Walk of life
How brain state, spatial, and social context affect neural processing in rat perirhinal cortex, hippocampus, and sensory cortices
Bos, J.J.

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Chapter 1

Introduction
Preface

In order to thrive in our ever-changing surroundings, we need to constantly monitor and adapt to our environment. To sample our shifting environment, we use our different senses; touch, vision, audition, taste and smell. Even though we use different sensory organs to perceive the outside world, these sensory modalities are seldom used separately. It is a rare case where we only use vision and not any of the other senses to perceive something. Instead, inputs from multiple senses are naturally combined to enhance perception, where the whole is more than the sum of its parts (Ghazanfar and Schroeder, 2006; Meredith et al., 1992; Meredith and Stein, 1986). For instance, when cycling we use both vision and audition to follow other traffic, all the while using vision and haptic feedback to make sure we do not fall while crossing a tram track. Thus, despite entering the brain using different pathways, sensory information is rapidly and abundantly combined to form multisensory percepts.

Another example of brain areas working together is memory. The memory system is remarkably resilient to lesions of many different brain areas. In his seminal study, (Lashley, 1950) lesioned different brain areas in search of the seat of memory. His surprising conclusion was that it was not the location of the lesion per se, but more the extent of the lesions which best predicted memory deficits. Larger cortical lesions resulted in more severe deficits, irrespective of the location. While we do currently have evidence for memory specific regions, this example does show a remarkable resilience of brain function to damage.

These are but two of many examples which indicate the importance of interactions between different areas in the brain.

This is one of the main topics of this thesis: investigating how different brain areas interact in functions such as perception and memory. During my PhD, we recorded neuronal activity in the brains of rats. Recordings were made using tetrodes. Tetrodes are bundles of 4 micro-wires (electrodes) twisted together (Gray et al., 1995). The tetrodes were held in a small device, which was fixed on the skull of the animal. This method allowed us to record, both single unit activity and local field potentials (LFP), while the animal performed behavioural tasks. One of the main innovations of my PhD project was the development and initial use of a newly designed tetrode holding device, called the quad drive. The quad drive, developed by the Technology Centre of the University of Amsterdam, provided an important advance over the old recording devices. It holds more than twice the number of tetrodes of the previous devices, 36 vs 14, while still being lighter than the old version. Having 36 tetrodes to record with, we now had enough tetrodes to viably record 4 brain areas at the same time.

Traditionally multi-areal research is restricted more to the macro scale, using methods like scalp electroencephalography (EEG), magnetoencephalography (MEG), electrocorticogram (ECoG) or functional magnetic resonance imaging (fMRI) to record multiple parts of the brain simultaneously. Compared to
these methods recording 4 brain areas is still quite limited. However, tetrodes recordings have the marked advantage that one can record signals from individual neurons at a microsecond timescale. Being able to record single units from these multiple areas simultaneously will provide a unique opportunity to answer new questions regarding network dynamics (interactions between different brain areas).

Introducing my experiments

In this thesis, I will present work from two different studies, which we performed during my PhD. The primary experiment was called ‘Touch & See’. The second experiment we conducted had the intriguing working title ‘Rat Robot’. Both experiments were conducted by myself together with another PhD-student; Martin Vinck.

Being social animals, it is important for rodents to keep track of the whereabouts of others in addition to oneself. This is the central question of the Rat Robot project; Can the hippocampal place field system (O’Keefe and Dostrovsky, 1971; O’Keefe, 1976) be used to keep track of other moving agents in a shared environment in addition to keeping track where I am myself. Building on the finding of mirror neurons, we set out to investigate whether similar mirror type properties could also be expressed by hippocampal place fields.

Mirror neurons were originally found in monkey premotor cortex (Gallese et al., 1996; Rizzolatti et al., 1996). These neurons respond not only when the animal performs an action, but also when the animal observes another agent (e.g. the experimenter) perform the same action. Interestingly these mirror neurons function across species (monkey-ape) and can also generalize to machines/robots (Gazzola et al., 2007; Oberman et al., 2007).

Could hippocampal place cells be used in a similar way? Hippocampal place cells are neurons which are selectively active when the subject is at specific locations (place fields) within an environment. By keeping track of the activity of these hippocampal place cells, one can abduce the location of the animal in space. In the Rat Robot experiment we aimed to test if these hippocampal place fields also respond to other agents.

If found, mirror place cells would be defined as place cells which not only fire when the animal itself is at a specific location, but which would also activate when the animal observes another agent move through the same location. Mirror place fields could be an elegant way of reusing the highly developed hippocampal place field system for different, but related functions.

To investigate this, we used in vivo electrophysiology tetrode recordings from the CA1 sub-region of the hippocampus. In this experiment we trained rats to observe the movements of a small robot (e-puck; Acerbi et al., 2007; Mondada et al., 2009) driving around on the maze compartment in order to obtain reward, see Figure 1A-D. The animals had to indicate the movement direction of the robot by making a nose poke in the reward well corresponding to the same side of
the robot movement; nose poke in the right reward well for a rightward robot movement and left reward well for leftward movements. Using previously existing 14 tetrode recording devices (mono-hyperdrive), we recorded LFP and single units from CA1 while the rats were performing this task. To investigate whether responses for the robot resembled the place field responses for the rat itself, every recording started and ended with the rat exploring maze compartment.

Touch & See was the experiment revolving around the newly developed ‘quad drive’ (Technology Centre, University of Amsterdam). We were the first to use the upgraded recording devices. The addition of the extra tetrodes allowed us to target more brain areas at the same time, while still maintaining a sufficient
number of tetrodes per area. In the touch and see experiment we recorded from four areas; the visual cortex, barrel cortex, perirhinal cortex, and CA1 of the hippocampus.

Touch & See was designed to investigate how inputs from different senses are combined to form unitary percepts and stored in memory. We aimed to investigate how different areas along a pathway interact. To investigate this, we made simultaneous recordings from two primary sensory areas; monocular primary visual cortex (V1M), and the barrel subfield of the primary somatosensory cortex (barrel cortex, S1BF), which processes inputs from the whiskers. Higher up in the processing pathway we recorded from the perirhinal cortex which is important for combining sensory information and acting as a relay station to the hippocampal formation (Burwell, 2000; Witter et al., 2000; Witter et al., 2000; Furtak et al., 2007a). Finally, we included the dorsal CA1 subfield of the hippocampus. The hippocampus serves an important function in episodic and declarative memory (Scoville and Milner, 1957; Tulving and Markowitsch, 1998; Burgess et al., 2002; Lisman and Redish, 2009) and spatial navigation (place fields).

Combined recordings from these four brain areas allowed us to sample different subsections of the sensory pathway important for combining unisensory information into multisensory percepts.

In order to engage this sensory pathway during recordings, rats performed a visual discrimination task in a figure-8 maze, see Figure 1E-G. In this task the rats started in the middle arm of the figure-8 maze. After the intertrial interval, the animals could initiate stimulus presentation by positioning themselves in front of the Plexiglas blocking wall facing the screens positioned at the start of each side arm. Stimuli consisted of wingdings pictures of diamonds and an airplane, one on each screen. Animals were trained with either the airplane or the diamonds as the target conditioned stimulus (CS+). If the animals chose the arm containing the CS+ they received a food pellet reward at the end of the side arm. The size of the rewards was determined by the sandpaper the animal encountered at the initial part of the side arm. Rough sand paper indicated a larger reward and smooth sandpaper corresponded to a smaller reward. We switched the sandpaper every 10 trials to avoid a side bias due to the differences in reward size.

In this thesis, I will present some of the results we have found to date in our two unique datasets. In this thesis, I will present data from both experiments. The first three papers (chapters 2-4) pertain to results from the Touch & See experiment and the last experimental chapter (chapter 5) relates to the Rat Robot experiment. These four papers touch upon different topics and different types of analysis. This means that there is a lot to introduce. As such in the brief span of this thesis I will have to make multiple jumps between topics. I will make an effort to present the stories in a common framework, however I also do not wish to claim connections where there are none.
Chapter 1 | Introduction

Episodic memory
Memory is a very broad topic which by itself can and does fill thick books. Memory can be subdivided into many sub parts. The first major split is between short term and long-term memory. A second major subdivision is between explicit or declarative memory and implicit memory. Explicit memory refers to memories we can recall consciously. Implicit memory is memory where the recall is unconscious. The most common form of implicit memory is procedural memory. I know what a bike is and I can describe what a bike looks like. I even know how to ride one, but I could not give you the details of how I keep my balance while driving. Nevertheless, I do.

Explicit memory is broadly made up of semantic and episodic memory (Tulving, 1972). Semantic memory is the memory of facts, while episodic memory is memory for autobiographical events. These types of memory are not fully separate from each other. Semantic memory, for instance, will provide information of properties of items within episodic memories. Semantic knowledge is thought to be distilled from many different episodes. A child growing up with a calico cat may think that all cats are tortoise shelled, however over time that child will encounter all different types of cats and as such will learn to define a cat by different, more generalized, features.

A defining feature of episodic memory is that it is a multifaceted memory, which includes a “what” (persons, objects, events), “where” (locations) and a “when” (time). As such it is a complex collection of information spanning different domains. Take my very first memory. I was 3 and my twin sister and I were at kindergarten. It was a normal day until suddenly a small panic broke out because there was a bear outside and we all hid under the table. It is a vivid memory painting a complete scene, with other people (my sister, other kids, the teachers), a bear outside, a location (under the table in kindergarten in the centre of Amsterdam) and a time element (very long ago when I was three).

Studies of patients who suffered brain lesions following trauma or stroke or who had parts of their brains surgically removed to alleviate symptoms of disorders like epilepsy have yielded insight into which brain areas could be important for episodic memory. The classic study by Scoville and Milner (1957) showed that bilateral resection of the medial temporal lobe (MTL) resulted in severe memory loss. In 9/10 patients the size of the bilateral resection correlated with the severity of the deficit. Patient 1, belonging to the group of people who were most impaired, was the famous patient Henry Molaison (better known as H.M.).

Following surgery, he was unable to create new memories of events, even without a loss of general intelligence. H.M. could have a conversation or keep daily events, names or items in active working memory, but any distractions would cause a complete loss of recollection. Not only was H.M. unable to create new memories, he displayed retrograde amnesia for autobiographical memories up
to 3-11 years prior to his surgery. Autobiographical memories from his early childhood were maintained, but they were presented more as facts rather than as episodes (Corkin, 1984, 2002; Steinvorth et al., 2005).

Even though he could not consciously recall previous training, H.M. was still able to learn procedural memory tasks, like mirror drawing and, albeit very slowly, he did acquire some semantic knowledge during his post-surgery life. For instance, later in life he was able to draw a floorplan of the house he moved in 5 years after his surgery. With some help he could complete the names of people who had become famous after his surgery, and he could recognize pictures 24 to 6 months after extensive learning (Corkin, 2002; Squire, 2009).

Lesions in H.M. encompassed the anterior ~5 cm of both his temporal lobes. This medial temporal lobe region includes the hippocampus and adjacent, entorhinal, perirhinal and parahippocampal cortices (the latter is called the postrhinal cortex in rodents), subiculum and amygdala. Investigation of other patients with more restricted lesions (Zola-Morgan et al., 1986; Victor and Agamanolis, 1990; Squire and Zola-Morgan, 1991) showed that damage restricted to the CA1 subfield of the hippocampus is enough to cause anterograde amnesia without changes in personality or general intelligence.

Lesions which on top of the hippocampus also included the surrounding MTL areas, however, resulted in more severe deficits, see Figure 2A-B. Interestingly, lesions which were restricted to the perirhinal and parahippocampal cortices, but spared the hippocampus and the amygdala also caused severe memory deficits. Deficits found in monkey with combined perirhinal and parahippocampal cortex lesions were comparable to deficits in animals with extensive damage to hippocampal and parahippocampal areas and more severe than deficits caused by hippocampus-only lesions (Zola-Morgan et al., 1989; Squire and Zola-Morgan, 1991; Zola et al., 2000).

These results indicate that both the hippocampus and its surrounding areas serve an important role in episodic memory. In monkeys, not only lesions to the MTL, but also lesions to the adjacent inferior temporal cortex can lead to severe memory deficiencies (Mishkin and Ungerleider, 1982). This deficit is, however, more likely due to a general failure in processing visual information than a memory impairment (Buffalo et al., 1999, 2000).

More detailed research on the distinct roles of the hippocampus and the parahippocampal areas has revealed that they play complementary yet different roles in recognition memory.

Episodic memories are very complex. Sometimes the full memory cannot (immediately) be retrieved. In those cases, there can be a stage of familiarity (“I have seen this person before”), without specifically knowing from what, when, or where. Mention of a name or additional event can subsequently allow you to fully recollect where you know the person from. Familiarity and recollection are collectively called recognition memory.
The perirhinal cortex is deemed to be important for familiarity judgements, while the hippocampus is required for recollection (Brown and Aggleton, 2001; Kealy and Commins, 2011; Eichenbaum et al., 2012; Suzuki and Naya, 2014). Selective lesions to the hippocampus impaired recollection memory, but preserved performance on tasks which could be solved using familiarity or recency judgements (Mayes et al., 1999; Brown and Aggleton, 2001; Yonelinas, 2002; Aggleton and Brown, 2006; Bartko et al., 2007; Eichenbaum et al., 2007; Bowles et al., 2016). Making an objective distinction between familiarity and recollection is not trivial. The most common way to distinguish between familiarity and recollection in humans is by using confidence ratings during a recognition task. Low confidence scores point towards familiarity, while high confidence ratings indicate recollection.

fMRI studies in humans show a tendency for increased activity in the hippocampus and posterior parahippocampal gyrus (analogue to monkey parahippocampal cortex and rodent postrhinal cortex) in contrasts designed to highlight recollection. Using contrasts for familiarity these structures show little changes in activity. A reverse pattern was seen in the anterior parahippocampal gyrus (anal-
logue to the perirhinal cortex in monkeys and rodents). In the anterior parahippocampal gyrus, increased activity during encoding and decreased activity during retrieval correlate with higher familiarity as reported by the subjects (Ranganath et al., 2003; Daselaar et al., 2006; Montaldi et al., 2006; Eichenbaum et al., 2007).

Rodents and monkeys cannot provide verbal confidence ratings. Instead these animals are often tested on delayed non-match to sample tasks (DNMTS). In these tasks animals are initially presented with a sample object during the sample phase. After a delay the animals are presented with two objects during the recognition phase. One of the objects is the previously encountered object and the other is new. The animal is rewarded for making a response towards the novel object (or towards the familiar object in delayed match to sample tasks).

Single unit recordings from rat and monkey perirhinal cortex show object specific responses. Repeated presentation of the same stimuli usually decreased firing rates, with respect to their initial presentations (Zhu and Brown, 1995; Young et al., 1997; Brown and Banks, 2015; von Linstow Roloff et al., 2016). Similar responses have also been observed in the lateral rat entorhinal cortex (LEC; Young et al., 1997), but not in the postrhinal cortex (parahippocampal cortex (PHC) in monkeys), the medial entorhinal cortex (MEC) or the hippocampus (HPC; Otto and Eichenbaum, 1992; Suzuki and Eichenbaum, 2000; Eichenbaum et al., 2007).

Hippocampal neurons do show object related responses. These single neuron responses are not modulated by repetition, but they are modulated by space (Otto and Eichenbaum, 1992; Wood et al., 1999; Manns and Eichenbaum, 2009). This is line with the extensive body of literature about spatially responsive cells in the MEC (Hafting et al., 2005) and HPC (O’Keefe and Dostrovsky, 1971).

Immediate early gene studies show a similar pattern. Transcription of immediate early genes starts following strong activation of neurons and can be used as a marker for activation of brain areas. In rats fos expression was increased in PRH following novel stimuli, while POR and HPC neurons only show increased activity when the stimuli are rearranged in space (Vann et al., 2000).

These response patterns indicate a double dissociation where PRH and LEC code object properties, while the POR, MEC and HPC code for its location. Tasks which involve judgements of both object identity and location require successful interaction of both the PRH and the HPC (Brown and Aggleton, 2001; Brown et al., 2010).

**Pathways in the medial temporal lobe**

The brain is a highly interactive and dynamic organ. As such referring to a pathway of information flow is by definition a gross over-simplification. There will always be a multitude of branches, parallels and back-projections. However, that does not mean there are not certain pathways which are more pronounced than others.

Tracing studies have shown that there is a strong connection from the per-
irhinal and parahippocampal/postrhinal cortices to the entorhinal cortex. Perirhinal and postrhinal cortex efferents constitute approximately two thirds of all cortical inputs to the entorhinal cortex in monkeys (Insausti et al., 1987; Suzuki and Amaral, 1994; Dolorfo and Amaral, 1998; Naber et al., 1999; Burwell, 2000). In rats, where cortical afferents are generally more distributed, PRH and POR efferents comprise around 17% of the total entorhinal inputs (Burwell and Amaral, 1998).

The entorhinal cortex in turn provides the main input to the hippocampal formation. The hippocampal formation is a highly organized structure which is conserved throughout phylogeny. The main route through the hippocampus goes from entorhinal cortex to dentate gyrus to CA3 to CA1 and back to the entorhinal cortex via the subiculum. Despite this highly organized structure there are many “short cuts”, see Figure 2C.

The main input pathway to the hippocampus is the called the perforant pathway. In the perforant pathway axons from layers II/IV of the entorhinal cortex project to both the dentate gyrus and CA3. Within the hippocampus, the dentate gyrus projects to CA3 via mossy fibres. CA3 in turn projects to CA1 via the Schaffer Collaterals. The main hippocampal output projection starts in CA1 and projects back to the entorhinal cortex via the subiculum. In addition to the perforant pathway, there are many smaller projections. A notable example is a direct projection from the perirhinal cortex to CA1 mainly originating in layers II and III (Naber et al., 1999; Witter et al., 2000).

The functional dissociations found between object or spatial judgements have initiated a flurry of neuroanatomical studies. These studies have found that, following the functional similarity between PRH and LEC, the PRH is preferentially connected to the LEC, while the PHC/POR is most densely connected to the MEC. Both the LEC and MEC project to the hippocampus, but their projections to the DG and CA1 target different septal-temporal/proximal-distal locations (Burwell, 2000; Witter et al., 2000; Furtak et al., 2007a).

As such two main pathways arise. The PRH/LEC pathway which is thought to be more associated with object information and the POR(PHC)/MEC pathway which is associated with space, see Figure 2D. The presence of this anatomical differentiation has cemented the functional differentiation between objects and space to a point that nearly every textbook now refers to the ‘what’ and ‘where’ pathway. Despite their names, these pathways are not fully differentiated and there are many cross-overs between the pathways along the way (Burwell, 2000; Witter et al., 2000). Still, these clearly defined pathways provide a basis to investigate information transfer between areas since we know that the areas within a pathway are heavily interconnected. In the Touch and See experiment we used this knowledge for selecting the four areas we recorded from. Firstly, we selected touch and vision as sensory modalities to be studied. This was done, because it is relatively easy to manipulate both visual and haptic information, but also because these sensory modalities are naturally integrated: rodents rely heavily on their whiskers while moving around (Grant et al., 2012; Arkley et al., 2014). The
visual and barrel cortex both have direct and indirect connections with the PRH, which may serve as an intermediate integration area (Naber et al., 2000; Burwell, 2001; Furtak et al., 2007b). The PRH in turn sends both direct and indirect projections to CA1. In this way, we can investigate both lateral (between visual and barrel cortex), bottom up and top down interactions as well as long (inter-area) and short range (intra-area) connections.

**Behavioural states: Sleep**

Sleep after learning something new or after recalling something previously learned can enhance subsequent recollection, whereas disruption of sleep after learning disrupts recollection (McGaugh, 2000; Rasch and Born, 2013; Wixted, 2004).

How sleep facilitates retention is still poorly understood. Traditionally the role of sleep was thought to be mostly passive. However, sleep adds a timing dependent benefit to remembering previously learned stimuli. Sleeping immediately after learning provides a larger benefit than sleeping a few hours later (Gais et al., 2006; Rasch and Born, 2013). During sleep, sensory inputs towards the cortex are largely quenched by the thalamus (Campbell, 2010; Steriade, 2005). The lack of new inputs could reduce interference, allowing time for the previously encountered information to be consolidated.

In addition to benefits from reduced interference, recent studies emphasize an active role of sleep in consolidation. During sleep, previous experiences are repeated, to store them into memory. This repetition of memory traces can be observed as reactivation of previous patterns of neural activations. Neurons which are co-activated during learning are co-activated during sleep, presumably to strengthen the (new) connections which started to form during the experience (Ji and Wilson, 2007; Lansink et al., 2009; Lee and Wilson, 2002). Gupta et al. (2010) have shown that reactivation not only includes experienced activity patterns, but also includes novel sequences. These sequences combined different trajectories, resulting in valid paths which the rats could have, but never did experience. As such reactivation aids learning, not by passive replay of experienced events, but also by integrating them.

This effect of sleep on previous experiences is not a homogeneous process; sleep does not influence every experience equally. Emotionally charged events are preferentially stored over non-emotional ones (Walker, 2009; Payne et al., 2012). Additionally, future relevance (van Dongen et al., 2012) or anticipated reward (Fischer and Born, 2009; Wilhelm et al., 2011) can selectively enhance consolidation in humans. In the study by van Dongen et al. (2012) subjects learned two sets of picture-location associations. After learning they were told that only one of the sets would be retested. When, after a 14 h delay, both sets were retested subjects performed better at the cued set if they had had a night of sleep during the delay. If they did not sleep in the 14 hours between learning and testing there was no difference in performance between the cued and non-cued training set.

Consolidation can also be actively influenced during SWS by exposing sub-
Sleep in mammals consists of two main stages; slow-wave sleep (SWS) and rapid-eye-movement (REM) sleep, which alternate throughout the sleep period, see Figure 3A. Initial sleep contains more SWS. As the night progresses REM sleep becomes more abundant. These sleep stages are differentiated by different electrophysiological patterns, which vary between different brain areas.

SWS states are characterized by slow, high-amplitude delta (0.5-3 Hz) oscillations in electroencephalography (EEG) and local field potential (LFP) data of the neocortex. These slow oscillations are expressed as up- and down-states at the cellular level. During up-states the membrane potential of the cell is depolarized, facilitating neural firing, while during down-states the membrane potential is hyperpolarized, suppressing firing.

In addition to delta waves, the initial period of neocortical SWS is characterized by K-complexes (Amzica and Steriade, 1997; Colrain, 2005) and sleep spindles (Kandel and Buzsáki, 1997; Steriade et al., 1993; Steriade and Timofeev, 2003). K-complexes are very large cortical waves consisting of a single large up and downswing in the delta range. K-complexes are often followed by bursts of spindles (7-14 Hz) lasting for 0.5 to 3 s. During a k-complex neural cortical activity is depressed, akin to a single down state (Ji and Wilson, 2007; Cash et al., 2009).

Sleep spindles occur predominantly during light sleep stages. They are the result of strong excitatory thalamic inputs to the cortex and are associated with the loss of awareness.

Sharp-wave ripple complexes (SWRs) can be observed in the hippocampus at the onset of SWS, during periods of immobility, and following reward. Sharp waves are short irregular high amplitude deflections in the LFP. Ripples occur during sharp waves and are short (~100 ms) high frequency (150-250 Hz) oscillations. CA1 ripples are caused by population bursts in CA3 (Buzsáki et al., 1992; Chrobak and Buzsáki, 1996).

REM sleep is characterized with electrophysiological patterns resembling those of wakefulness. In the cortex REM sleep shows fast, low-amplitude EEG patterns, while in the hippocampus REM sleep is accompanied by a strong theta rhythm. During wakefulness, hippocampal theta is mainly observed when rodents are actively moving around.

The occurrence of both sleep spindles and SWRs are positively correlated with memory performance (Gais et al., 2006; Johnson et al., 2010; O’Neill et al., 2010), while disruption of both spindles and SWR has been shown to be detrimental for memory (Smith and MacNeill, 1994; Girardeau et al., 2009; van de Ven et al., 2016).
Figure 3 | Sleep and the two-stage model for memory. A) Overview of sleep stages in humans, top, and rodents, bottom. In rodents, sleep is subdivided in 2 sleep stages: NREM and REM sleep. In humans NREM sleep is subdivided in 4 sleep stages: S1-2 are also referred to as light sleep (LS), showing more K-complexes and spindles, while stages 3-4 show the large slow oscillations associated with slow wave sleep (SWS). EEG patterns during REM sleep more closely resemble EEG traces during wakefulness than other, NREM sleep patterns. In rodents REM sleep is also associated with an increase in theta oscillations in the hippocampus. Throughout the night humans show clear sleep patterns. During the first half of the night, sleep is dominated by deep sleep stages or slow wave sleep. During the second half REM sleep becomes more prevalent. In contrast, rats, which are nocturnal animals, sleep mostly during the day and show very fragmented sleep. Image was adapted from Genzel et al. (2014). B) Graphic representation of the two-stage model for memory. Memories initially require a large interaction between the hippocampus and cortical areas. Over time the intracortical connections strengthen, while hippocampal-cortical interactions slowly deteriorate - until finally, memories become hippocampus-independent. Image was adapted from Frankland and Bontempi (2005).
atory neurons are activated. Despite the large number of simultaneously activated neurons, activation during ripples is not random. During exploration, neurons with adjacent place fields fire in predictable patterns, based on the animal’s progression through the place fields. Neurons which sequentially activate during a task have been found to have a larger probability to fire together during post-task ripples (Foster and Wilson, 2006; Gupta et al., 2010; Lee and Wilson, 2002; Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994).

This reactivation or replay is hypothesised to be off-line rehearsal of important sequences, in line with Hebb’s learning rule; ‘neurons wire together if they fire together’ (Hebb, 1949; Löwel and Singer, 1992). During ripples the sequences are replayed in a time compressed manner (Wilson and McNaughton, 1994; Nádasdy et al., 1999; Euston et al., 2007). Even though REM sleep has not been as extensively studied as SWS, it been hypothesised to be involved in offline learning and replay (Hars et al., 1985; Louie and Wilson, 2001). Sequences are not only replayed during sleep. Hippocampal replay has also been shown during quiet wakefulness (Carr et al., 2011; Foster and Wilson, 2006; Pfeiffer and Foster, 2013).

The two stage model for memory consolidation is one of the leading current theories about memory formation (Frankland and Bontempi, 2005; Marr, 1971; Rasch and Born, 2013; Squire and Alvarez, 1995). In the two-stage model new information is initially stored in the hippocampus before it is transferred to the cortex, see Figure 3B. This framework fits with observations from patients like H.M. who suffer from time-limited retrograde amnesia following surgery or trauma.

Sleep could provide a window during which information can be transferred from the hippocampus to the cortex. According to this view, reactivation should not be limited to the hippocampus. fMRI studies show that this could indeed be the case. Hippocampal activation decreased with the age of the memory, while activity in cortical areas increased (Smith and Squire, 2009; Takashima et al., 2009). In addition, replay of neuronal sequences has been observed between HPC and the visual cortex (Ji and Wilson, 2007), the nucleus accumbens (Lansink et al., 2009), and the prefrontal cortex (Benchenane et al., 2010).

**Behavioural states: Wakefulness**

Above it was already mentioned that sleep can be segmented into different states (SWS and REM) based on the rhythms and events in the LFP. Similarly, wakefulness also is a heterogeneous state, which can be segmented by differences in attentiveness and behavioural activity.

Changes between these states can occur very rapidly. In the space of a few seconds we can go from an attentive state (e.g. reading) to an unconscious state (e.g. sleep), while a single loud noise can cause a transition from a quiescent to a hyperattentive state.

The speed of these transitions exceeds the speed with which the brain structurally changes neuronal connectivity. Instead, the way neurons communicate
through the existing connections is changed. This is exemplified by a study from Massimini et al. (2005) in which they stimulated the premotor cortex of human subjects using TMS (transcranial magnetic stimulation) and measured the spread of activation (EEG) during quiet wakefulness (QW) and sleep, see Figure 4. During QW the activity spread to connected areas, both in the same hemisphere and contra-laterally and lasted around 300 ms. A similar activation during SWS showed an increased initial response but almost no spreading and the response was much shorter (~150 ms). These results indicate changes in effective connectivity between sleep and wakefulness. Behavioural state influences how activations in one area propagates to other areas.

The functional interaction during different states can be modulated by changing the activity of neurons; the correlation between the firing rates of neurons and their locking to different oscillations (Harris and Thiele, 2011; McCormick et al., 2015). Following the example from Massimini et al. (2005) these changes can be local or global and they can differentially affect interactions within or between areas. Steriade et al. (2001) showed that during SWS all different neuronal subtypes they measured in the neocortex of cats were entrained by the slow waves in the LFP. During SWS there were periods of hyperpolarization in the membrane potential during which cells did not fire (‘down states’). In between the silent periods - during depolarization phases (‘up states’) - units showed firing rates which were equivalent to awake firing rates. This indicates that sleep is not simply a result of decreased cortical activity. Instead, it seems that it is the interaction between neurons or the duration of activations which is important to create a sleep state. A host of studies has since refined the effect of behavioural state on neuronal pro-
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Sensory processing (Gentet et al., 2010; Goltstein et al., 2015; Hirase et al., 2001; Hirase et al., 2001; Klausberger et al., 2003; Lansink et al., 2010; McCormick et al., 2015; McGinley et al., 2015b, 2015a; Perrenoud et al., 2016; Steriade et al., 1993; Vinck et al., 2015).

These studies show that sensory processing is highly state-dependent. For instance, sensory processing is highly influenced by changes in arousal, where intermediate levels of arousal lead to improved perception compared to both high and low arousal (McGinley et al., 2015b, 2015a; Vinck et al., 2015; Vyazovskiy et al., 2011).

GABAergic interneurons

The barrel cortex has been widely used as a model system to study sensory processing in rodents. Rodents use their whiskers in a similar way in detection as primates use their fingers and they are capable of perceiving similarly fine-grained textures as we can, using our fingers (Carvell and Simons, 1990; Lak et al., 2010). The barrel cortex is a highly-organized structure in which the inputs from each whisker terminate onto a specific patch of cortex. The layout of these patches or ‘barrels’ closely follows the arrangement of the whiskers, with adjacent barrels receiving inputs from adjacent whiskers (Brecht et al., 2004; Petersen, 2007; von Heimendahl et al., 2007; Diamond et al., 2008).

Using targeted dual whole-cell recordings in mice Gentet et al. (2010) looked into the interactions of excitatory and inhibitory neurons in S1BF during different awake states. Firing rates of both excitatory and inhibitory neurons differed strongly depending on whether the animal was quiescent or actively whisking. Concurrent with previous literature there is a tight co-firing between the excitatory and inhibitory neurons (Atallah and Scanziani, 2009; Haider and McCormick, 2009; Okun and Lampl, 2008). However, different subtypes of GABAergic interneurons were recruited during different brain states in rat hippocampus (Klausberger et al., 2003; Lapray et al., 2012) or mouse cortex (Gentet et al., 2010). Fast-spiking GABAergic neurons in mouse barrel cortex were most active during quiet wakefulness, while non-fast-spiking GABAergic neurons were most active during whisking. This indicates distinct roles for different GABAergic interneuron subtypes during different behavioural states (Gentet et al., 2010; Klausberger et al., 2003; Lapray et al., 2012).

GABAergic interneurons are the most prominent source of inhibition in the brain. Without inhibition, recurrent excitatory activity can quickly spiral out of control (Moore et al., 2010). In addition to preventing over-excitation, GABAergic interneurons are also important for the generation of the different rhythms observed in the LFPs (Buzsáki and Chrobak, 1995).

Using extracellular tetrode recordings from rodent somatosensory and prefrontal cortex, it is possible to make a putative split between fast and narrow spiking inhibitory neurons and slow broadly spiking pyramidal neurons (Barthó et al.,
2004). Trying to distinguish between the different subtypes of GABAergic neurons extracellularly will quickly bring us into the realm of speculation. However, this lack of understanding should not excuse researchers using extracellular recording techniques to stop trying to identify differences within the (inter)neuron population.

Using morphology and staining for different peptides, GABAergic neurons can be divided into many different subgroups (Gentet, 2012). The largest of these subgroups are the group most classically associated with the interneuron phenotype; the “narrow” or fast spikers (FS) which are parvalbumin positive (PV+). The large majority of FS are basket cells, which make up about 50% of GABAergic interneurons in S1BF (Perrenoud et al., 2013) and provide local perisomal inhibition. PV+ neuron activity is closely coupled to the activity of excitatory neurons (Freund and Buzsáki, 1996; Klausberger and Somogyi, 2008; Sohal et al., 2009; Gentet, 2012; Kepecs and Fishell, 2014).

A second large class of interneurons are the somatostatin expressing (SSt+) Martinotti cells. SSt+ neurons show a very different activity pattern from PV+ cells. Whereas the activity of PV+ cells increases during activity (active whisker touch in the case of S1BF), SSt+ neuron firing decreases. Additionally, where PV+ firing is tightly coupled to excitatory firing, coupling of SSt+ activity is more variable (Kvitsiani et al., 2013). Gentet et al. (2012) showed that SSt+ neurons are the only type of interneuron recorded from the superficial layers of the barrel cortex which was not excited by active touch. SSt+ activity is therefore hypothesised to be important for gating top-down information coming in through the superficial layers. Optogenetic inhibition of SSt+ neurons results in increased bursting activity in nearby excitatory neurons, suggesting a role for SSt+ neurons in preventing burst firing under baseline conditions.

The third and final group of interneurons I would like to mention here are the vasoactive intestinal polypeptide (VIP+) expressing neurons, which make up about 5% of GABAergic interneurons. VIP+ neurons are deemed to be important for modulating other types of GABAergic neurons through disinhibition, in which they primarily target SSt+ neurons (Pi et al., 2013). This disinhibition could serve a role in facilitating behavioural transitions (Kepecs and Fishell, 2014).

Most GABAergic neurons project locally, but there are also long range GABAergic connections. These connections are very diverse and can have different targets in different areas or have both local and distal targets. Caputi et al. (2013) suggested that inter-cortical connections and connections from the cortex to the hippocampus preferentially express SSt+, while hippocampo-cortical connections seem to express more PV+. Both cortico-hippocampal and hippocampo-cortical long range GABAergic projections preferentially target other GABAergic cells (Caputi et al., 2013; Melzer et al., 2012).
Oscillations

GABAergic interneurons also play an important role in oscillatory activity (Buzsáki and Chrobak, 1995; Aarne Ylinen et al., 1995; Chrobak and Buzsáki, 1996; Csicsvari et al., 1999; Blatow et al., 2003; Stark et al., 2013; Bosman et al., 2014; Stark et al., 2014). Oscillations are usually measured in the extracellular medium (LFP) or on the skull using EEG or MEG. The oscillations are deemed the result of synchronized dendritic input in a specific area. The strength of the rhythm is determined by the number of units involved and their orientation with respect to each other. Neatly organized neurons like in the neocortex or especially the hippocampus can generate larger LFPs.

While the precise functions of oscillations remain unknown, it is widely hypothesised that oscillations serve an important function in providing a timing element. Much like during the previously mentioned up and down states associated with SWS, during which pyramidal firing is normal or quenched, faster oscillations can also create windows of facilitated or suppressed firing. Faster oscillations do however contain less energy and as such this modulatory effect is not as pronounced as during the slow delta waves. Smaller windows created by faster oscillations do however enable more precise timing of inputs.

Matching windows of facilitated firing within or between areas is a proposed way to facilitate information transfer (see Figure 5A; Salinas and Sejnowski, 2001; Fries, 2005, 2015; Sejnowski and Paulsen, 2006). Different oscillatory cycles or different phase segments of the oscillations (upswing, peak, downslope, trough) can also help to segment time and information. Concentrating inputs and outputs could enhance communication through processes like spike-timing-dependent-plasticity (STDP, see Figure 5B).

In STDP connections between pre- and postsynaptic neurons are updated based on the relative timing of their activity. If a presynaptic neuron consistently fires before the postsynaptic neuron, the connection is strengthened, while connections in which the presynaptic neuron fires after the postsynaptic neuron are weakened. This is similar to the Hebbian learning rule described above (Levy and Steward, 1983; Song et al., 2000; Dan and Poo, 2004).

We have already mentioned the very slow delta oscillations (<4 Hz) characteristic for SWS. Other important rhythms in the rodent brain are theta (4-7 Hz), beta (12-30 Hz) and gamma (30-90 Hz). Across different species the definitions of frequency bands change a bit. The most noticeable change is the seeming absence of alpha (~8-12 Hz) in rodent research.

Alpha is a frequency range which is widely investigated in human and monkey literature and is most prevalent during relaxation with closed eyes. Decreased alpha is associated with increased visual attention and improved performance on sensory discrimination tasks (Foxe et al., 1998; Goldman et al., 2002; Haegens et al., 2011). The lack of alpha is not because rodents do not display oscillations in the classically defined alpha range. Instead, it is the result of history. Initial recordings from the hippocampus of rabbits and cats (Jung and Kornmueller, 1983).
1938; Green and Arduini, 1954) reported sustained 5-6 Hz oscillations (thus within the theta range: 4-7 Hz). Later studies in rodent hippocampus displayed similar strong sustained oscillations, however these oscillations were slightly faster, between 7-9 Hz (Green, 1964; Whishaw and Vanderwolf, 1973). By that time hippocampal theta had already become an engrained concept in the literature. As a result, the definition of rodent theta has been stretched to include frequencies which in non-rodent literature would likely be referred to as alpha.

In this thesis, we will present results from rodent data and as such we will stick to rodent definitions, with the full knowledge that the different definitions of theta make the comparison between rodent and human/monkey data more difficult.

The neat organization of neurons in the hippocampus allows for the generation of very large amplitude rhythms, which can be maintained for hours (Whishaw and Vanderwolf, 1973). Theta is observed in CA1 when rats engage in active behaviours like running, rearing, grooming, but also during REM sleep. It is mostly absent when the animal is immobile. Theta is associated with, but not a result of running. Theta has been shown to initiate just before movement onset and it is modulated by the speed of movement, where faster running corresponds to higher theta frequencies (Whishaw and Vanderwolf, 1973; Stawinska and Kasicki, 1998; Muir and Bilkey, 2003; Ledberg and Robbe, 2011). During navigation and decision-making theta is hypothesised to be important for regulating spike timing (Sirot et al., 2008; Buzsáki and Moser, 2013; Siegle and Wilson, 2014). Theta is

![Figure 5 | Two examples of how spike timing can facilitate or inhibit neural communication. A) The communication through coherence hypothesis. Oscillations provide windows of facilitated and depressed firing. If neurons receive input during the excitable phase this will enhance firing, red and blue neurons. If on the other hand activations are timed with the depressed state, the same activation does not lead to enhanced activation of the target neuron (blue and black). Image was adapted from Fries (2005). B) Spike time dependent plasticity is another example of how timing of input and output signals can influence connectivity. Timing of presynaptic and postsynaptic action potentials can strengthen (with presynaptic activity before postsynaptic activity, blue) or weaken (with presynaptic activity after postsynaptic activity, red), connections. Image was adapted from Butts and Kanold (2010).](image-url)
also thought to have a role in memory, where it could facilitate separating encoding from retrieval (Hasselmo and Stern, 2014; Hasselmo, 2005). In addition, there was a positive correlation between theta oscillations during running and temporal offsets during preplay (Diba and Buzsáki, 2007).

It is unclear how hippocampal theta is generated. The diagonal band of Broca in the medial septum has an internal theta rhythm (Mitchell and Ranck, 1980; Jeffery et al., 1995). Long range cholinergic and PV+ inhibitory connections between the medial septum and the hippocampal formation are hypothesised to be the source of hippocampal theta (Hangya et al., 2009). Lesioning medial septo-hippocampal connections largely abolishes theta in CA1 of the hippocampus (Lee et al., 1994).

In addition to these inhibitory inputs, theta in the hippocampus is also driven by excitatory inputs from the entorhinal cortex. Pathways for the induction of theta reach CA1 both via direct connections from the entorhinal cortex and through the perforant pathway via CA3 (A Ylinen et al., 1995; Buzsáki, 2002). Lesioning the entorhinal cortex abolishes most theta. Any remaining theta is thought to originate from the septohippocampal connections and recurrent connections in CA3 (Bragin et al., 1995; Buzsáki, 2002).

Septal/entorhinal theta and theta intrinsically generated in CA3 work in concert to drive theta oscillations in CA1 (Cutsuridis and Hasselmo, 2012). Gu and Yakel, (2017), argue that the interaction between the medial septum, entorhinal cortex and hippocampus could induce a theta rhythm even in the absence of a clear pacemaker. Interaction between entorhinal and CA3 generated theta leads to gradients in both amplitude and phase across the different layers of CA1. In contrast theta amplitude and phase are similar along the longitudinal axis of CA1 (Buzsáki, 2002).

Relative to theta and gamma, beta rhythmicity (12-30Hz) has been much less investigated. Especially in rodents, which have a very dominant hippocampal theta rhythm, beta in the hippocampus is often referred to as the first harmonic of theta. It is hard to distinguish beta from theta harmonics, since increases in hippocampal beta, similarly to theta, are correlated to running speed and beta peaks are small with respect to peaks in the theta frequency (Terrazas et al., 2005). Despite these complications, efforts are made to show that hippocampal beta is an independent rhythm (Berke et al., 2008; Rangel et al., 2015). Lansink et al. (2016) have found beta to be more prominent during cued exploration, which includes an element of reward expectancy. Beta has also been shown to be involved in long range coupling between CA1 of the hippocampus and either the ventral striatum (Lansink et al., 2016) or the entorhinal cortex (Igarashi et al., 2014).

In different species with less prominent hippocampal theta, like monkeys and humans, beta has been more widely studied. Here beta has been linked to feedback mechanisms from higher order areas to the deep layers of the visual (Bastos et al., 2012; van Kerkoerle et al., 2014; Bastos et al., 2015; Michalareas et al., 2016) auditory (Haenschel et al., 2000) or sensorimotor cortices (Brovelli et al., 2012).
2004; van Ede et al., 2010). This function of beta is not restricted to these species. It has also been hypothesised to play a similar role in rodents (Grossberg, 2009).

Finally, a brief introduction to gamma (30-90 Hz) is in place. Like theta, gamma has been abundantly studied and has been shown to be involved in active processing and feed forward routing in diverse areas like monkey visual cortex (Bosman et al., 2012; van Kerkoerle et al., 2014), monkey posterior parietal cortex (Buschman and Miller, 2007), mouse barrel cortex (Cardin et al., 2009), rat entorhinal cortex (Chrobak and Buzsáki, 1998), rat hippocampus (Bragin et al., 1995; Harris et al., 2003) and rat thalamus (Pinault and Deschênes, 1992; for gamma reviews, see: Jensen and Lisman, 1996; Bartos et al., 2007; Fries, 2009; Colgin and Moser, 2010; Buzsáki and Wang, 2012; Bosman et al., 2014; Vinck and Bosman, 2016). The fast gamma frequency can help to precisely time neuronal firing. The small windows provide specific moments for synchronisation to which the activity of neuronal ensembles can be anchored (Fries, 2009; Buzsáki and Wang, 2012).

Gamma is generally presumed to be locally generated (Bragin et al., 1995; Sirota et al., 2008). However, long range intrahemispheric (V1-V4 in monkeys; van Kerkoerle et al., 2014) and interhemispheric (hippocampus; Chrobak and Buzsáki, 1998) gamma coherence also exist. This could be accomplished through long range interneurons carrying the gamma rhythm (Buzsáki et al., 2004) or through non-gamma rhythmic signals which drive new gamma oscillations in the target area (Sohal et al., 2009).

Cardin et al. (2009) showed that gamma can be induced in mouse barrel cortex by optogenetically stimulating PV+ interneurons. Stimulating PV+ interneurons at frequencies between 8-200 Hz resulted in gamma synchronization in the LFP, with a peak around 40Hz. In contrast stimulating regular spiking pyramidal neurons did not induce gamma.

Based on the importance of interneurons in the generation of gamma rhythms, two competing models have arisen to explain cortical gamma: the ING (interneuron network gamma) and the PING (pyramidal interneuron network gamma). In the ING theory network gamma is generated by cyclic mutual inhibition of interneurons. In this model, asynchronous excitatory inputs are enough to engage inhibitory activity which can generate a gamma rhythm (Whittington et al., 1995; Bartos et al., 2007). In the PING network the activity of interneurons is driven by pyramidal neurons (Wilson and Cowan, 1972; Csicsvari et al., 2003). Here, excitatory activity precedes the inhibitory activity. An excitatory volley will activate inhibitory interneurons. Increased activity of inhibitory interneurons then decreases excitatory firing. The reduced excitatory drive in turn reduces inhibitory activity and as a consequence excitatory firing will increase again. The absence of gamma resulting from excitatory activation in the above experiment by Cardin et al. 2009 point towards ING mechanisms. There is however also evidence for PING mechanisms provided by studies reporting that pyramidal firing precedes inhibitory firing by a few milliseconds in rat hippocampus (Csicsvari et al., 2003; Hájos and Paulsen, 2009).
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Whether these two mechanisms compete with each other or whether they function in concert, both theories point towards the importance of PV+ type fast spiking interneurons (“basket cells”). These basket cells are deemed important for the generation of gamma by providing peri-somatic inhibition of pyramidal cells. PV+ positive interneurons are not only important for the generation of the gamma rhythm. Multipolar bursty PV+ positive interneurons have been shown to drive other rhythms like theta (Blatow et al., 2003; Stark et al., 2013).

The fast nature of gamma makes it very suited to synchronize local spike timing. For long distance coupling fast gamma oscillations provide some challenges. Gamma oscillations contain very little energy compared to lower frequency oscillations. As such gamma oscillations are not volume conducted very well. In order to couple two areas gamma needs to be actively maintained in both areas. Conductance delays due to long distances between areas can hinder communication by skewing the timing of the small spiking windows provided by gamma.

One way to get around the long-range gamma synchronization problem is by combining fast gamma oscillations with slow oscillations. The timing of gamma activity can be coupled to a specific phase of these slower frequencies (e.g. theta): cross-frequency coupling (Bragin et al., 1995; Buzsáki and Chrobak, 1995; Sirotta et al., 2008). By using the slow frequencies to time gamma activity, fast synchronous activations can be achieved over long distances.

Analysis methods

The proposed function of all of these different oscillations is to modulate communication between neurons, both within and between brain areas. To test how oscillations modulate information transfer between areas, we need multi area recordings from behaving animals. Getting simultaneous multi-area single unit and LFP recordings is one challenge. Another is using and developing analytic methods to detect these oscillations and to compare coherence between oscillations or timing of spikes with each other or with respect to these oscillations.

For this purpose, Martin Vinck and colleagues have developed two metrics which improve on previous methods used to calculate either LFP-LFP coherence or spike-LFP coherence, the weighted phase lag index (WPLI, Vinck et al., 2011) and the pairwise phase consistency (PPC, Vinck et al., 2010, 2012), respectively. The exact mathematical explanation and validation of these measures is beyond the scope of this thesis, but I will briefly introduce them below.

LFP-LFP coherence is traditionally calculated using the phase locking value (PLV, Lachaux et al., 1999). To calculate the phase locking value, first the relative phase difference between the oscillations of two input sources is calculated. The phase-lead/lag value is the resultant length of all recorded phase differences.

There are several pitfalls associated with using PLV in LFP-LFP coherence. The WPLI is designed to improve on the phase-locking value by correcting for these complications. The first problem with PLV is the problem of volume con-
duction. Activity generated by a third source can drive the oscillations of both LFP traces one wishes to compare. These common inputs will result in an apparent high phase synchronization.

Biological rhythms are never pure oscillations of a single frequency. Instead, recorded oscillations are compounds of oscillations of many different frequencies. Fourier transform can be used to disentangle the frequencies and calculate the contribution of a certain frequency in the complex oscillation.

The Fourier transform returns a complex number. While sounding like a higher mathematical concept, using and performing basic calculations with complex numbers is quite simple. A complex number is made of a real (the kind of numbers we are all familiar with) and an imaginary part. An imaginary number (i) is a mathematical construct whereby the square of an imaginary number is negative (e.g. \( i^2 = -1 \) and \( 5i^2 = -25 \)), thus allowing us to take the square root of a negative number. Complex numbers can be easily visualized when drawn in an xy-plane (Cartesian plane), where the real number is represented on the x-axis and where the y-axis is imaginary. A complex number like \( 2 + 3i \) can be drawn as vector starting at \((0,0)\) and ending at \((x = 2, y = 3)\), see Figure 6A. The length of this vector can be calculated using Pythagoras’s theorem and represents the amplitude of the oscillation. The angle between the vector and the positive x-axis represents the phase of the oscillation with respect to a cosine function.

If two sources are driven by a third source the phase difference between these two sources will be close to zero degrees since both sources receive the same in-phase noise input from the third source (or 180 degrees (phase reversed) depending on the orientation with respect to the dipole of the third source). This means that common noise inputs will reside on or close to the x-axis at 0 or 180 degrees. Especially under situations of small or no correlation between the signals, small amounts of volume conduction will have a big effect on the resulting phase locking value. At 0 and 180 degrees, the imaginary component is zero. As such, Nolte et al. (2004) proposed that only using the imaginary part of the Fourier transform can help mitigate the problem of volume conduction from a common noise source.

Noise measured at two different recording points is however not always fully correlated. Noise biases arise when there are multiple sources of noise or when the noise affects the measuring points differently, resulting in non-zero difference in phase delay at the measuring points.

In 2007 Stam et al. proposed the phase lag index (PLI), which looked at the asymmetry between the phases of two input traces, like EEG or LFP. PLI only uses the imaginary component and looks at the distribution of positive and negative phase differences. All phase differences, with positive imaginary components, get an imaginary value of 1, while all negative phase differences, with negative imaginary components receive an imaginary value of -1, irrespective of the size of the leads or lags.

The PLI is more robust and less sensitive to noise than Nolte’s imaginary component (ImC) measure (Stam et al., 2007; Vinck et al., 2011). The PLI, how-
ever, is sensitive to jitter around the real axis. Adding noise to phase differences around the real axis (0 and 180-degree phase difference) can flip the sign of the value from -1 to 1 or vice versa. If the noise is not equally distributed it can cause the relative differences to be rotated with respect to the real axis. This distribution change can cause the PLI to increase (more positive phase differences) or decreased (more negative phase differences). To correct for this the WPLI has added a weighting factor, based on the size of the imaginary component. This decreasing the influence of phase differences around the real axis (phase differences of 0 and 180 degrees), see Figure 6B.

Another problem is the problem of sample size. This refers to the limited number of samples of phase differences which can be recorded and used to estimate coherence. The problem here is that with fewer samples coherence (ImC and WPI) tends to get overestimated. One way to get around limited numbers of samples is by using pairwise comparisons between trials. Instead of calculating an average of weighted imaginary components across trials, an average is calculated across all pairs of trials.

The second measure of rhythmic synchrony is called the pairwise phase consistency (PPC, Vinck et al., 2012, 2010). This metric is mainly used to calculate spike-LFP coherence. In spike-LFP coherence measures we look at the locking of spikes to LFP oscillations measured from electrodes other than the one on which the spikes were recorded. Voltage changes caused by spikes can bleed through in the LFP filtered signal and as such influence the measured angle of a given frequency.

Unlike LFP-LFP analyses, spike-LFP coherence is not biased by volume conduction, since the LFP phase is only determined once; at the time point of the spike. However classical spike-LFP coherence measures like the phase locking value are sensitive to sample size (Lachaux et al., 1999; Vinck et al., 2010). The PPC reduces the sensitivity to the total number of spikes of a given neuron by making pairwise comparisons. For each spike the corresponding phase of a given LFP trace is calculated. The PPC is calculated by taking the cosine of the absolute difference between the phases of two spikes. The PPC is made to resemble the PLV, but it has the advantage that it converges to a stable prediction using very few observations/trials.

Vinck et al. (2012) refined the PPC metric in two ways. First, spikes are not statistically independent from each other. Neurons have refractory periods and some types of neurons can fire bursts of action potentials. A second problem is that the probability of spiking and LFP phase are not independent from each other. Different events across trials can affect firing rates, leading to an uneven distribution of spikes across trials. The first problem is circumvented by comparing only pairs of spikes from different trials. The second problem is solved by averaging combinations within a pair of trials before averaging across all different trial combinations. This way each pair of trials will receive the same weight.
Walk of life | How brain state, spatial, and social context affect neural processing in rat perirhinal cortex, hippocampus, and sensory cortices

Figure 6 | Advanced analysis methods. A) Visual representation of an imaginary number in a cartesian xy plot. Brain oscillations are comprised of many different oscillations. To investigate the contribution of different frequencies in a complex oscillation, several different methods can be used (i.e. fast fourier transform). These methods return complex numbers to denote the frequencies. The x-axis represents the real numbers while the imaginary numbers are shown on the y-axis. Complex numbers can be drawn as a vector in this x/y plane. The length of the arrow represents the amplitude of the measured oscillation. The angle between the positive real axis and the vector shows the phase of the oscillation. B) Visual explanation of the difference between the phase lag index (PLI) and the weighted phase lag index (WPLI). Every line represents the cross spectrum between two signals at a specific frequency, i.e. LPF traces of two trials (this represents the phase difference, following conjugate multiplication of the complex outputs of the fourier transform). In the PLI every blue line (phase lead) will get a weight of 1 and every red line (phase lag) will get a weight of -1. Small jitter along the real axis can completely change the value of -1 to 1 or vice versa. In the WPLI the observed leads and lags are weighted by the magnitude of the imaginary component (magnitude along the y-axis). As such small changes in value around the real axis will have less of an impact on the estimate of the coherence. Image was adapted from Vinck et al. (2011). C) Examples of linear and non-linear correlations. Each dot represents a sample of the population. For the top row linear correlation measures (orange values, left top) accurately describe the x-y relationship of the signals. For the bottom row, linear correlation measures fail to capture the more complex non-linear correlations, resulting in 0 values. Mutual information (purple values, right top) does capture both the linear and non-linear relationships between x and y. Image was adapted from Ince et al. (2017).
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After discussing LFP-LFP and spike-LFP interactions, we will now take a look at spike-spike interactions. Spike-spike interactions are most commonly investigated using linear correlation measures. One of the most commonly used correlation measures is the cross-correlation between spikes of two neurons (Perkel et al., 1967; Barthó et al., 2004; Lansink et al., 2009). Cross-correlations show whether the neurons have a propensity to fire together and if so in which temporal order. A second way to use correlations in spike data is to check whether firing rates of different units fluctuate synchronously over time. Using linear correlation measures like Pearson’s R, we can check for simple patterns. If unit A and B are positively correlated an increase in firing rate of A will be accompanied by an increase in the firing rate of B. The more A fires, the more B will fire as well. However, in biology we often do not see simple linear interactions like this. An example of this is the inverted ‘U’ shaped curve representing the effect of many neurotransmitters (Baldi and Bucherelli, 2005). What if two neurons have an inverted U-shaped interaction pattern? When neuron X increases its firing rate, neuron Y will initially increase as well. However, given very high firing rates of X, Y will decrease its firing. The firing rates of both neuron pairs A-B and X-Y may be coupled to each other. The firing rates of A and B have a linear relationship. The firing rates of X and Y are not linearly correlated, but they are non-linearly correlated, see Figure 6C. Both these types of interactions contain information. Knowing the firing rates of A and X both allows one to predict B and Y. Neurons show both types of interactions. For this reason, we used mutual information measures, which measure both linear and non-linear interactions to determine the dependency between pairs of neurons.

We use two information measures. The first is conditional mutual information (cMI) and the second conditional delayed auto-mutual information (cDAMI). cMI (Steuer et al., 2002; MacKay, 2003) was computed to measure the functional coupling between pairs of neurons across behavioural states in chapter 3. In chapter 4 we use cMI to calculate the mutual information between the current firing rate of a neuron and the position of the robot, while controlling for the position of the rat (different rat positions constitute the conditions). For cMI one can choose the constituents of the pairs. Two neurons from the same area can be selected to look at mutual information within an area, or one neuron from area 1 and another from area 2 can be chosen to assess the mutual information across these two areas, or one neuron and the position of the robot.

The second measure, cDAMI (Pompe et al., 1998; MacKay, 2003), looks at recurrent connections of neurons. In other words, it quantifies how well the current activity of a neuron predicts its future activity. This interaction is determined for each behavioural state. Here we did not distinguish between direct monosynaptic self-feedback (autaptic connections) and polysynaptic feedback, which is determined by other neurons.
Questions in this thesis

In chapter 2 we look into oscillatory activity within S1BF of the rat. Gamma oscillations are associated with local operations, and Cardin et al. (2009) have shown that gamma oscillations can be induced in S1BF by optogenetically stimulating PV+ interneurons. Under naturalistic conditions gamma oscillations have not always been observed. Using intracellular recordings Poulet and Petersen (2008) did not find gamma oscillations in S1BF. A few studies using extracellular recordings have reported increased gamma power during whisking or vibrissae stimulation (Jones and Barth, 1997; Zagha et al., 2013). However, these studies did not investigate spike-LFP or LFP-LFP gamma-phase synchronization to investigate the specificity of the gamma responses.

In this chapter we first ask the question: Are there behaviourally coupled gamma oscillations in S1BF? This question applies to both intra- and extracellularly recorded signals. If there is gamma activity during natural behaviours, is this gamma coherent across different recording sites? To investigate if neuronal firing is locked to gamma oscillations in the LFP, we first segmented our recorded single units in different subgroups (e.g. excitatory and inhibitory) before calculating gamma phase locking. Because segmenting extracellular activity is not trivial, we contrasted our extracellular classification with classification results of intracellular whole cell recordings of defined populations (Excitatory, PV+, SS+t+ and non-fast spiking cells; Gentet et al., 2010, 2012). Based on the phase locking results, can we distinguish between ING and PING type mechanisms for the generation of gamma?

We are not only interested in neuronal communication within S1BF using gamma oscillations. Other rhythms, like theta and beta have also been shown to be important for intra- and inter-areal communication. Making use of the simultaneous multi-area recordings we next investigated at which frequencies we can find phase locking (LFP-LFP and spike-LFP) within and between S1BF, V1M, PRH and CA1.

In chapter 3 we look into intra- and inter-areal connectivity during different behavioural states; active wakefulness, quiet wakefulness, and non-REM sleep. As found by Massimini et al. (2005) connectivity between brain areas can change across behavioural states. The transition from wake to sleep significantly changed the extent of the spread of neural activity following TMS activation of the premotor cortex. Activation during wakefulness resulted in sustained widespread activity throughout the brain, while this same activation during slow wave sleep only induced local short-lasting activity.

In this chapter we will investigate state-dependent changes in spike-spike interactions, both within and between areas. We will use mutual information measures to estimate functional connectivity in the Touch and See dataset. The advantage of using these measures is that they include both linear and non-linear correlations between firing rates of pairs of neurons. Having simultaneous record-
ings from four areas, we compared mutual information across different areas.

Similar to chapter two, we divided the neural activity in an excitatory and inhibitory population. This allowed us to investigate if functional connectivity is altered across behavioural states, between these populations.

Finally, we divide our neuronal population based on task-related activity. Are neurons which show behavioural correlates to the task more strongly correlated to each other? Do they form a functional network? Is the interaction within these networks maintained across different behavioural states or are they state-dependent?

In chapter 4 we explore perirhinal function and compare it to HPC, S1BF, and V1M. The perirhinal cortex is widely regarded to be important in object recognition, but it is also deemed to serve an important function in learning complex configurations. In this chapter we will explore a surprising finding involving the perirhinal cortex in the spatial segmentation of the task environment.

In the final experimental chapter, we display results from the Rat Robot experiment. Being social animals, it is important for rats to not only pay attention to themselves, but also to others. Here we investigate if neurons in CA1 can function perform a function similar to mirror neurons found in pre-motor areas. Can CA1 neurons also code for the position of another moving agent in addition to the position of the self. In this experiment the “other” was a small robot (e-puck). Can neurons in CA1 also keep track of other moving agents? If so, do they show mirror-like place field activations or is the other represented by modulations of place field activity (rate or global remapping)?

References


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How brain state, spatial, and social context affect neural processing in rat perirhinal cortex, hippocampus, and sensory cortices
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Walk of life | How brain state, spatial, and social context affect neural processing in rat perirhinal cortex, hippocampus, and sensory cortices
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