iron	willow	banana	valley	silver
walnut	orange	crater	sodium	hickory	plum	waterfall	potassium
teak	watermelon	tundra	sulfur	aspen	raspberry	cellar	zinc
angelfish	blizzard	hall	los angeles	eel	tornado	kitchen	chicago
carp	mist	ceiling	rome	trout	thunder	livingroom	mumbai
mackerel	storm	stairway	montreal	blowfish	sleet	o?ce	tokyo
catfish	sunshine	floor	moscow	shark	hail	chimney	berlin
swordfish	wind	room	stockholm	sturgeon	rain	wall	jerusalem
marlin	flood	closet	cairo	goldfish	lightning	cruiseship	vancouver
silk	afghanistan	sailboat	fly	mohair	cuba	speedboat	ant
velvet	switzerland	yacht	spider	polyester	norway	rowboat	bee
fleece	finland	canoe	mosquito	flannel	egypt	tugboat	beetle
satin	iraq	motorboat	ladybird	suede	brazil	paddle	grasshopper
leather	ethiopia	submarine	butterfly	cashmere	australia	boat	wasp
wool	iran	battleship	moth	lycra	india	kayak	cockroach
knee	rollingpin	alabama	nissan	ear	blender	kansas	corvette
lung	spatula	tennessee	mitsubishi	rib	tongs	massachusetts	cadillac
hand	sieve	mississippi	subaru	eye	toaster	kentucky	chrysler
fir	cantaloupe	bay	copper	maple	cherry	volcano	chlorine
cypress	papaya	ocean	neon	spruce	raisin	cave	calcium
birch	honeydew	glacier	aluminium	redwood	mango	mountain	lithium
minnow	drizzle	window	madrid	sardine	fog	bedroom	copenhagen
tuna	clouds	hearth	naples	cod	typhoon	cupboard	brussels
salmon	snow	corridor	venice	halibut	frost	bathroom	paris
corduroy	mexico	raft	caterpillar	cotton	canada	titanic	firefly
linen	venezuela	barge	centipede	spandex	sweden	catamaran	dragonfly
tweed	peru	dinghy	flea	denim	portugal	pontoon	hornet
vapor	empire	boundary	powder	navy	refuge	rattle	circle
witness	district	sandwich	anchor	acid	journal	record	fountain
herald	letter	ticket	captain	water	spirit	canvas	machine
traffic	captive	monkey	orchard	station	pillow	fortune	penny
kitten	puzzle	market	poem	event	dinner	heaven	angle
echo	wagon	agent	temple	minute	career	soldier	survey
feather	motor	errand	couple	product	gesture	level	model
basket	resort	barrel	carriage	button	banner	device	patent
butter	arrow	tunnel	chapter	helmet	pitcher	blanket	credit
armour	habit	total	needle	moment	acre	bubble	cannon
ribbon	treasure	column	picture	candle	college	contest	marble
blessing	fever	prison	contract	tiger	project	novel	concert

PPID: ______________________ VISIT #:______ DATE:______________ CIRCLE: AM / PM

TRAIL MAKING

Part A

SAMPLE



15 17 21

20 19

16 18

4 22

5

13 6

Begin

7 1 24

14

2

8 10

3

9

End

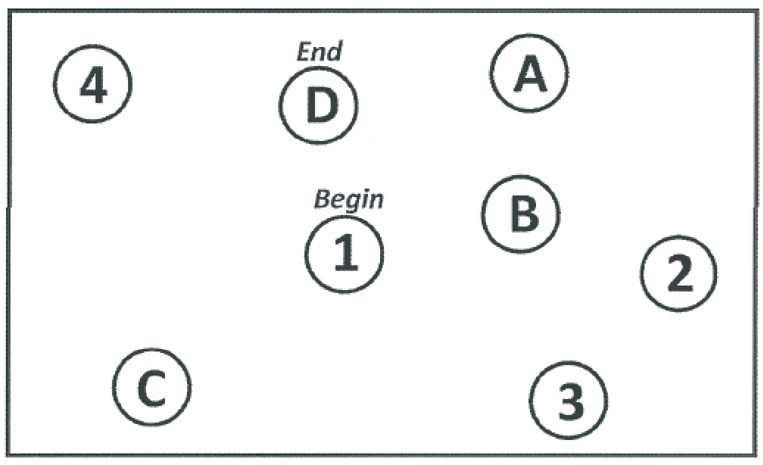
11 25

12 23

PPID: ______________________________ VISIT #:______ DATE:______________ CIRCLE: AM / PM

TRAIL MAKING TEST B

SAMPLE B



End
13 10
8 9 I D
4
B
3
Begin
7 1
5
H
C
12
G
A J
2 6
L
E
F
K 11

St. Mary's Hospital Sleep Questionnaire

This questionnaire refers to your sleep over the past 24 hours. Please try and answer every question.

PPID: ______________________ VISIT #:_____ DATE:__________ CIRCLE: AM / PM

At what time did you;

1) settle down for the night? ....

2) fall asleep last night? ....

3) finally wake this morning? ....

4) get up this morning? ....

5) Was your sleep (tick box)

- 1 very light ☐
- 2 light ☐
- 3 fairly light ☐
- 4 light average ☐
- 5 deep average ☐
- 6 fairly deep ☐
- 7 deep ☐
- 8 very deep ☐

6) How many times did you wake up? (tick box)

- 0 not at all ☐
- 1 once ☐
- 2 twice ☐
- 3 three times ☐
- 4 four times ☐
- 5 five times ☐
- 6 six times ☐
- 7 more than six times ☐

How much sleep did you have

7) last night? ....hrs.........mins

8) during the day, yesterday? ....hrs.........mins

9) How well did you sleep last night? (tick box)

- 1 very badly ☐
- 2 badly ☐
- 3 fairly badly ☐
- 4 fairly well ☐
- 5 well ☐
- 6 very well ☐

If not well what was the trouble (eg restless, etc)?

1____

2____

10) How clear-headed did you feel after getting up this morning? (tick box)

- 1 still very drowsy indeed ☐
- 2 still moderately drowsy ☐
- 3 still slightly drowsy ☐
- 4 fairly clear-headed ☐
- 5 alert ☐
- 6 very alert ☐

11) How satisfied were you with last night's sleep? (tick box)

- 1 very unsatisfied ☐
- 2 moderately unsatisfied ☐
- 3 slightly unsatisfied ☐
- 4 fairly satisfied ☐
- 5 completely satisfied ☐

12) Were you troubled by waking early and being unable to get off to sleep again? (tick box)

NO ☐ YES ☐

13) How much difficulty did you have in getting off to sleep last night? (tick box)

- 1 none or very little ☐
- 2 some ☐
- 3 a lot ☐
- 4 extreme difficulty ☐

14) How long did it take you to fall asleep last night? .... hrs............. mins

APPENDIX H:

PPID: ______________________________ VISIT #:_____ DATE:_____________ CIRCLE: AM / PM

Participant Identification Number:
Name of Researcher:

PANAS Questionnaire

Title of Project: **TARGETING DOPAMINE TO TREAT IMPAIRED MEMORY CONSOLIDATION IN NEURODEGENERATIVE DISEASE**

To be filled in the evening and in the morning after recall

....

This scale consists of a number of words that describe different feelings and emotions. Reach each item and then list the number from the scale below next to each word. Indicate to what extent you feel this way **right now**, that is, at the present moment.

Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
1	2	3	4	5

To be filled by the volunteer:

1	________	Interested	11	________	Irritable
2	________	Distressed	12	________	Alert
3	________	Excited	13	________	Ashamed
4	________	Upset	14	________	Inspired
5	________	Strong	15	________	Nervous
6	________	Guilty	16	________	Determined
7	________	Scared	17	________	Attentive
8	________	Hostile	18	________	Jittery
9	________	Enthusiastic	19	________	Active
10	________	Proud	20	________	Afraid

To be filled by the investigator:

Added score for items 1, 3, 5, 9, 10, 12, 14, 16, 17, and 19: ________________

Added score for items 2, 4, 6, 7, 8, 11, 13, 15, 18, and 20: ________________

APPENDIX I:

PPID: ______________________________ VISIT #:______ DATE:______________ CIRCLE: AM / PM

TORONTO HOSPITAL ALERTNESS TEST

This questionnaire tries to establish how alert you feel. In reporting your feeling, we would like you to consider your <u>last week</u>. Using the following scale, please choose one response for each question.

During the last week I felt:	Not at all	Less than ¼ of the time	¼ to ½ of the time	½ to ¾ of the time	More than ¾ of the time	All the time I was awake
1. Able to concentrate						
2. Alert						
3. Fresh						
4. Energetic						
5. Able to think of new ideas						
6. Vision was clear noting all details (e.g., driving)						
7. Able to focus on the task at hand						
8. Mental facilities were operating at peak level						
9. Extra effort was needed to maintain alertness						
10. In a boring situation, I would find my mind wandering						

Leeds Sleep Evaluation Questionnaire

Please dissect the line where appropriate.
A line dissected in the middle indicates no change to normal.

How would you describe the way you currently fall asleep in comparison to usual?

More difficult than usual	______________________________	Easier than usual
Slower than usual	______________________________	More quickly than usual
I feel less sleepy than usual	______________________________	I feel more sleepy than usual

How would you describe the quality of your sleep compared to usual?

More restless than usual	______________________________	Calmer than usual
With more wakeful periods than usual	______________________________	With less wakeful periods than usual

How would you describe your awakening in comparison to usual?

More difficult than usual	______________________________	Easier than usual
Requires a period of time longer than usual	______________________________	Shorter than usual

How did you feel when you woke up?

More tired than usual	______________________________	More alert

How do you feel now?

More tired than usual	______________________________	More alert

How would you describe your balance and coordination upon awakening?

More disrupted than usual	______________________________	Less disrupted than usual

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Email: elizabeth.coulthard@bristol.ac.uk
www.bristol.ac.uk

Version 3

07.07.2017

Visit Number: Date:

Participant Identification Number:

BLINDING VERIFICATION FORM:

To be filled at the end of each visit by volunteer and all experimenters

Title of Project: **THE DOPAMIND STUDY**

Name of Researcher:

Which study condition would you guess you completed during this visit? Please circle the appropriate option.

1. Drug

2. Placebo

RēMemBr Group

1 of 1

School of Clinical Sciences

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Email: elizabeth.coulthard@bristol.ac.uk
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Version 3

07.07.2017

Visit Number:

Participant Identification Number:

BLINDING VERIFICATION FORM:

To be filled at the end of final visit by volunteer and all experimenters

Title of Project: **THE DOPAMIND STUDY**

Name of Researcher:

Which study condition would you guess you completed on each visit?

Please circle the appropriate option.

1st visit

- Placebo
- Drug

2nd visit

- Placebo
- Drug

RMemBr Group

1 of 1

DARet1 Testing SOP

All files stored on:

N:\Doctors Folder\Neurology\BRACE neurology\Research Projects\Dopamine & Retrieval Site File

The explanation of the task and sample words to say to the participants to explain it are in the file 'Task Explanations.docx'.

Testing

Computer setup – same for all sessions

1. Turn on & log in (socs-stop and st87df_### for Dell laptops)
2. Open matlab, and make sure 'current folder' is 'C:\Users\socs-stop\Documents\DARet1\Data\X' where X is the ppID for this participat

Day 1 session

1. Answer any questions the patient has
2. Ask them to sign the consent form
3. Go through the screening form
4. Give them the patient information card
5. Fill in the front of the pro-forma (incl medications + dosages)
    a. The versions should already be filled out for the later pages

Begin Testing

GainLoss

1. Give participant instructions about the task
2. Say 'you're going to start with the Practice trials' and give them the instructions
3. Run GLPratice(ppID,sesNo, versNo) with the relevant numbers
4. Ask if they have any questions.
    a. Don't talk through trials, but do keep an eye on them to make sure they understand how to do the task
5. Now run GLLearn(ppID,sesNo,versNo)

6. Novel Pairs Test
    a. Give instructions
    b. Run GLTestA(ppID,sesNo,versNo)

Remember Know

7. Give learning instructions
8. Run RKNLearn(ppID,sesNo,versNo)

30 min delay – paper tests

9. Now complete paper tests (record versions & times on pro-forma)
    a. MoCA
    b. Digit span
    c. LARS
    d. BIS

GainLoss delay

10. Now say 'we're going to test you again on the symbols task. Give instructions
11. Run GLTestB(ppID,sesNo,versNo)

Remember-know delay

12. Give instructions
13. Run RKNTestA or B with (ppID,sesNo,versNo)

Sleep questionnaire

14. Give SMHSQ

Admin

15. Confirm time & travel arrangements for tomorrow
16. Get details for taxi if booking is needed

####################

Day 2 session

You need to have someone not involved in the testing for this participant perform the drug/placebo preparation. The instructions are in 'Drug Preparation SOPV2.docx'.

Begin Testing

13. Sign consent form
14. Go through continuance criteria

Drug/placebo administration 1

15. Go to day ward and measure blood pressure and heart rate (t0)
16. Administer domperidone/placebo (purple drink)
17. 30 minute delay
18. Can't be unattended, but can be left in day ward if other staff members are around. Check on them occasionally

Drug/placebo administration 2

19. After 30 minutes take BP and HR again (t30 – 30 minutes after baseline)
20. Administer madopar/placebo (orange drink)
21. Can't be unattended, but can be left in day ward if other staff members are around. Check on them occasionally
22. Wait 30 minutes and check BP and HR again (t60)
23. Wait another 30 minutes and check BP and HR again (t90)
24. Begin testing session

Testing session

GainLoss

25. Give instructions
26. In Matlab, run GLTestC(ppID,sesNo,versNo)

Remember-know

27. Give instructions
28. Run RKNTestB or A (ppID,sesNo,versNo)

Paper assessments

29. Now complete paper tests (record versions & times on pro-forma)
    a. Digit span
    b. SMHSQ
    c. Any tests not done on day 1 (these can also be given during medication delays if needed)

Long-term remember-know learning

30. This is for the long-term RKN tests which will be done over the phone
31. Confirm they are OK to do the phone calls and set times for them (record on sheet + calendar)
32. Give instructions (same as before)
33. Run LongTermRKNLearnA or B with ppID and sesNo (no versNo)
34. Now give the testing instructions
35. Run LongTermRKNTestA or B with ppID, sesNo and versNo

Admin

Make sure the phone calls are booked in

36. Check how they are feeling
37. Confirm the date for the next sessions
38. If returning by taxi, see them into the taxi and pay for it

Phone calls

1. 1 day (24 hours) after day 2 LongTermRKNLearn
    a. Call them
    b. Ask how they were after yesterday's session
    c. Open up the 'long term test version A.xlsx' or version B, as appropriate
    d. Give instructions
    e. Read each word loudly and clearly
    f. Record their response
        i. You need a response for OLD/NEW and then REMEMBER/KNOW/GUESS or SURE/GUESS
    g. Confirm the time of the next call
    h. Thank them

2. 3 days (72 hours) after day 2 LongTermRKNLearn
    a. Same as previous call (except for b.)
3. 5 days (120 hours) after day 2 LongTermRKNLearn
4.

Days 3 and 4 + phone calls

Same as days 1+2+phone calls session, except different versions of task and digit span given.

Digit span and SMHSQ are done each day, but all other paper assessments are done just once.

To transfer the data to the hard drive

1. Plug the hard drive in to the computer
2. Copy all the .txt, and .mat files created for this participant in the working folder you selected back at the beginning
3. Copy these to the N drive folder
   N:\Doctors Folder\Neurology\BRACE neurology\Research Projects\Dopamine & Retrieval Site File\Data\
   and to the hard drive
4. If there isn't already a folder for that participant, copy the 'PPID' folder and rename it
5. SAFELY EJECT THE HARD DRIVE!!!!!!!!!!!!

Normality tests

All behavioural measures (tagged, non-tagged and tagging effect for D' across L-DOPA and placebo) were normally distributed. For stage 2, spindle density on placebo and frequency change between L-DOPA and placebo were the only measure where kurtosis was >2, other measures (duration and frequency) were normally distributed. For slow wave sleep spindles all characteristics except spindle amplitude and density change were normally distributed.

Pearson's correlations between slow wave and stage 2 spindles against memory performance on L-DOPA and on placebo revealed no further associations between spindles and memory.

L-DOPA spindle amplitude against memory

		Stage 2	Slow wave sleep
Non-tagged	Pearson r	-0.037	-0.059
	p-value	0.844	0.753
Tagged	Pearson r	-0.004	-0.026
	p-value	0.984	0.891
Tagging effect	Pearson r	0.029	0.023
	p-value	0.878	0.903

Placebo spindle amplitude against memory

		Stage 2	Slow wave sleep
Non-tagged	Pearson r	0.281	0.283
	p-value	0.125	0.123
Tagged	Pearson r	0.243	0.224
	p-value	0.188	0.226
Tagging effect	Pearson r	0.004	-0.024
	p-value	0.984	0.899

L-DOPA induced changes in spindle characteristics against memory

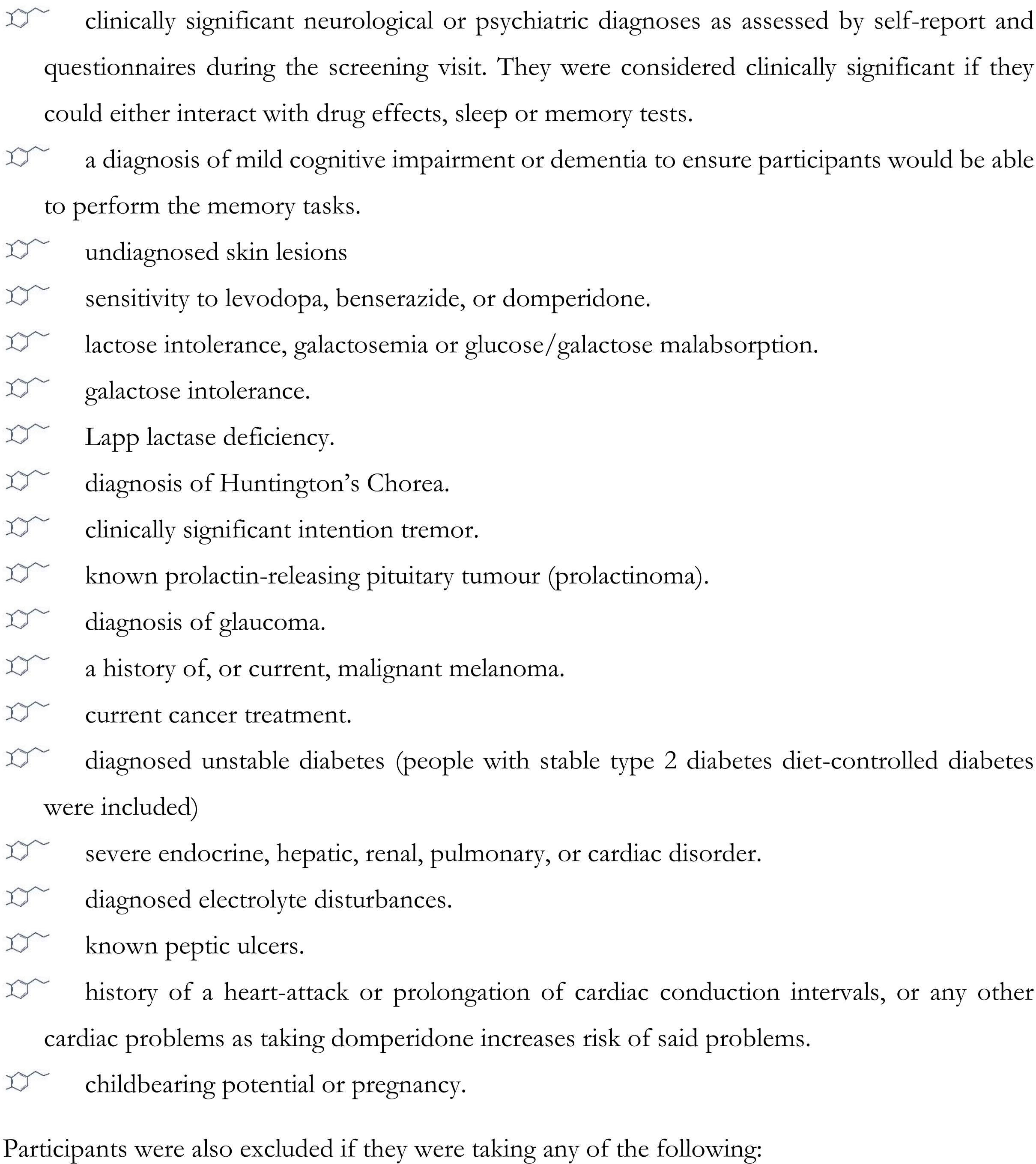
No effects.

Stage 2		Tagging effect	Non-tagged	Tagged
Density	Pearson	0.085	-0.215	-0.072
	p-value	0.648	0.245	0.700
Duration	Pearson	-0.302	0.351	-0.063
	p-value	0.099	0.053	0.735
Frequency	Spearman's rho	-0.010	-0.050	-0.167
	p-value	0.957	0.791	0.370

Slow wave sleep		Tagging effect	Non-tagged	Tagged
Density	Spearman's rho	-0.088	0.147	0.052
	p-value	0.635	0.430	0.779
Duration	Pearson	-0.245	0.224	-0.098
	p-value	0.185	0.226	0.601
Frequency	Pearson	-0.145	0.166	-0.032
	p-value	0.437	0.373	0.863

Exclusion criteria

Participants did not have:



- clinically significant neurological or psychiatric diagnoses as assessed by self-report and questionnaires during the screening visit. They were considered clinically significant if they could either interact with drug effects, sleep or memory tests.
- a diagnosis of mild cognitive impairment or dementia to ensure participants would be able to perform the memory tasks.
- undiagnosed skin lesions
- sensitivity to levodopa, benserazide, or domperidone.
- lactose intolerance, galactosemia or glucose/galactose malabsorption.
- galactose intolerance.
- Lapp lactase deficiency.
- diagnosis of Huntington's Chorea.
- clinically significant intention tremor.
- known prolactin-releasing pituitary tumour (prolactinoma).
- diagnosis of glaucoma.
- a history of, or current, malignant melanoma.
- current cancer treatment.
- diagnosed unstable diabetes (people with stable type 2 diabetes diet-controlled diabetes were included)
- severe endocrine, hepatic, renal, pulmonary, or cardiac disorder.
- diagnosed electrolyte disturbances.
- known peptic ulcers.
- history of a heart-attack or prolongation of cardiac conduction intervals, or any other cardiac problems as taking domperidone increases risk of said problems.
- childbearing potential or pregnancy.

Participants were also excluded if they were taking any of the following:

- dopaminergic medications.
- noradrenergic, serotonergic, or anticholinergic medications started or changed within the past 3 months.

- monoamine oxidase inhibitors (MAO-I), except if selective MAO-A or MAO-B inhibitors are given alone. MAO-A and MAO-B inhibitors given together are equivalent to non-selective MAO-inhibition and therefore volunteers taking both MAO-A and MAO-B were not included.
- cholinesterase inhibitors, except if the participant was on stable treatment (at least 3 months).
- antihypertensives containing reserpine.
- ferrous sulphate on the day of dosing.
- opioids or sympathomimetics (e.g. amphetamines, epinephrine/adrenaline) unless if the participant was able to abstain on the day of dosing.
- diazepam or other benzodiazepines, unless none taken for prior 3 days or stable dose was maintained for more than 3 months.
- ketoconazole, erythromycin or CYP3A4 inhibitors (e.g. fluconazole, voriconazole, clarithromycin, amiodarone, telithromycin).
- antibiotics, if taken to treat an active infection.
- hormone replacement therapy.
- anti-fungal agents (pentamidine).
- anti-malarial agents.
- antihistaminics unless stable dose for 3 months or none for 3 days prior to testing sessions.
- AIDS/HIV medications.
- Any QTc prolonging medicinal products.
- if a participant took antacids or antisecretory agents they were required not to be taken at the same time as L-DOPA

Participants were asked to provide a list of medications and supplements they were taking, including alternative medication. A clinician verified each volunteer's eligibility and that none of these medications interfered with co-beneldopa, or domperidone.

Majority of the exclusion criteria were set to reduce risk of unwanted side effects from taking either levodopa or domperidone. The exclusion criteria were set based on the summaries of product characteristics for the medications uses in this thesis, expert advice from the study PI (Liz Coulthard, consultant neurologist) and from neurologists Dr Claire Rice and Dr Catherine

Pennington, as well as members of the NHS ethics board and upon regulatory authority recommendation.

Additional exclusion criteria for DOPAMIND

For the DOPAMIND study, I excluded volunteers with clinically significant sleep problems in the past year. I consider a sleep disorder to be clinically significant when it results in fewer than 6 hours' sleep per night regularly, in the estimation of the volunteer, and when they self-reported impaired sleep. Sleep disorders that required intervention (including equipment or medication) likely to interfere with our protocol or people with diagnosed sleep disorders who require intervention but who are unable to or have decided not to have the intervention (e.g. people with sleep apnoea for which a mask was recommended but who could not tolerate the mask) were also excluded. One volunteer was excluded for being a wheelchair user, as it was not possible not accommodate for a carer to stay with the volunteer.

Magnetic resonance

Participants could take part in other aspects of the DOPAMIND trial even if they were not eligible to be scanned in the MRI. For the MRI, participants' suitability was assessed case-by-case if they had any metal in their body, such as:

- a pacemaker
- an implantable cardioverter-defibrillator (ICD)
- a nerve stimulator or a drug pump implant
- a cochlear implant
- brain aneurysm clips
- metallic fragments in or near eyes or blood vessels (common in people who do or have done welding or metalwork for a living).

- prosthetic (artificial) metal heart valves
- penile implants
- eye implants
- an intrauterine device (IUD), i.e. contraceptive coil
- artificial joints
- dental fillings or bridges
- tubal ligation clips
- surgical clips or staples used to close wounds after an operation

Decisions about eligibility to scan were made by the first operator of the MRI scan (typically Hanna Isotalus) referring to established guidelines (e.g. MRIsafety.com). Where there was uncertainty, an expert radiographer was consulted.

Randomisation and blinding

Treatment order was randomised in blocks of 6 using an Excel randomisation program written in Excel. A block randomisation approach was used to allow for interim analyses and safety monitoring. Six blocks were randomised in one go (n = 36) and no repeat blocks were allowed.

Different preparation, randomising and blinding procedures were used for the two studies as DOPAMIND drug provision, randomisation and blinding was performed by the pharmacy while in DARet these were performed by members of the ReMemBr group.

DOPAMIND

The UBH Pharmaceuticals, Bristol Royal Infirmary, performed the randomisation and issued drugs and placebos in opaque pre-labelled bottles together with sealed unblinding envelopes. The bottles were labelled by participant and visit number. The capsules were not perfectly matched as due to copyright – a small imprint ('Roche') on the co-beneldopa capsule could not be printed on the placebos. The drugs were administered by a study doctor, who was blind to treatment allocation, with no investigators in the room. The doctor did not administer any of the experimental tasks. Participants were encouraged to pop the capsules directly from the bottle into their mouth without inspecting them.

Unblinding envelopes were sealed and stored on site at CRICBristol in St Michael's Hospital, and copies were stored in the ReMemBr group office in Southmead hospital. Unblinding was performed by two researchers after the 1st and 5th blocks of participants, and at the end of the study. The first unblinding was done due to adverse events (Appendix A), and the second (after 5th block) to allow data to be used for MSc and PhD student projects and conference presentations.

Initially, the DOPAMIND study also had a ropinirole (D2-agonist) arm. However, this was given to participants in a dose that induced nausea despite the use of anti-emetic. For this reason, this arm of the study was pulled out after first 6 participants had been tested. Further details are available in the appendices.

DARet

The randomisation was performed by a member of the ReMemBr group (Miss Brogan Knight), who had no other role in this study. She prepared two envelopes for each participant – one for both (placebo and treatment) sessions – and a master unblinding envelope for the entire study and for each block separately. A member of the ReMemBr group, who had no other role in this trial, unsealed the envelope and mixed either the placebo or the medication in accordance to detailed instructions (Appendix B). The unblinding envelopes were sealed and stored on site. At each visit, the person who mixed the cordial held onto the unblinding information until the participant was sent home. The master envelopes were opened after 3 blocks – for MSc dissertations – and again at the end of the study by two researchers.

Treatment

In the DOPAMIND trial, participants received encapsulated co-beneldopa controlled release containing 200mg of L-DOPA. This form of L-DOPA is active in the system for up to ~12h.

In the DARet trial, participants received a 150mg L-DOPA dispersible. This type of L-DOPA reaches tMax in ~1.5h.

Drug doses were selected based on previous research (Chowdhury et al., 2012). Both studies used co-beneldopa and placebo together with the anti-emetic Domperidone. Co-beneldopa contains both L-DOPA and benserazide. L-DOPA is a dopamine precursor that is absorbed into the blood stream and that passes the blood-brain barrier. It is converted to dopamine by DOPA decarboxylase centrally and in the periphery. Benserazide is a DOPA decarboxylase enzyme inhibitor. As benserazide does not cross the blood-brain barrier, its coadministration reduces peripheral L-DOPA conversion into dopamine increasing dopamine availability centrally.

Nausea caused by peripheral dopamine binding is a common side effect of dopaminergic medications. The antiemetic domperidone, a peripheral dopamine-inhibitor, was used to reduce nausea.

DOPAMIND

On each testing session, domperidone (10mg) was administered in tablet form. 30 minutes later, the volunteers were given a single oral dose of either co-beneldopa controlled-release (CR; 200mg/50mg administered as 2x capsules of 100mg/25mg), or inert powder (2x microcrystalline cellulose in capsule form Figure 45 A. The time to reach maximum plasma concentration (tMax) is approximately 3h and the peak serum concentration (cMax) is approximately 58% for co-beneldopa CR. Figure 45 B provides basic pharmacokinetic information based on the Summary

of Product Characteristics and does not present measurements made by mass spectroscopy. Instead, a linear rise of tMax was assumed.

The drugs and the placebo were issued by United Bristol Healthcare Pharmaceuticals. The placebo was matched to the co-beneldopa tablet in size and colour.

A

B

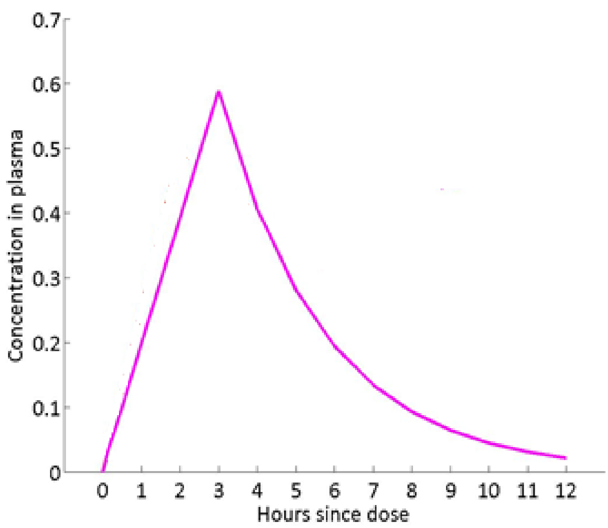


Figure 45: Co-beneldopa capsules and half-life curve

Figure 45: Co-beneldopa capsules and half-life curve
Visual guide to blinded capsules and half-life curve for co-beneldopa controlled release
A) The co-beneldopa capsules had an imprint while the placebo capsules did not. This is a guide to demonstrate blinding only and does not correspond exactly to the medicinal products. (B) The area under the curve demonstrates an estimate of the total exposure to L-DOPA over time, i.e. the drug concentration in plasma as a factor of time.

DARet

In the L-DOPA condition, volunteers were given 10ml of Domperidone suspension (liquid form), and 37.5/150mg co-beneldopa dispersible. The half-life of this form of co-beneldopa is ~ 112 minutes in the elderly population according to the Summary of Product Characteristics. The Domperidone was measured using an oral syringe and diluted into 40ml of blackcurrant cordial to mask its taste and colour. Co-beneldopa was diluted into 40ml of orange cordial. In the placebo condition 40ml blackcurrant and orange cordials with Vitamin C (250mg) mixed into the latter were given. Residue from the vitamin mimicked that from co-beneldopa. All mixtures were then diluted into a small amount of water. The blackcurrant and orange drinks were used as their distinct colours prevented the investigators from accidentally dosing the volunteer with co-beneldopa first.

The domperidone and co-beneldopa were purchased from the North Bristol Trust pharmacy, Brunel Building, Southmead Hospital, Bristol, and the Vitamin C from a supermarket.

Monitoring

Participants were carefully monitored throughout the study visits (Appendix C), and study drugs (/placebo) were only administered where physiological monitoring was within safe limits. Where there was uncertainty the Consultant Neurologist (EJC) was consulted. If a volunteer showed signs of nausea following the administration of the L-DOPA, another dose of Domperidone was given immediately.

	PPID136	PPID135	HKI	OR	RW	Night cover	Study Dr	
17:30			Food	Setup	Setup			17:30
17:45			Arrive					17:45
18:00	Arrive & consent	Arrive & consent	Consent					18:00
18:15	PSG	PSG	PSG	PSG	PSG			18:15
19:30	Dinner	Dinner	Drugs	Dinner	Dinner			19:15
20:00	PSG	Baseline	PSG	PSG	PSG		Baseline	20:00
20:15		PA Learn		Test				20:15
20:31	Baseline	RKN Learn					Baseline	20:31
20.45	PSG	t(0) DOMPO & Monitoring	PSG	PSG	PSG		Dose & Monitor	20.45
20:50								20:50
20:55		MST Learn		Test				20:55
21:00	PA Learn				Test			21:00
21:15	RKN Learn	t(30) monitoring + IMP					Monitor & dose	21:15
21:30	t(0) Domperidone	PSG	PSG	PSG	PSG		Dose	21:30
21:35	t(05) monitoring						Monitor	21:35
21:40	MST Learn				Test			21:40
21:45		t(60) monitoring					Monitor	21:45
21:50		PSG						21:50
22:00	t(30) monitoring + IMP						Monitor & dose	22:00
22:05	PSG							22:05
22:15		t(90) monitoring					Monitor	22:15
22:20		PA test		Test				22:20
22:30	t(60) monitoring	RKN test					Monitor	22:30
22:35	PSG	PANAS and THAT						22:35
22:50		PSG Calibration		PSG				22:50
23:00	t(90) monitoring	Wind-down					Monitor	23:00
23:05	PA test				Test		Sign and go home	23:05
23:15	RKN test	t(150) Lights out						23:15
23:20	PANAS and THAT			Leave				23:20
23:35	PSG Calibration				Leave	PSG		23:35
23:45	Wind-down							23:45
00:00	t(150) Lights out							00:00

Study visit schedule
An example of a sleep visit evening schedule for the DOPAMIND study for two volunteers tested on the same night.

Note that the times are not to scale and that the t(0) in this schedule refers to domperidone and not L-DOPA. The first and last column denote target times for events. The 2nd and 3rd columns denote the schedule for each participant. Columns 4-6 denote responsibilities allocated to the three researchers carrying out the study visit (HKI (me), OR (Oliver Radtke) and RW (Rachel Williams). Column 7 denotes the arrival time and responsibilities for the research assistant who stayed up to monitor the night. Column 8 contains the timetable for the study doctor. Up to two participants could be tested once.
Abbreviations are explained in table 4.