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Zpracování informace o sociálních interakcích hipokampálními neurony potkana.

The Processing of Social Information by Neurons in the Rat's Hippocampus.

Diplomová práce

Vedoucí diplomové práce: RNDr. Eduard Kelemen, Ph.D.

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Podpis

Poděkování

V první řadě děkuji svému školiteli, RNDr. Eduardu Kelemenovi, Ph.D., za vedení mé práce, za nebývalý vhled do dané problematiky a ochotu se o něj podělit, za trpělivost. Také bych rád poděkoval svým kolegům, jmenovitě Mgr. Tereze Rydzykové, za cenné rady ohledně stereotaktických operací a behaviorální části pokusu, a dále Nikhilovi Ahujovi, Msc, Ph.D., za nemalou pomoc s elektrofysiologickou částí mého experimentu. Konečně chci poděkovat své rodině, bez jejíž podpory by byla jak diplomová práce, tak celé magisterské studium téměř nemyslitelné.

ABSTRAKT

Schopnost vnímat a zpracovávat sociální podněty v prostředí je životně důležitá napříč téměř celou živočišnou říší. Hipokampální formace byla vždy tradičně spjata s prostorovou pamětí. Nedávný výzkum však poukázal na roli hipokampu, konkrétně CA2 oblasti, ve zpracování sociální informace a kódování sociální paměti. Kromě jiného se objevily nové behaviorální i elektrofysiologické studie vyzdvihující význam spánku pro fysiologické zpracování sociální informace. Ve své diplomové práci jsem se zabýval zpracováním sociální informace neurony v CA2 oblasti hipokampu u potkana. Nahrával jsem jednotkovou aktivitu z hipokampu bdělých zvířat během behaviorálního experimentu, ale také ve spánku. Můj experiment zahrnoval nové paradigma, při kterém byl experimentální potkan vystaven sociální stimulaci v podobě dvou stimulačních potkanů na čtyřramenném bludišti. Pozoroval jsem, že aktivita některých neuronů byla v bludišti prostorově organisována. Prostorová aktivita neuronů se změnila nejen poté, co byl experimentálnímu potkanovi presentován sociální kontext, ale i poté, co byli stimulační potkani přemístěni do jiných ramen. Konečně jsem pozoroval také změnu aktivity neuronů ve spánku následujícím behaviorální úlohu. Výsledky mé práce prohlubují dosavadní znalosti neuronálních mechanismů týkajících se kódování sociální informace v hipokampu.

Klíčová slova: hipokampus, sociální paměť, jednotkové nahrávání, spánek.

ABTRACT

In order to survive, an animal must be able to integrate vital information about it's surroundings, such as information about the environment and the social interactions therein. Decades of research have established the hippocampal formation as a structure indispensable for spatial memory. It was only recently, though, that evidence has emerged suggesting that the hippocampus, most notably the dorsal CA2 region, also supports the encoding of social information. New behavioural as well as electrophysiological evidence appeared, highlighting the importance of sleep for the processing of social information. In my thesis, I used microelectrodes to record the electrophysiological activity of individual CA2 neurons from freely-moving rats, during wake as well as in sleep. In order to study the processing of social information by hippocampal neurons, I employed a novel experimental paradigm in which social stimulation, in the form of two rat conspecifics, was presented in a spatial context. I report that the discharge of some CA2 neurons was organised within the experimental maze, even after social stimulation was added. Moreover, I observed that the spatial activity of neurons changed after the addition of social stimuli, and that it further changed when the location of the two conspecifics was shuffled. Finally, I found that the firing rates of neurons changed in post-learing sleep. The results of my work aim to deepen the knowledge of the neuronal mechanisms involved in the encoding of social information within the hippocampus.

Key words: hippocampus, social-recognition memory, single-unit recording, sleep.

ABBREVIATIONS

Avpr1b = Vasopressinergic 1b receptor

CA1 = Cornu Ammonis Area 1

CA2 = Cornu Ammonis Area 2

CA3 = Cornu Ammonis Area 3

DAC = Digital-analog converter

DG = Dentate Gyrus

EC = entorhinal cortex

FRM = Firing Rate Map

GABA = Gamma-aminobutyric Acid

IEG = Immediate Early Gene

lEC = lateral Entorhinal Cortex

LFP = Local Field Potential

LTP = Long-term Potentiation

mEC = medial Entorhinal Cortex

MWM = Morris Water Maze

PCP4 = Purkinje Cell Protein 4

PV = Population Vector

SWS = Slow-wave sleep

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1. INTRODUCTION

The hippocampal formation is a unique structure of the animal brain. It has been studied extensively by neuroscientists during the past decades, mostly in context of spatial navigation and memory. Indeed, neurons were identified within the hippocampus which appear to code for space, maybe even represent space. With time, however, it became clear that the hippocampal neuronal networks are able to process other types of information, and that the hippocampal function is much more complex.

Most notably, it was recently shown that some hippocampal regions, specifically dorsal CA2, play an essential role in the processing of social information. Any animals must be able to distinguish its conspecifics from other species, as well as it must be able to discern one conspecific from another. It was demonstrated that social recognition memory, the life-depending aptitude of an animal to remember one of its own kind, relies on the dorsal CA2. New evidence has emerged, suggesting that the neurons in the rat's CA2 indeed process social information, and what is more, they do so differently in sleep and in wake.

In my thesis, I recorded electrophysiological activity of neurons from the rat's dorsal CA2, in wake as well as in sleep, to study social coding in a spatial context. My experimental paradigm included two social stimuli, positions of which were shuffled around the maze, in order to differentiate whether a neuron processes social or spatial information. I inquired (a) whether the activity of neurons is organised within the maze, (b) whether this spatial organisation changes with the addition of social stimulation, (c) whether the shuffling of social stimuli affects the organised discharge of neurons, and (d) whether the neuronal activity will change in sleep. As such, my thesis comprised four major pars:

First, in the *theoretical background*, I review the current state of knowledge there is about the processing of information by neuronal ensembles in the rat's hippocampus. Some essential computational features of hippocampal neuronal networks are discussed. Then, in greater detail, I write about the encoding of spatial information by hippocampal neurons as well as neuronal ensembles. The last part of the theoretical background is devoted to the relatively thinner evidence we have about the coding of social information within the hippocampus in wake and also in sleep.

Second, in *materials and methods* and in *data analysis,* I clarify the methodology that was used in my thesis. I describe the recording of single units by tetrodes from freely-moving rats and I explain how do the single units give evidence about the activity of individual neurons. All analyses used in my experiment are elucidated in this part.

Third, in *specific aims,* I describe the questions I had about the processing of social information by the hippocampus, and then, in *results*, I explain how were these inquiries resolved.

Finally, in *discussion*, the results of my work are broken down in detail. I collate what was discovered with the work of other researchers, and I hypothesize about how my observations relate to the function of hippocampal neuronal networks.

2. THEORETICAL BACKGROUND

2.1 Hippocampal Anatomy and Circuitry

Distinct Types of Neurons in the Hippocampus.

Electrophysiologists have long observed two distinct kinds of units in the hippocampus: *complex-spike cells* and *theta cells* (Ranck, 1973; Fox and Ranck, 1975, 1981). Theta cells, earning their name for their increase of firing during hippocampal theta oscillations, can be distinguished by high average (>8 Hz) as well as maximal (>30 Hz) firing rate (Ranck, 1973; Fox and Ranck, 1981). These firing rate values are almost 10x higher than those of complex-spike cells and are consistently present during various behaviours of the animal, in wake and also during sleep (Fox and Ranck, 1981).

The main feature of complex-spike cells is their ability to discharge multiple subsequent spikes in a very short time (5-10 ms) (Ranck, 1973). Complex-spike cells may or may not exhibit this feature whereas theta cells never fire in complex spikes, only in single spikes (Ranck, 1973). What is more, the extracellular spikes of complex-spike cells were observed to be longer (300-500 μs) than those of theta cells (150-250 μs).

While theta and complex-spike cells are electrophysiological concepts, they are deeply related to the hippocampal anatomy. From an anatomical and histological perspective, there are the *pyramidal* or *principal neurons*, whose somata lie in the stratum pyramidale of the Cornu Ammonis (CA) areas, and the *granule neurons*, whose bodies reside in the stratum granulosum of the Dentate Gyrus (DG), both of which are glutamatergic (Andersen et al., 1964). Furthermore, there are the *inhibitory interneurons* located in the vicinity of the pyramidal and granule cells (Ribak et al., 1978). Eventually it became apparent that these neuronal populations overlap; it has been demonstrated that the pyramidal and granule cells correspond to the complex-spike units observed on electrophysiological recordings and, in a similar fashion, the theta cells most likely correspond to the inhibitory interneurons (Fox and Ranck, 1975, 1981). Throughout this text the terms "pyramidal cells" and "interneurons" are used to describe both anatomical and electrophysiological characteristics of those neurons.

Connections between Regions of the Hippocampus.

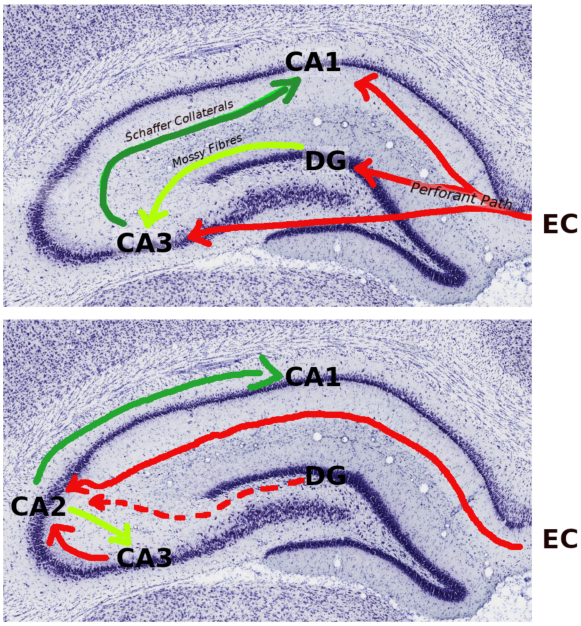

***Figure 1**: major connections in the rat's dorsal hippocampus. **Top**, the trisynaptic circuit. **Bottom**, projections to and from the CA2. CA1, Cornu Ammonis Area 1. CA2, Cornu Ammonis Area 2. CA3, Cornu Ammonis area 3. DG, Dentate Gyrus. EC, Entorhinal Cortex. PP, Perforant Pathway. MF, Mossy Fibres. SC, Schaffer Collaterals. The path from DG to CA2 is depicted by dashed line to highlight the fact that evidence for it is not abundant. From Brainmaps.org, modified.*

Before explaining any topic of systems neuroscience, it is essential to grasp at least a basic notion of the brain's circuitry. This is especially true for the hippocampus, as this allows, in combination with electrophysiological studies, to hypothesize about the direction of flow of information (Treves and Rolls, 1992; McClelland et al., 1995).

When it comes to the connectivity between hippocampal regions, the *trisynaptic pathway* is traditionally mentioned (Andersen, 1975). CA1, CA3 and DG pyramidal neurons receive afferent inputs from the EC via the *Perforant Pathway*. Furthermore, the granule cells in DG project to the dendrites of the CA3 pyramidal neurons via *mossy fibres*, which in turn project to the dendrites of CA1 pyramidal neurons via *Schaffer collaterals* (see fig. 1, top).

As the CA2 was the main target for my recording, it is important to know how it relates to the established trisynaptic circuit (see fig. 1, bottom). In a fashion similar to CA1, the CA2 receives afferent input both from the EC and the CA3 (Chevaleyre and Siegelbaum, 2010; Jones and McHugh, 2011). It also extends efferent projections to the CA1 (Jones and McHugh, 2011). Even projections from the DG to the CA2 have been reported by a solitary paper (Kohara et al., 2014). What is more, studies using adenoviral vectors have demonstrated that there is a reciprocal projection from the CA2 to the CA3 (Cui et al., 2013). This relatively new anatomical knowledge suggests that the CA2 plays a considerable role in the processing of information in the hippocampus.

Autoassociative Connections within the Hippocampus.

All subregions of the hippocampus share one common feat in terms of the wiring of principal cells and interneurons. The principal neurons (pyramidal in CA regions or granule in the DG) send out excitatory projections to the neighbouring interneurons, which, in turn, feed inhibitory projections to the very same pyramidal or granule neurons (Redish, 1999). Thus the interneurons provide a feedback loop by inhibiting the very neurons which excited them in the first place (Redish, 1999).

Apart from this similarity, there are profound differences among the regions. The pyramidal neurons within the CA1 rarely connect to one another, whereas the pyramidal cells within the CA3 are much more interconnected (Ishizuka et al., 1990, 1995; Amaral et al., 1991; Redish, 1999). It has been postulated that a CA3 pyramidal neuron sends excitatory projections to 30 - 70% of the CA3 population (Li et al., 1994). Recent evidence suggests that some kind of interconnection is also present among pyramidal neurons of CA2 (Cui et al., 2013; Okamoto and Ikegaya, 2019). Those interconnecting projections take the form of recurrent collaterals in the sense that a pyramidal cell sends out excitatory projections to almost every other pyramidal cells in the region, including itself, thus effectively creating an autoassociative network (see fig. 2) (Treves and Rolls, 1992). Such factuality greatly impacts the region's computational capabilities.

In systems theory, an *attractor* is a state which a system tends to evolve into and remain in (Hopfield, 1982). Theoretical models have shown that the autoassociative network in the CA3 possesses attractor properties (Rolls et al., 2008). Let us presume that, for example by a simple process of Hebbian learning (Hebb, 1949), some connections in the network are made stronger than others, i.e. when a neuron fires, it activates some neurons more while others not so much (see fig. 2). When external input is introduced into the network, after a brief period of disorganized activity, only the neurons with strong autoassociative connections among each other will remain active. Such network has evolved into its attractor state represented by this specific pattern of neuronal activity.

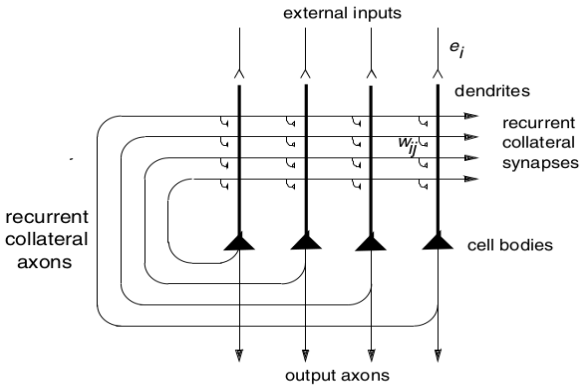

***Figure 2**: schematic depiction of an autoassociative network. w_{ij} represents the i-th synapse of a j-th neuron. Each synapse can be tuned so that a firing of a cell excites the cells it projects to differently. From Rolls et al., 2008.*

Now, let us imagine that there are multiple attractors in the network. When the external input is introduced, the network will evolve into only one of its attractors. This property is called *pattern separation*: the network has evolved into the attractor activity patterns which resembled the input activity pattern the most (Guzowski et al., 2004; Rolls, 2013). Pattern separation itself, however, describes only half of what an attractor network is capable of. It is important to stress out that the external input has to *resemble* an attractor state in order to steer the network towards it, by no means does the input have to be identical to the attractor. This property is called *pattern completion*: an attractor state is achieved even though the corresponding input is incomplete and only partially corresponds to the attractor (Guzowski et al., 2004; Rolls, 2013).

In the case of CA3, the external input would be the Perforant Path from the EC and the mossy fibres from the DG. The activity pattern in the CA3 network might represent some information about the animals surroundings. According to hypotheses, such attractor properties would allow the CA3 network not only to temporally store a representation, but also to compare a previously stored representation with a new one (Rolls et al., 2008; Rolls, 2012). Indeed, there is evidence suggesting that the CA3 (and also the DG) might be performing such

computations with spatial information in rats (Leutgeb et al., 2004, 2007; Vazdarjanova and Guzowski, 2004) (also see chapter 2.2: Processing of Spatial Information by Hippocampal Ensembles.). What remains an open question, though, is whether the processing of information in the CA2 could abide by the same attractor dynamics as does the processing of information in CA3 and DG.

2.2 Spatial Coding in the Hippocampus

Over the years, both human and rat hippocampus has been extensively studied in the context of spatial navigation. There is vast evidence for the hippocampus being crucial for spatial memory (Morris et al., 1982; Zola-Morgan et al., 1986; Bolhuis et al., 1994). It has been demonstrated that rats with lesions to the hippocampus are unable to learn and perform in spatial navigation tasks (Morris et al., 1982). Damage to the rat's hippocampus impairs previously acquired spatial memory (Bolhuis et al., 1994). Not only do full hippocampal lesions cause retrograde amnesia, they also disrupt the ability to reactivate spatial memory when the animal is re-learning a previously known spatial task (Bolhuis et al., 1994; Martin et al., 2005). The amassing evidence has led electrophysiologists to search for the neuronal substrate of spatial information processing in the hippocampus.

Place Cells.

Indeed, neurons which fire in response to the animal's location were found in the CA1 and CA3 hippocampal regions (O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978; Muller et al., 1987). They were named *place cells* (Muller et al., 1987). Discovered by John O'Keefe in 1978, the cell's response was originally attributed to a combination of the rat's location and spatial orientation on a linear track (O'Keefe and Dostrovsky, 1971). Only more thorough analyses have revealed that it is the spatial information which is predominantly processed by these cells (Muller et al., 1987).

There are several interesting properties to place cells. A place cell is the most active when the rat is situated within a specific portion of the rat's environment; this specific location is called a *firing field* or *place field* (Muller et al., 1987). It has been shown that a place cell may exhibit different firing fields in different environments: it may undergo the *remapping of firing fields* (Muller et al., 1987). Some place cells can remap completely in that they cease firing in some environments and resume firing in others, while some place cells may not remap at all and have stable firing fields throughout varying environments (Muller et al., 1987). While retaining similar firing field in different environments, place cells may still change their firing rates inside those firing fields. The phenomenon of a place cell exhibiting different firing rates while having identical firing fields in different environments is called *rate remapping* (Sanders et al., 2019).

The size of a firing field is affected by the size of the environment a rat navigates in (Muller et al., 1987; Fenton et al., 2008). The same cell may display a

single, large firing field in a small cylindrical arena while having multiple smaller firing fields in a chamber three times the size of the arena (Fenton et al., 2008). Being backed by decades of research, the processing of spatial information is arguably better understood than the processing of social information in the hippocampus. The ability to compare the relatively well-understood spatial coding with the novel topic of social coding forms a pivotal aspect of my work. In my thesis, I inspect a neuron's spatial properties in a social context in order to delineate the neuron's ability to encode social information.

Measuring Spatial Coding of Neurons.

There are several ways of looking at a cell's activity with respect to space. Three such methods were applied in my thesis and will be briefly described in this section.

Probably the most straightforward method to visualize the spatial activity of a neuron is by creating a *firing rate map* (FRM) (see fig. 3) (Muller et al., 1987). Although the FRM has seen many modifications since it was first introduced in 1987, in my work it is applied in its original form. At first, the arena which the rat navigates in is decomposed into pixels. Then the *spike map* is created by counting the amount of spikes in a pixel and assigning a colour to that pixel (fig.

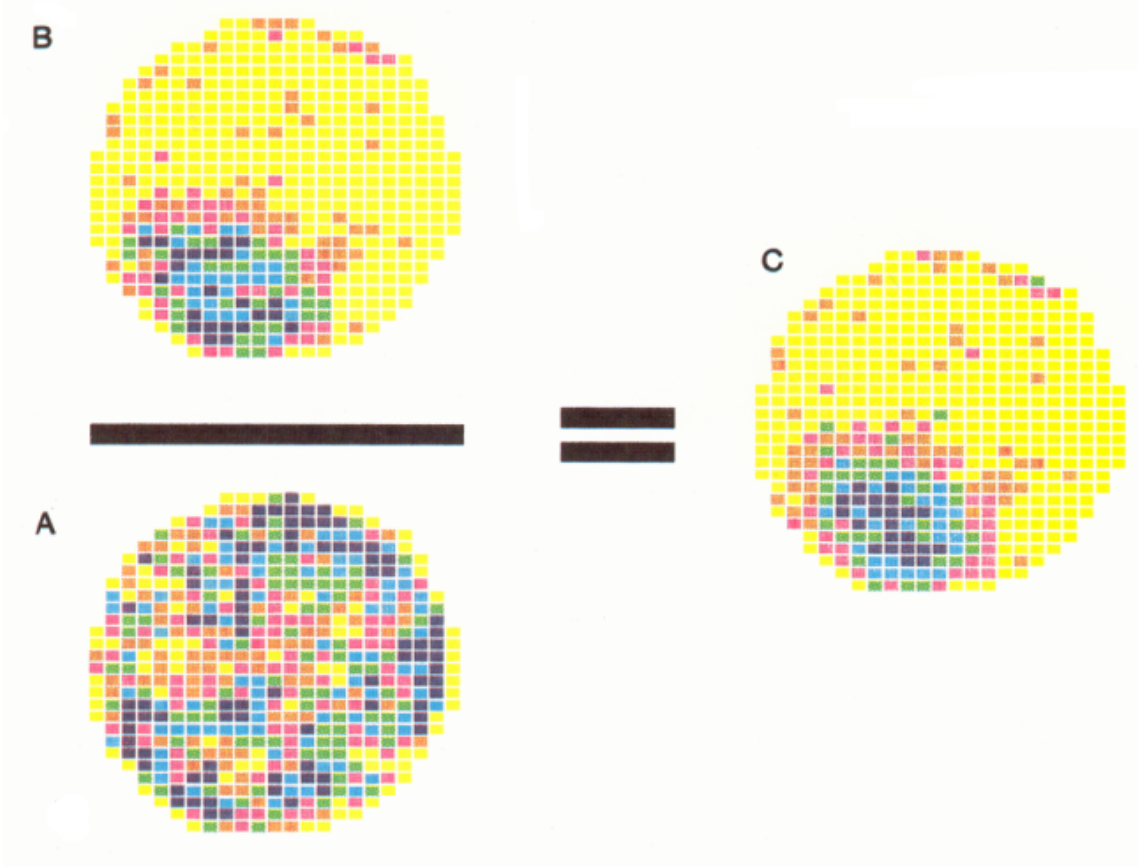

Figure 3*: a firing rate map (FRM) (shown in C) is created by normalizing the spike count in a pixel by the time the rat has dwelt in the corresponding pixels. A, dwell map. B, spike map. Colder colours indicate higher firing rate while warmer colours denote lower firing rate. Yellow means that the neuron did not discharge at all in the corresponding pixel. From the firing rate map (and also from the spike map) it is apparent that a firing field clearly stands out in the lower part of the arena. From Muller et al., 1987.*

B), ranging from yellow (no activity to purple (the highest activity). Afterwards, a *dwell map* is created in a similar fashion as the spike map, except that the colour of each pixel denotes the amount of time a rat has spent in there (fig. 3A). Finally, each pixel of the spike map is divided by the corresponding pixel of the dwell map, thus creating a map of firing rates (fig. 3C). The darker the colour on the FRM, the more spikes per second did the neuron discharge in that pixel. If a neuron is a place cell, it's firing field should be visible from the FRM.

With the introduction of FRMs came the need to enumerate the quality of those maps. Such measure, called *spatial coherence* or *firing rate map coherence* was soon introduced (Muller and Kubie, 1989). To calculate spatial coherence, first a list of firing rates in each pixel is created. Then, a similar list is made, containing mean firing rates in 8 pixels neighbouring to each corresponding pixel. Finally, the values are z-transformed and correlated using Pearson's correlation. As such, spatial coherence is a measure of orderliness or compactness of the FRM.

Another approach, based on the application of information theory (Shannon, 1948) in neuroscience, is the calculation of *spatial information* (Skaggs et al., 1992). Let us presume that a single spike of an ideal neuron carries exactly one bit of information about the rat's surroundings. Let λ be the mean firing rate of a neuron, $\lambda(x)$ be the firing rate of a neuron at location x and p(x) be the probability that a rat is currently at location x. Then spatial information of a neuron's spike is given by:

$$I = \sum_j \lambda(x_j) \log_2\left(\frac{\lambda(x_j)}{\lambda} p(x_j)\right)$$

eq. 1

The spatial information *I* describes how much information about the rat's surrounding does a single spike of a neuron convey relative to the spike of and ideal neuron. It is measured in bits per second. In contrast with the FRM, spatial information allows for the quantification of spatial data and therefore allows for the comparison of spatial coding of multiple neurons at once.

Hypotheses of How Place Cells Code for Space.

In the past few decades, it has been proven that hippocampal neurons process spatial information. Many opinions have stated that hippocampal place cells *represent* space. A more pressing question in neuroscience, though, is *how* do they do it. This question breaks down to two major parts: (a) how is spatial information encoded in a single neuron's activity and (b) how is spatial information encoded in the activity of an ensemble of neurons.

A place cell increases its firing rate when the rat enters the respective firing field, therefore it has been postulated that the spatial information is encoded by the cell's firing rate (Muller et al., 1987). However, it appears that there are other ways than the so called *rate coding* (please note that rate coding a different concept from the previously mentioned rate remapping). Theta waves, LFP oscillations with frequency between 7 and 10 Hz, are prominent in the rats

hippocampus during periods of locomotion (McFarland et al., 1975). It has been demonstrated that place cells also encode spatial information by means of synchronization with the hippocampal theta rhythm, in a process named *theta phase precession* (O'Keefe and Recce, 1993; Maurer et al., 2006). When a rat enters a firing field, the respective place cell begins firing in synchrony with hippocampal theta, i.e. the cell always discharges in a specific moment in the theta cycle. As the rat nears the centre of the firing field, the respective place cell fires progressively sooner in the theta cycle, eventually being almost synchronized with the previous theta cycle (O'Keefe and Recce, 1993; Maurer et al., 2006). Hence it may be said that it is not only the firing rate, but also the temporal relationship with hippocampal LFP oscillations that codes for space.

The phenomenon of theta phase precession is also informative of the relation between a pyramidal cell and an interneuron. The general opinion is that it is the pyramidal neurons which have the properties of place cells (Moser et al., 2008). It was discovered, however, that the interneurons may also exhibit spatial modulation (Kubie et al., 1990; Maurer et al., 2006). It has been demonstrated that the phenomenon of theta phase precession also occurs in the interneurons which are in the vicinity of place cells (Maurer et al., 2006). This is in concurrence with the observation that the pyramidal cells directly and monosynaptically excite their neighbouring interneurons (see 2.1.Autoassociative Connections within the Hippocampus.).

Up until now, the means by which a single neuron may convey information about space were discussed. The way place cells are often presented may encourage the idea that the hippocampal network simply decomposes the surrounding environment and that the (in)activity of every single cell represents one piece of that environment. According to such view, a single neuron would code for one and only one factuality. In the case of place cells, a single place cell would increase it's firing rate when the rat enters a specific part of the arena; only the discharge of the singular place cell would represent this exact location. While it may work this way in some cognitive systems (Quiroga et al., 2005), it appears that other networks function differently (Abeles, 1991; Kelemen and Fenton, 2013).

It appears that in other systems a single factuality is not encoded by the activity of a single neuron, rather by the integrated activity of a neuronal ensemble *(Abeles, 1991; Abeles et al., 1993)*. If place cells followed the ways of *ensemble coding*, a rat's specific location in an arena would be encoded not by a single place cell, but by the synchronized discharge of multiple place cells. The synchronized firing of a unique subset of the place cell ensemble would represent a specific location.

Indeed, there is evidence which speaks in favour of ensemble coding in the hippocampus (Kelemen and Fenton, 2010, 2013). A behavioural task exists in which a rat must navigate with respect to two different sets of spatial cues, two different spatial reference frames (Fenton et al., 1998). It was demonstrated that those place cells which coded for location in the same reference frame were more likely to discharge as close as 15 ms together in time (Kelemen and Fenton, 2010, 2013). Such observations suggest the existence of at least two ensembles of place cells, one for each reference frame.

To conclude, the amassing evidence suggests that it is equally important to study the activity of individual hippocampal neurons as it is to focus on the integrated activity of a neuronal ensemble.

The Spatial Properties of Neurons Differ Across the Hippocampus.

Spatial coding is, to a certain extent, noticeable in almost all pyramidal neurons of the hippocampal formation (Moser et al., 2008; Oliva et al., 2016). Nevertheless, the spatial qualities of those neurons vary (Oliva et al., 2016). Differences in the features of place cells have been observed not only between the main hippocampal regions, but also within those regions (Oliva et al., 2016). The following terminology is used in this text to describe the sub-regions of the hippocampus: *lateral CA1* and *lateral* CA3 denote the portions of CA1 and CA3 which are closest to the CA2, while *medial CA1* and *CA3* are the sub-regions which are closer to the mid-sagittal plane and farther from the CA2. As a rule of thumb, it could be stated that many properties of neurons related to spatial coding follow a unitary trend along the mediolateral axis of the hippocampus. Starting in the medial portion of the CA1, they intensify and increase until reaching the CA2; in the CA2 and lateral CA3 they diminish, only to increase again in medial CA3 (Henriksen et al., 2010; Lu et al., 2015; Mankin et al., 2015; Alexander et al.,

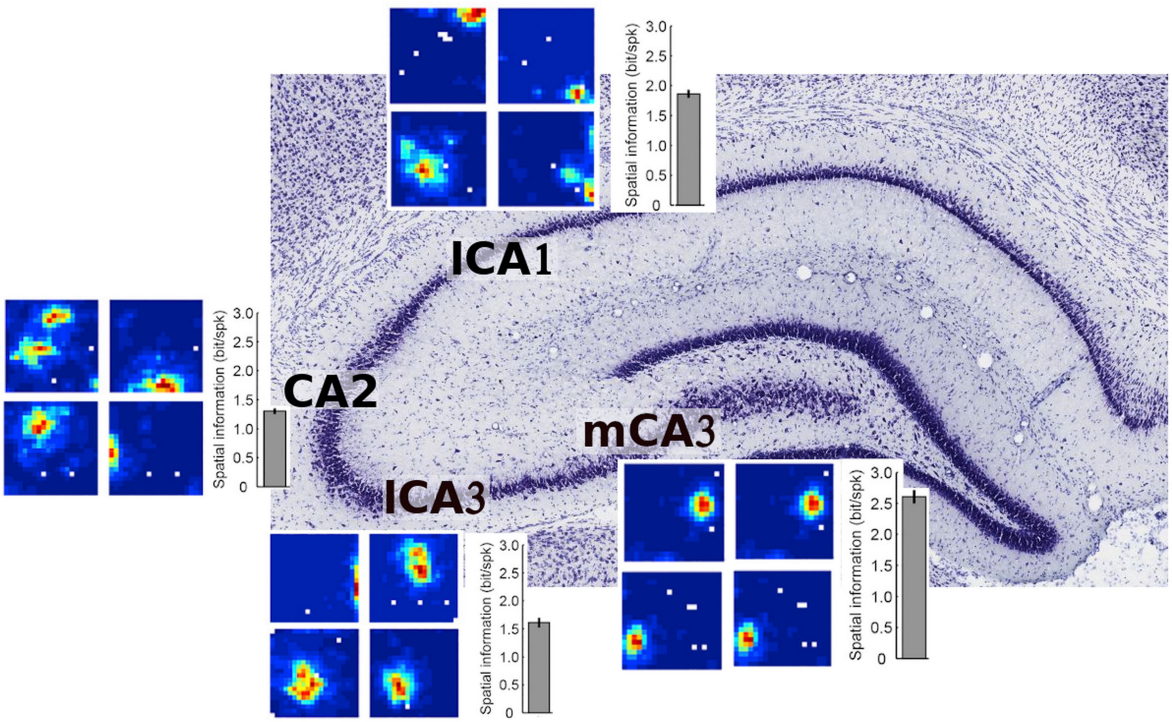

Figure 4*: examples of FRMs and mean spatial information of place cells from medial CA3 (mCA3), lateral CA3 (lCA3), lateral CA1 (lCA1) and the CA2 depicted in context of a rat's left dorsal hippocampus. Four examples of place cells and their respective FRMs are shown for every hippocampal (sub)region. Note the single small firing fields in medial CA3 (mCA3) contrasting the relatively poorer spatial coding (multiple, larger firing fields) in the CA2 and also the large firing fields in lateral CA3. The firing fields of neurons in the lateral CA1 are slightly larger than of those in the CA3, yet only single firing fields are present. The FRMs shown here were "smoothened" (Skaggs et al., 1992). From Lu et al. 2015, and Brainmaps.org, modified.*

2016; Oliva et al., 2016; Donegan et al., 2020). The following section will compare the spatial properties of neurons across those subregions.

Place cells throughout the hippocampus differ when it comes to the size and quantity of firing fields (see fig. 4). It was observed that, in general, the neurons in the CA3 and especially in the CA2 tend to have more than one firing field when compared to the CA1 (Oliva et al., 2016). The CA1 itself is not homogenous in that aspect; the place cells of medial CA1 tend to have on average slightly more firing fields than those of lateral CA1, which usually display a single firing field (Henriksen et al., 2010; Oliva et al., 2016). In contrast to the small firing fields of medial CA3 and lateral CA1 cells, the largest firing fields were found in the neurons of the CA2 and also of lateral CA3; some authors have quoted them to be almost 2.5 times larger (Lu et al., 2015) while others have noted only a cca 30-40% increase in size (Oliva et al., 2016). In concordance, spatial information follows a similar trend (fig. 4). For place cells in the CA1 the overall spatial information is higher (almost 2.5 bits/spike in lateral and 2.1 bit/spike in medial CA1) (Henriksen et al., 2010) whereas the spatial information of CA2 place cells may fall short of 1.2 bits/spike (Oliva et al., 2016). The medial CA3 neurons were also reported to have higher spatial information than the neurons in lateral CA3 (Lu et al., 2015). It can be concluded that the medial CA3 and lateral CA1 populations display the most accurate coding for space, as opposed to the relatively poorer spatial coding of the CA2 and lateral CA3 neurons.

It was observed that the major hippocampal regions also differ in firing rates. The general agreement is that place cells in the CA2 manifest higher mean firing rate than place cells in the CA3 and CA1 (Mankin et al., 2015; Oliva et al., 2016). Some have mentioned that the peak firing rates follow a similar disposition (Oliva et al., 2016) while others have stated that there is no significant difference between peak firing rates across the three regions (Mankin et al., 2015). This discrepancy may be explained by different behavioural protocols used in the respective experiments.

Individual place cells in the CA2 are much more prone to the remapping of firing fields (Lu et al., 2015). When the rats were tested in 11 plainly distinct environments, the CA3 place cells displayed stable firing fields in 1-2 of the environments. The CA2 place cells, however, discharged almost in every single environment and had unstable firing fields (Lu et al., 2015).

Theta phase precession is detectable in all place cells of the hippocampal formation, albeit the slope of the precession appears steeper for lateral CA1 neurons than for CA2 and lateral CA3 neurons (Oliva et al., 2016). It seems that all neurons exhibit rate remapping, but again this is less apparent for cells in lateral CA3 and the CA2 (Lu et al., 2015).

The knowledge of spatial properties of neurons from distinct hippocampal (sub)regions, notably the CA2 and lateral CA1, is imperative to my work. The aforementioned observations were made in rats foraging in an open field or running on linear tracks, i.e. under experimental conditions which are different from the one used in my thesis. Nevertheless, it provides me with valuable information which can be compared with the data obtained in my experiment.

Processing of Spatial Information by Hippocampal Ensembles.

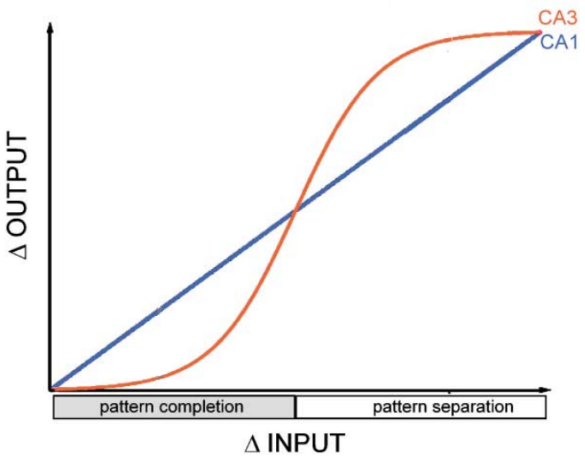

***Figure 5**: schematic illustration of the proposed function of the CA3 network and it's difference to CA1. The x axis symbolizes the change in an environment (input), the y axis symbolizes a continuum of attractors (output). The inflexion point illustrates a threshold. Below the threshold, input has not changed significantly and so the output will be very similar to the input, thus the CA3 network is performing pattern completion. If the change of input exceeds the threshold, the output will predominantly change, thus performing pattern separation. The CA1 is thought to work in a more linear fashion.*

As it was explained above, the spatial properties of individual neurons are diverse throughout the hippocampus. To fully understand the problem of spatial coding, however, one must study the diversity also on the level of neuronal networks. Indeed, there is evidence suggesting that the neuronal ensembles in CA1 and in CA3 regions process spatial information differently (Guzowski et al., 2004; Lee et al., 2004; Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004). A convenient way of studying ensemble activity is to study the expression of *immediate early genes* (IEGs). Being important cellular transcription factors, elevated cytoplasmic levels of IEG transcripts have been correlated with the increase of synaptic activity; some IEGs have also been implied in learning and memory (Guzowski et al., 2001). Both electrophysiological and IEG studies have shown that CA1 and CA3 place cell populations show distinct responses to a changing environment (Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004; Wintzer et al., 2014).

In an experiment in which the animal's surroundings were gradually transformed so that some spatial cues in a session were dissimilar to the ones in the previous session, overlapping population of CA1 neurons were recruited, i.e. a fraction of neurons which were active in the previous arena was also active in the next one (Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004). Significantly lower overlap between sessions was observed in the CA3 population, suggesting that the CA3 strictly discerns the subtlest of difference in an environment (Leutgeb et al., 2004). On the other hand, when the gradual transformation of environment was measured by different means, e.g. the progressive rotation of distal versus proximal spatial cues, different observations were made (Lee et al., 2004). In those experiments, the CA3 ensembles did display a high degree of overlap, even higher than the CA1 (Lee et al., 2004; Vazdarjanova and Guzowski, 2004). Indeed, it appears that under some circumstances, the changing environments are perceived by the CA3 as being completely different while under other circumstances, the changing environments are interpreted as being similar (Lee et al., 2004; Leutgeb et al., 2004; Vazdarjanova and Guzowski, 2004).

As was described above, the pyramidal cells in the CA3 region are extensively interconnected in an autoassociative manner (Ishizuka et al., 1990; Redish, 1999), which has important consequences for the ensemble's computational abilities. The presented evidence therefore corroborates the theory that the

hippocampal CA3 region functions as an autoassociative network and, as such, is capable of pattern separation as well as pattern completion (fig. 5) (Guzowski et al., 2004). It seems that there is a certain threshold for similarity levels between two distinct environments: the CA3 will perform pattern separation for wholly dissimilar environments but will also display pattern completion when the two environments are somewhat alike.

The discovery of distinct computational abilities of CA3 and CA1 network has inspired thoughts about the flow of spatial information in the hippocampus. It is a fact that while both regions receive afferent input from the EC, only CA1 receives additional projections from the CA3 and not the other way around (Andersen, 1975). It has been proposed that the CA3 is an upstream region in the CA3-CA1 network. The medial EC is an important component of the hippocampal spatial navigation system as it also accommodates neurons which process spatial information (Moser et al., 2008). Therefore it has been hypothesized that, after obtaining novel spatial information from the EC, the CA3 compares the information with the stored spatial representation, performs either pattern separation or pattern completion, and then feeds the "yes/no" result to the CA1 (Vazdarjanova and Guzowski, 2004). The CA1 finally integrates informational streams from the CA3 and EC (Vazdarjanova and Guzowski, 2004).

Substantial attention has been given to the computational properties of CA1 and CA3. However, only recently it was studied how the CA2 ensembles react to the gradual change of spatial cues (Wintzer et al., 2014). It was discovered that the CA2 spatial representations had remapped completely with every subtle difference in spatial cues, and, what is more, they had remapped more extensively than the CA1 or even the CA3 (Wintzer et al., 2014). It would be tempting to assume that this is evidence of the CA2 performing pattern separation, given the fact that recurrent projections are also present in the CA2 (Cui et al., 2013). However, LTP studies have revealed that the CA2 is not very plastic; protocols which induced LTP in CA1 failed to do so in the CA2 pyramidal cells (Zhao et al., 2007). This suggests that if the CA2 indeed were an attractor network, it would be a fairly rigid one (Rolls, 2013). It remains an open question, then, to what extent could the CA2 and the processing of social information therein be modelled as an attractor network.

2.3 Social and Temporal Coding in the Hippocampus

The discovery of place cells commenced a prominent line of research into the hippocampus' ability to code for space. The hippocampus indeed seems to process spatial information if space is what the scientist inquires about in their experiment. When a rat navigates in an environment containing no other stimuli than spatial cues, the activity of neurons in the hippocampus changes, hence it was concluded that the neurons must process spatial information. Yet this does not necessarily mean that space is the *only* thing that hippocampal neurons encode. The way we

interpret the function of hippocampal ensembles is, by its very nature, biased by how an experiment is designed.

As it turns out, the hippocampal neurons also change their activity in response to non-spatial variables, such as the passing of time (Pastalkova et al., 2008; MacDonald et al., 2011; Mankin et al., 2012, 2015). For example, and experiment was conducted in which running on a linear track was interposed by a short period of the rat being stationary. It was observed that novel neuronal ensembles were recruited during these stationary periods and that within those ensembles, individual neurons discharged in a fixed temporal order (MacDonald et al., 2011). It was therefore ascertained that hippocampal neurons may also encode the temporal aspects of a rat's experience and that the hippocampus plays a role in the planning of future actions (Pastalkova et al., 2008; MacDonald et al., 2011). Although the aforementioned experiments concerned the neurons in the CA1 and CA3 regions, it was eventually revealed that this topic is also highly relevant for the CA2 (Mankin et al., 2012; Lu et al., 2015).

The Effect of Time on the CA2.

In general, it appears that the CA2 spatial representations are volatile in time, and what is more, are volatile to an even greater extent than the representations in the CA1 and CA3 (Lu et al., 2015; Mankin et al., 2015). This effect is evident at the level of individual neurons as well as at the level of neuronal ensembles.

The firing fields of individual place cells were studied in two different environments presented in a sequence, i.e. one environment was being alternated with the other (Mankin et al., 2012). Unsurprisingly, it was noted that throughout the sequence, CA1 and CA3 place cells displayed two different firing fields, one for each environment. What was more surprising, though, was the fact that the firing fields of CA2 place cells have remapped almost every time, regardless of the kind of environment presented. Indeed, when the experiment was repeated except only one kind of environment was presented, the CA2 place cells proved unstable as opposed to the CA1 and CA3 place cells (see fig. 6A) (Mankin et al., 2015).

These findings were also true at the population level (Lu et al., 2015; Mankin et al., 2015). An interesting way how to describe the activity of a place cell ensemble in an environment is by *population vectors* (PV). In brief, the firing rate of every neuron of the ensemble at a given location is stored into a PV; a set of PVs for every location then describes the ensemble's activity in that environment (Mankin et al., 2015). It was demonstrated that when the environment is constant, the PVs of CA1 and CA3 ensembles may be correlated for up to 31 hours while the CA2 ensembles are much less stable, becoming less correlated after 30 minutes and completely uncorrelated after 6-7 hours (fig. 6B and C) (Lu et al., 2015; Mankin et al., 2015).

Although the temporal coding in the CA2 was not the main focus of my thesis, it bears great significance to it. The presented evidence suggests that the CA2 is highly sensitive to the passing of time. It seems that the CA2 ensembles can reliably encode a static spatial representation for a rather short period of time, and one does not have to go far to make a similar assumption about social coding

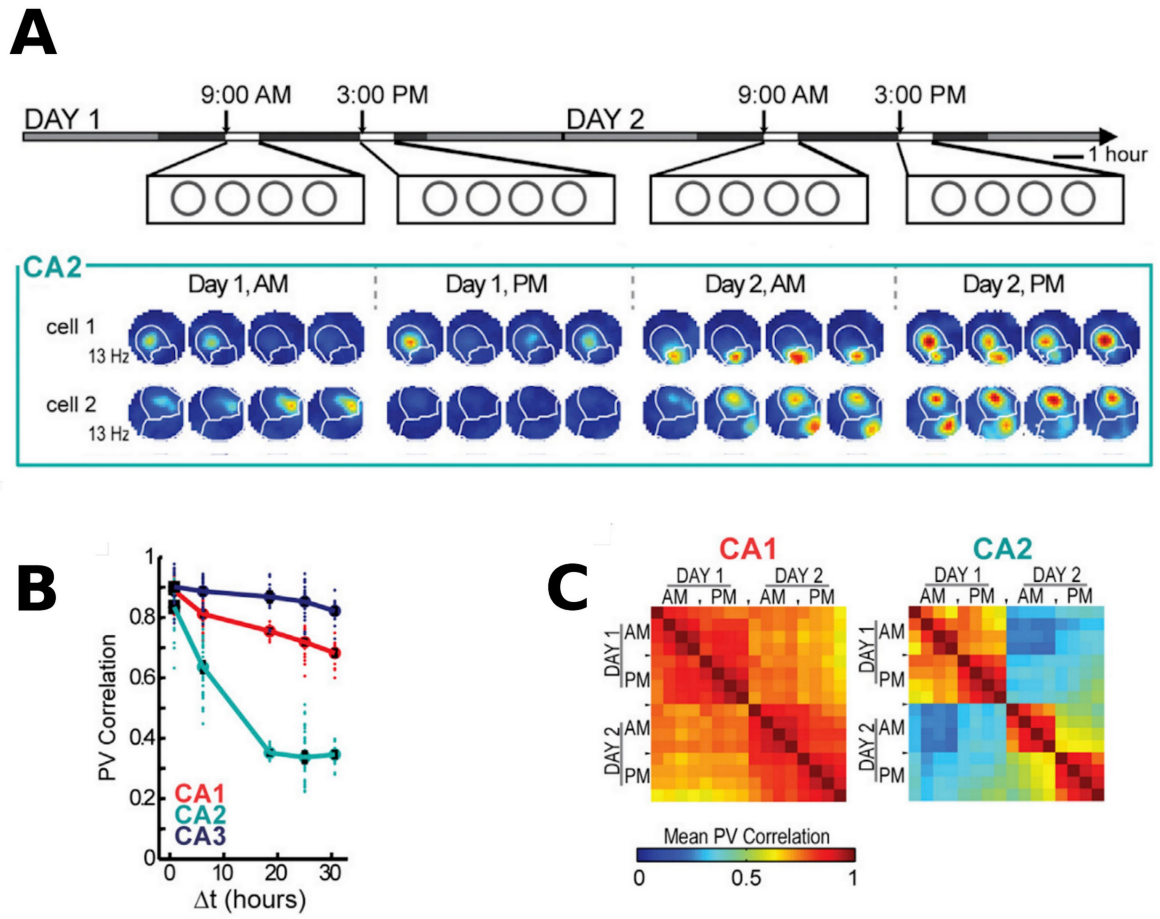

Figure 6*: neuronal activity in the CA2 changes with time.* ***A****, an experimental paradigm in which one environment was presented repeatedly throughout the course of 31 hours (top row). Bottom row shows FRMs of two model place cells recorded from the CA2.* ***B****, a chart showing the change of PV correlation as a function of time for population in the CA2, CA1 and CA3. Each dot represents the averaged Pearson's correlation between all PVs in a pair of sessions. Note the rapid decrease for the CA2.* ***C****, colour-coded matrices of averaged Pearson's correlation values between all pairs of sessions for CA2 and CA1. Warmer colours denote high correlation whereas cold colours mean low correlation. Note the stability of CA1 ensemble coding for space as opposed to the CA2. From Mankin et al., 2012, 2015.*

(Donegan et al., 2020). Therefore it should be advantageous to view the CA2 as a structure which processes the information about an animal's surroundings in a highly dynamic manner, integrating space, time and also social stimuli (Alexander et al., 2016; Donegan et al., 2020; Oliva et al., 2020).

The CA2 is Essential for Social Memory.

Social memory or *social recognition memory* in rats is defined as the animal's ability to recognize its conspecific (Thor and Holloway, 1982). The quantitative measurement of social memory often relies on the rat's inherent drive to explore. It is observed that the time spent by olfactory exploration of an unknown conspecific is greater than that of a familiar one, therefore it may be said that the rat remembers better the one conspecific which it does not explore as much (Thor and Holloway, 1982).

In contrast to other hippocampal regions, the CA2 is relatively difficult to discern by optical microscopy. Only the discovery of specific molecular markers which could differentiate the CA2 from the CA1 and CA3 enabled biologists to study the region in more detail (Lein et al., 2005; Young et al., 2006). For example, *Purkinje Cell Protein 4* (PCP4) was located in the CA2 and also in DG, but not in other regions (Lein et al., 2005). More importantly, however, the pyramidal neurons of the CA2 were found to be the only hippocampal cells to express the *vasopressinergic receptor 1b* (*Avpr1b*) (Young et al., 2006), a receptor which is a part of the vasopressinergic afferents coming from the paraventricular nucleus of hypothalamus (Cui et al., 2013). Avpr1b is the major mediator of vasopressinergic modulation in mammalian brain and, as such, has many functions, some of which include a role in social recognition and social aggression in rats (Young et al., 2006). This is in agreement with the observations that the CA2 might be implied in social aspects of some neuropsychiatric disorders, such as schizophrenia (Benes et al., 1998; Chevaleyre and Piskorowski, 2016).

A fairly convincing evidence was brought by experiments involving genetic manipulation and inactivation of CA2 neuronal populations (Hitti and Siegelbaum, 2014; Pagani et al., 2015). Aggressive behaviour is often seen in rodents when an unfamiliar conspecific intrudes into another's territory (Miczek and O'Donnell, 1978). It was demonstrated that such aggressive behaviour is reduced in Avpt1b-knockout mice, yet reappears when Avpr1b is re-introduced into CA2 pyramidal neurons via lentiviral vectors (Pagani et al., 2015). Moreover, the selective inactivation of CA2 pyramidal cells by tetanotoxin fused with adeno-associated viral vector resulted in a deficit of social recognition memory in rats (Hitti and Siegelbaum, 2014). Interestingly enough, this inactivation of CA2 did not impair canonical spatial memory as tested on a MWM, neither did it affect hippocampus-dependent fear memory nor anxiety-like behaviour (Hitti and Siegelbaum, 2014). Those results (coupled with the knowledge presented in chapter 2.2) suggest that while the CA2 also plays a role in spatial coding, it's function is also significantly related to the processing of social information. Therefore I have recorded from this hippocampal region in order to study how neurons respond to social stimulation.

CA2 Firing Fields Remap in Response to a Social Stimulus.

Being a hot topic in neuroscience, several studies have already addressed the topic of social information processing in the CA2. Very few experiments, though, have examined the CA2's capacity for social coding directly by incorporating a social stimulus and recording from the neuronal populations (Alexander et al., 2016; Donegan et al., 2020; Oliva et al., 2020). The results of those experiments, albeit discordant in some aspects, shall be discussed thoroughly in the following sections.

Perhaps the most straightforward manner to explore how individual neurons respond to a social stimulus is by studying how the FRMs of these neurons change when a social stimulus is added into the paradigm. Most interesting are the observations made by Oliva and her colleagues (Oliva et al., 2020). Using an experiment in which two stimulus rats within a maze were being repositioned, they have discovered that some neurons remapped so that their firing fields were always in the vicinity of one of the stimulus rats (fig. 7). These so-called *socially-*

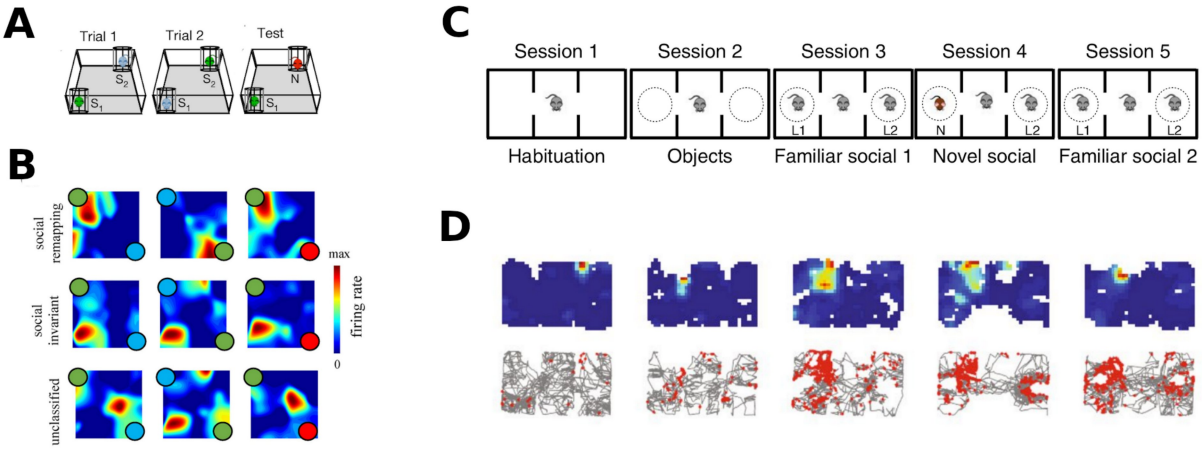

Figure 7*: the firing fields of CA2 neurons remap in response to a social stimulus.* ***A****, the experimental configuration made by Oliva and colleagues, in which the position of two familiar conspecifics is rotated between trials 1 and 2. One of the familiar conspecifics is then replaced by a novel one in the test session. There is a 2 hour period of sleep between trial 2 and test session (not shown).* ***B****, examples of kinds of CA2 neurons in the experiment which responded differently to the presentation of a social stimulus. Note the "socially-remapping" neuron, whose firing field appears to follow a particular stimulus rat.* ***C****, the experimental configuration (top row) by Donegan and colleagues, in which two familiar stimulus rats are added to the maze in the third session. In the fourth session, one conspecific is replaced by a novel conspecific. The fifth session is identical to the third one.* ***D****, the FRMs (top row) and spike-trajectory diagrams (bottom row) of a CA2 neuron created for each corresponding session. The red points on the spike-trajectory diagrams mean that a the neuron discharged at the given location. Please note that by looking at the spike-trajectory diagram, it is apparent that the neuron discharged in the vicinity of stimulus rat L1 in both the third and fifth session, yet there seem to be additional firing fields which are dissimilar between the two sessions. Hence in both sessions, the smoothened FRMs look rather different. From Oliva et al., 2020 and Donegan et al., 2020.*

remapping neurons accounted for cca 20% of all units that they have recorded from the CA2. 30% of the recorded units were identified as *socially-invariant* neurons, because they retained their firing fields regardless of the stimulus rats changing positions. The rest of the cells were unclassified, i.e. their firing fields changed every session without following the position of a particular stimulus rat (fig. 7B). These results are even more interesting considering the fact that they are in contrast to findings made by other laboratories. The majority of CA2 neurons recorded by the two other teams (Alexander et al., 2016; Donegan et al., 2020) also remapped in response to a social stimulus, however their firing fields did not seem to "follow" around any of the social stimuli, i.e. all would fall into the "unclassified" category reported by Oliva and colleagues (see fig. 7D).

Several explanations come to mind. First, having effectively rotated the positions of the stimulus rats between trials by 180°, the experiment made by Oliva and her colleagues was, by design, better suited for the discrimination of "socially-remapping" and "socially-invariant" cells. Another factor should be, however, taken into consideration, and that is time. As was mentioned above, the CA2 ensembles are highly sensitive towards the passing of time, much more than the CA1 or the CA3 (Mankin et al., 2012; Lu et al., 2015). The recording sessions of Oliva and her colleagues were shorter (5 minutes) than those made by others (10 minutes). It is therefore possible that in the longer experiments, the remapping in time overshadowed some neuron's response to the social stimulus (fig. 7). It may be hypothesized that as time passes, the neurons of CA2 adapt new firing fields which

may no longer be in the vicinity of a particular stimulus animal. Nevertheless, this further points towards the fact that temporal coding in the CA2 region is significant and should be taken into consideration when studying the processing of social information.

The Presence of a Social Stimulus in a Novel Context is Reflected by CA2 Ensemble Activity.

To further understand the computations made by the CA2, it is imperative not only to inquire about individual neurons, but also to look at the network as a whole. Two research teams did address the social code in CA2 from ensemble perspective, employing different methods yet yielding, to a certain extent, concordant results.

Using a straightforward approach, Alexander and her colleagues (Alexander et al., 2016) measured spatial correlation of firing rate maps in four different experimental conditions (fig. 8A). In short, each condition comprised two sessions. The first session in each condition had the experimental animal alone in an empty arena. Then, in the second session, either a familiar conspecific, novel conspecific or novel object was added to the arena. The fourth control condition consisted of two sessions of the experimental rat navigating in an empty arena. Although not detecting any significant change in average firing rate, they have observed that the FRMs between sessions of the first three conditions were significantly less correlated than the maps between sessions in the control condition (fig. 8B and C). Therefore they inferred that the CA2 undergoes global remapping in response to the addition of a social stimulus, but also in response to a novel object.

Equally interesting approach was taken by Donegan and her colleagues (Donegan et al., 2020). Their experiment consisted of 5 sessions (fig. 7C). After the first habituation session in an empty maze, two novel objects were added on each side in session 2. In session 3, two conspecifics replaced the objects. Session 4 had one of the conspecifics removed and a novel conspecific put in it's place. Finally, both conspecifics returned so that session 5 had the same configuration as session 3. On a side note, they also studied spatial correlations between each consecutive pair of sessions, only to learn that all pairs were equally uncorrelated, complying the findings of Alexander and colleagues. Most interestingly though, Donegan and her team employed a *support vector machine,* a linear classifier model, to train an artificial neural network to retrospectively decode various information about the experiment from the recorded ensemble activity. When it was attempted to decode the position of the experimental animal based on recordings from the CA2, the decoder failed to perform above chance levels. The decoder was, however, able to distinguish between individual sessions, indicating that the CA2 ensemble code for each session was unique. Such findings suggest that the CA2 network, rather than encoding social or spatial information separately, integrates both kinds of information as a contextual change.

Still, there is some evidence that the neuronal ensembles in the CA2 are tuned even more for social information. Donegan and her colleagues reported that cca 20% of the recorded CA2 neurons significantly increased their firing rates when social stimulus was introduced in session 3 (fig. 8E). Of those neurons, some

increased their firing rate even further in session 4, after the presentation of a novel conspecific. What is more, they focused on the changes of firing rate in the left part of their arena (fig. 8D) - the part where a novel conspecific replaced the familiar one in session 3. Surprisingly, it was found that the firing rates of almost all recorded CA2 neurons significantly increased in response to the novel conspecific in session 4, only to decrease again in session 5, when the familiar conspecific was returned (fig. 8F). Donovan and her colleagues performed this analysis on the last three sessions of their experiment. They do not, however, provide control to this portion of their experiment, e.g. having two familiar objects and one novel object in the last three sessions instead of the stimulus rats. Nevertheless those results hint that the CA2 neuronal ensembles react when the contextual change involves social stimulus, and react even more if this stimulus is unfamiliar.

Of important note, Alexander and her colleagues did not observe any change in firing rate (fig. 8C). I reckon that this was not due to the differences in experimental configuration but rather due to the differences in data analysis. Alexander and her team averaged firing rate across the whole ensemble and then compared it between experimental conditions, whereas Donegan and her team show normalised (z-scored) firing rate of every neuron in the ensemble. In any case, it may be so that the increased firing rate of some 20% of neurons in the CA2 is compensated by decrease of firing rate of the rest of the ensemble. Furthermore, when Donegan and colleagues reported that all CA2 neurons increased firing rate in response to novel conspecific, they also mentioned that many neurons increased firing rate only transiently (fig. 8C).

To summarise, there is emerging electrophysiological evidence which supports the role of CA2 in social and contextual coding. It appears that the CA2 is sensitive towards the change of context in general, and especially when such contextual change involves social stimuli. Nonetheless, there are many questions about the function of CA2 in social coding which are yet to be resolved.

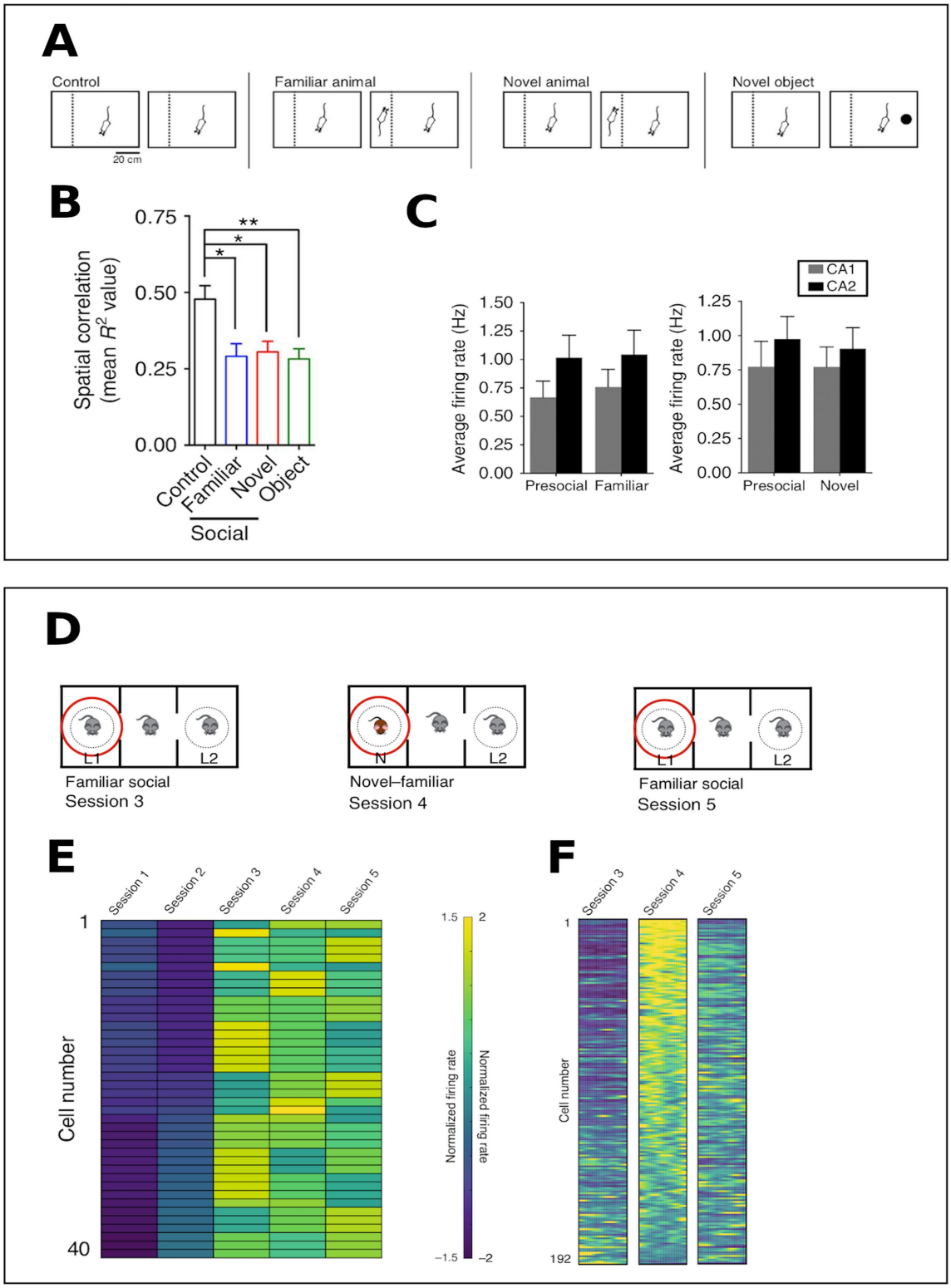

Figure 8*: CA2 neuronal ensembles respond to novelty of context, but especially when social stimulus is added.* ***A,*** *the experimental configuration of Alexander and her colleagues comprised four distinct conditions, in which either familiar conspecific, novel conspecific, novel object or nothing (control) was added to the maze.* ***B,*** *averaged spatial correlation values between session in the four distinct conditions in Alexander's experiment. Note the drop in correlation in all conditions except control.* ***C****, the averaged firing rates in first (pre-social) and second (social) session for CA1 and CA2 neurons recorded by Alexander and her team. Left chart concerns the addition of familiar conspecific, right chart depicts the change after adding novel conspecific. Note that no significant change in CA2 firing rate is detectable from the charts.* ***D,*** *the last three sessions of Donegan's experimental paradigm (shown in full in fig. 7C). The red circle denotes the area which Donegan and colleagues included in the firing rate analysis depicted in F - only spikes discharged in the red circle were analysed.* ***E, c****olour-coded firing rate matrix of 40 neurons which significantly increased their firing rates after the presentation of social stimuli in session 3 of Donegan's experiment. The colour palette represent normalized firing rate, with yellow indicating strong increase (+1.5 st. dev.) and purple indicating strong decrease (-1.5 st. dev.) of firing rate. Each row denotes a cell, each column denotes a session.* ***F****, colour-coded matrix of normalized firing rates of all CA2 neurons from Donegan's experiment, except only the spikes discharged in the left area of the maze (red circle in D) were analysed. Similarly as in E, rows correspond to cells and columns correspond to the last three sessions. The colour coding ranges from +2 st. dev. (yellow) to -2 st. dev. (purple). From Alexander et al., 2016 and Donegan et al., 2020.*

Processing of Social Information in Sleep.

Sleep is a unique state of mind. Since there are little to no sensory inputs during sleep, the brains neuronal networks may process information in ways which would be impossible in wake (Gais, 2004). Among the plethora of physiological processes, it was demonstrated that sleep plays a prominent role in memory formation (Diekelmann and Born, 2010). Most notably, sleep has been proven essential for memory consolidation, a process by which newly-formed memory traces are stabilised (Diekelmann and Born, 2010; Squire et al., 2015). It should be stressed, though, that memory consolidation in sleep does not consist only of the quantitative potentiation of information encoded during wake. Behavioural studies from animals as well as humans have shown that the encoded information undergoes qualitative change in sleep (Wagner et al., 2004; Ellenbogen et al., 2007; Tse et al., 2007, 2011; Verleger et al., 2013; Sawangjit et al., 2017). It appears that separate memory traces can become interconnected in sleep (Tse et al., 2007; Lewis and Durrant, 2011; Durrant et al., 2015).

Indeed, it seems that the integrative effect of sleep also concerns social recognition memory. Recently, evidence was presented which points towards an interesting effect of sleep on social and spatial memory (Sawangjit et al., 2017). In short, a stimulus rat was presented to the experimental rat at different parts of a radial four-arm maze, after which the experimental rat went to sleep. Then a test trial was performed, in which the experimental rat explored two conspecifics: the familiar rat from the previous sessions as well as a novel, unfamiliar rat. Sawangjit and colleagues showed that sleep enhanced social recognition memory for the familiar rat: the experimental rat explored the novel conspecific more than the familiar one. There was, however, an exception. The experimental rat did not explore the novel conspecific as much if the said conspecific was situated at the same location where the familiar conspecific was before sleep. Those results are most interesting because they point towards an interaction of social and spatial memory in sleep.

The fact that information is processed differently in sleep than in wake is also corroborated by electrophysiological evidence. It has been demonstrated that neurons which were active during awake experience can be reactivated in subsequent slow-wave sleep (Lee and Wilson, 2002; O'Neill et al., 2008). Many opinions have stated that this reactivation of neurons serves as the neuronal mechanism for memory consolidation in sleep (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010). In their pivotal work, the aforementioned Oliva and her colleagues (Oliva et al., 2020) not only demonstrated that the CA2 neurons encode social information, but also suggested that the social information is reactivated in sleep. Using interesting statistical methods, they showed that a significant portion of CA2 neuronal ensembles which were active during awake trials were reactivated during subsequent slow-wave sleep. They also reported that assemblies of neurons which were active during social exploration were more likely to be reactivated in sleep.

To summarise, both behavioural and electrophysiological evidence suggests that social information is processed in sleep. Furthermore, it appears that social information in sleep may be processed in an integrative manner and that it may interact with spatial memory. Therefore I also included sleep recordings in my

thesis, in order to study whether the neuronal activity in sleep will be affected by social stimulation during wake.

3. MATERIALS AND METHODS

3.1 Implant Construction

First, *tetrodes* were made by twisting four 12.5 together (Gray et al., 1995). The wires were supplied b The twisted wires were heated by hot air in order to slightly melt the wire insulation, thus making a tetrode. Second, a *Versadrive* was assembled from parts (supplied by *Axona Ltd*) to house the tetrodes (fig. 9) using custom 3D printed components. The tetrodes were inserted into thin steel cannulae, which were supported by small plastic holders. A small screw fit into a thread in the holder, thus allowing the holder with the tetrode to be moved vertically by turning the screw. The four wires of a tetrode were connected to four gold-plated pins, which the recording cable would plug into. A fully-fitted implant included 7 complete tetrodes (taking up 28 recording channels) and 4 standard ground points; the last tetrode was incomplete and comprised only 3 wires as one connection pin had to be used for a special LFP ground point.

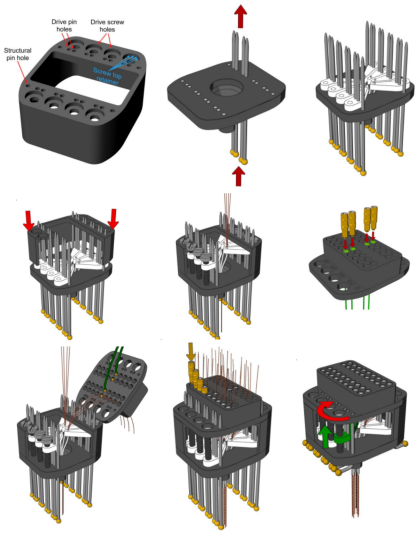

***Figure 9**: a simplified illustration of a Versadrive construction. The design enables vertical movement of tetrodes after surgery by turning of the screws.*

The night before surgery, the tetrodes were cut by sharp scissors so they would protrude cca 6 mm from the end of the cannula. The tetrode tips were then cleaned electrolytically: the tetrodes were connected to the negative terminal of a 9V power supply while submerged in 0,7% NaCl solution. A 20-30 s electrolysis usually lowered the impedance of a single electrode from 0,5 MOhm to 400 kOhm. Finally, the tetrode tips were gold-plated electrolytically by submerging the tetrodes in *gold plating solution* (supplied by Neuralynx, Inc.) and applying a couple of negative 9V pulses. This lowered the impedance even further, achieving 100 kOhms for each electrode.

3.2 Animals

9 male rats of the Long-Evans strain were used in the experiment, of which 6 were the *stimulus* rats and 3 were the *experimental* rats. The experimental rats (1-3 months old) were the ones which would later be operated and recorded from. After being handled for a few of weeks they underwent surgery at the age of 13 weeks.

The stimulus rats (5-15 weeks old) would later be used as social stimuli for the experimental rats. As the experimental design required them to be confined to one place, the stimulus rats were habituated for wearing a special harness (see 3.5 Experimental Paradigm, fig. 11D) in addition to the regular handling procedure. First they would just wear the harness without having their movement restricted. The following few days, they would wear the harness for 2-3 minutes while being confined to a specific arm of the maze. Only then were they integrated in the full-length experiment.

The experimental rats were also habituated to the maze in that they have visited the empty maze a couple of times during the week before the experiment. Finally, the experimental rats and the stimulus rats did encounter each other two times during the week before the experiment so that they would be habituated to each other's presence. Different pairs of stimulus rats was used for different experiments involving different experimental rats.

Both experimental and stimulus rats were fed 25 g of food pellets per day per animal. They were housed in a room with stable temperature (25°C) and a 12 hour light/dark cycle. All animals were supplied by *Velaz s.r.o.*

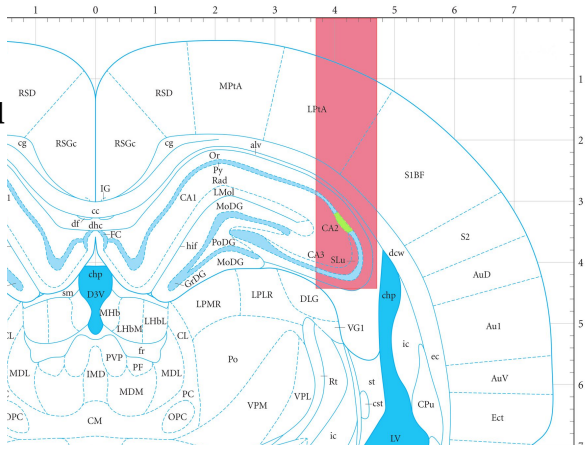

***Figure 10**: taking into consideration the discrepancy between stereotaxic atlas and a real rat brain, the coordinates were picked so that at least one tetrode hit the CA2. An excerpt from rat brain atlas (right hemisphere, bregma -3.72) is shown here. Red bar denotes the theoretical distance between the most medial and most laterally positioned tetrode. The CA2 stratum pyramidale is highlighted in yellow.*

3.3 Surgery

The experimental rats underwent stereotaxic surgery. As part of pre-operative care, the rats were administered 50 mg of ibuprofen and 0,85 mg of meloxicam to ease pain during and directly after the surgery. Then, before surgery, they were anaesthetized by inhaling a mixture of 5% isofluorane, 0,9% O_2 and 94% air. When fully anaesthetized, isofluorane was tuned down to and kept at 1,5% - 2,5% of the mixture. The skin was cut and the top of the skull was cleared of fasciae. If necessary, a few drops of adrenaline solution were applied locally to stop any excessive bleeding With the help of a stereotaxic apparatus, the desired implant location - the dorsal CA2 hippocampal region was located at -3.72 mm posterior and 4.2 mm lateral from bregma. The stereotaxic coordinates were chosen especially this way so that there was a high probability of at least one tetrode reaching the CA2 (see fig. 10). The tetrode tips were lowered 2.2 - 2.8 mm below the brain surface, depending on the rat. 3-5 small bone screws were driven in the skull above the cerebellum, which the implant ground points would later be soldered onto. Finally, the implant was secured to the skull by dental cement. After the surgery the animals were left to rest for 7 days. As part of post-operative care,

they were administered 0,85 mg of meloxicam a day for 3 days and 15 mg of enrofloxacin a day for 10 days.

3.4 Screening for Single Units and Probe Trials

After the experimental rats have recovered from surgery, the search for neuronal activity has begun. In order for the single units to be recorded, the tetrode tips need to be relatively close to the somata of hippocampal neurons so that the extracellular potentials of individual neurons can be differentiated (Henze et al., 2000). Since, as a safeguard measure, the tetrodes were implanted cca 0.4 - 0.8 mm above the estimated location of the thin CA2 stratum pyramidale, they had to be gradually lowered to the desired depth. Hence, in a daily process of *screening*, the tetrodes were lowered by 30 μm every 4 hours and highpass-filtered signal was checked (screened) for the extracellular potentials of neurons. During the screening procedure, the experimental rat moved freely on a wooden 1x1m platform so that the spatial activity of neurons could be detected. After 5-10 minutes, a stimulus rat was added on the platform so that any neuronal response to the social stimulus could be detected. Screening was carried out until reliable extracellular signal was found, usually for 20-60 days after surgery, with 1-3 screening sessions per day.

The extracellular signal of individual neurons began to stand out from physiological noise at the depth of cca 3.2 mm below brain surface, although such estimate is very crude as errors accumulated with every screening session. When this happened, the relevant tetrodes were shortly recorded from and the signal was analysed briefly in order to determine how many single units - the extracellular spikes of individual neurons - appeared on the tetrodes. If there were more than five single units on all tetrodes, the screening session was finished and the full-length experiment (see 3.5 Experimental Paradigm) was commenced.

This was, however, rarely the case. Most frequently only 1-2 single units across all tetrodes were found. Though not resulting in a full-length experiment, the screening sessions with such solitary cells were also recorded, resulting in what is denoted here as *probe trials*. Even though the vast majority of data presented in this thesis comes from the full-length experiments, a few probe trials were significant enough to be included in the final analysis.

3.5 Experimental Paradigm

The experimental paradigm in my thesis was designed in such a way that it could be differentiated if a neuron processes social or spatial information, or whether it simply responds to an inanimate object. There were three main phases

to the experiment: the pre-sleep phase, the awake phase and the sleep phase (see fig. 11).

The pre-sleep phase served as a control for the sleep recording. The experimental rat was put in it's home cage and observed until it was determined that it fell asleep (the rat was not moving and sharp waves were observed on the EEG). After that, several 10-minute recordings were made, from which only the best one, i.e. the one which the rat did not awake from, was chosen for subsequent analysis. The whole phase took at least 1.5 hours, sometimes more if the rat was having difficulties falling asleep.

The awake phase contained five 10-minute recording sessions in which the experimental rat explored a plus-like maze, either empty or populated with two other stimulus rats. The plastic maze, elevated at 60 cm above ground level, consisted of 4 arms, each 50 cm long and 9 cm wide. There was a small fence

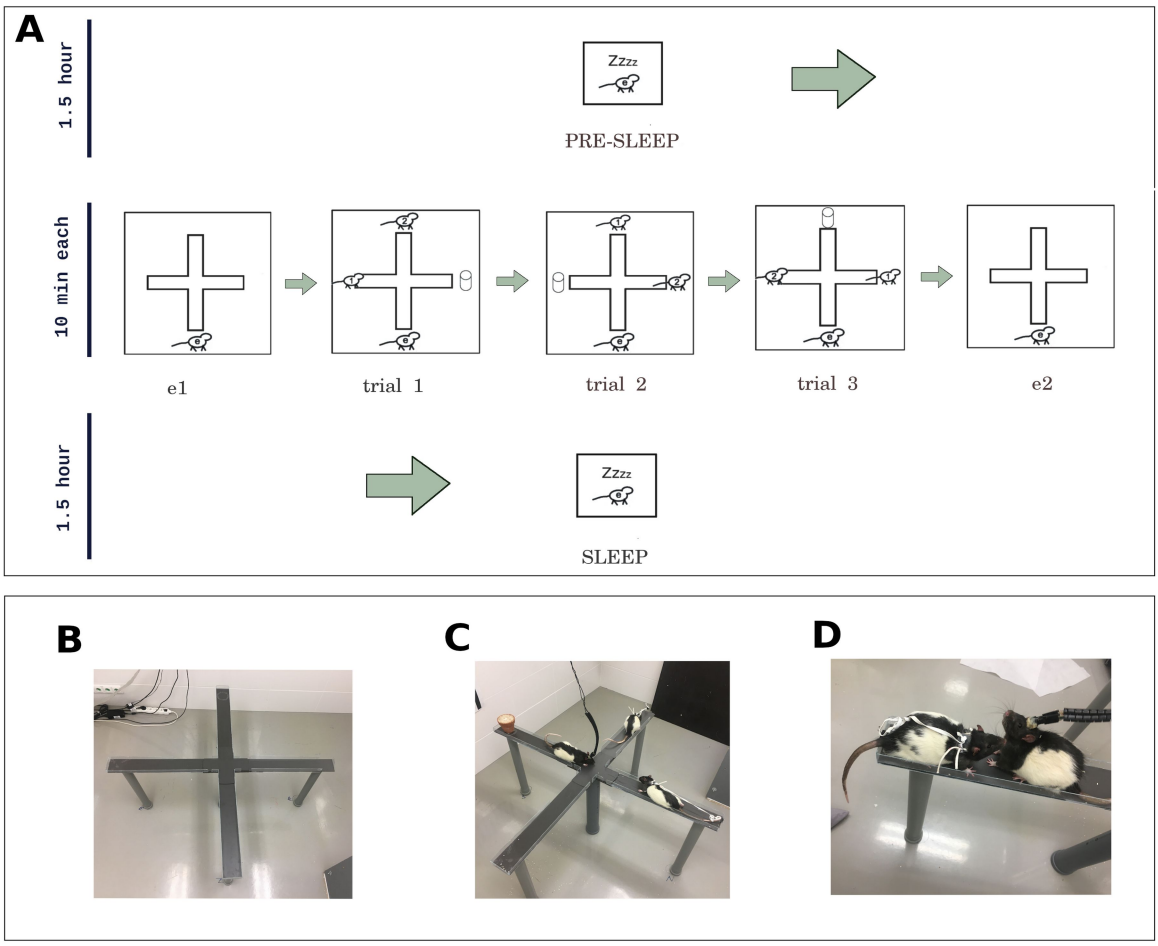

***Figure 11**: the experimental paradigm. **A**, the experiment comprised 3 phases: pre-sleep, awake and sleep phase. Pre-sleep was a control for the sleep phase. The awake phase consisted of 5 sessions: e1, trial 1, trial 2, trial 3 and e2. During e1 and e2 (as in "empty 1 and 2") the experimental rat explored an empty plus-like maze. Throughout trials 1-3, the maze was populated with two stimulus rats and one inanimate object, the location of which shifted every trial so that each stimulus rat would have been located at each arm exactly once. "E" inside the doodle of a rat denotes that it was the experimental rat; "1" and "2" inside the doodle denote stimulus rats 1 and 2. The stimulus rats wore a special harness so that they could not move away from the end of the arm. **B**, the empty maze as viewed from above. **C**, the experiment in progress. **D**, the experimental rat exploring one of the stimuli rats, which is constrained by a harness to the terminal part of the arm.*

along the arm's edges made of see-through plexiglass so that the rats would not fall down.

The first session was coined "e1" as it was the first time that the experimental rat navigated in the empty maze. This session facilitated the characterisation of spatial activity of neurons. Three stimulus sessions followed, named "trial 1," "trial 2" and "trial 3." The experimental rat, starting at the south arm of the maze, was allowed to explore two stimulus rats and an inanimate object, each positioned at the end of the remaining three arms. The stimulus rats wore special "harness" which was attached to the maze, thus restraining the stimulus rat to the respective arm. The position of the stimulus rats and of the object was changed every trial so that every possible combination of stimulus rat and place or object and place would have been tested. This shifting of position was essential as it was meant to distinguish whether a single unit processed spatial or social information. For example, if a neuron discharged always in the north arm regardless of which stimulus rat was in it, such neuron would respond only to place and thus would process spatial information. On the other hand, if a neuron was always active in the arm where one of the stimulus rats was located, it would respond to social stimuli and not to space. The inanimate object (a small plant pot) was there in case a neuron would simply change its activity in response to any object placed in the empty arm.

The last session of the awake phase, the e2 (as in "empty 2") had the experimental rat navigating the empty maze again. The e2 session was included so that the stability of neurons could be observed, i.e. whether the spatial response of neurons that were active in the e1 session would also be present in the e2 session.

The design of the sleep phase was identical to the pre-sleep phase. Thanks to the control provided by the pre-sleep phase it was possible to see which neurons became active only after the awake sessions.

To this day, electrophysiologists have studied social interaction only in the open field configuration (Alexander et al., 2016; Oliva et al., 2020). Our paradigm is novel in that it combines the element of a linear track with social stimulation. Each arm was designed to be only so wide that a single firing field of a putative place cell would fit into it. Not only is our configuration suited for the differentiation of social and spatial coding, the linearity makes it appropriate to study the reactivation of neuronal activity during sleep.

3.6 Histology

After a sufficient amount of data was recorded from a rat, it was necessary to verify the position of tetrodes in the hippocampus. The experimental rat was put to death by diethylether overdose. Then the brain was extracted and infused by 5% paraformaledyhe solution for 24 hours, after which it was stored in 30% saccharose solution for at least 7 consecutive days. When the brain was perfused sufficiently, it was frozen and cut into 50 μm thin sections using a cryotome.

The sections were dyed with Toluidine blue following a classic staining protocol. Before the procedure, a stock solution of Toluidine blue was prepared by dissolving 1 g of the dye in 100 ml of 70% ethanol. 20 ml of this solution were diluted by 180 ml of 1% NaCl solution, yielding a *working solution* of Toluidine blue. First, the saccharose was washed out of the sections by distilled water. Then, the sections were submerged in the working solution for 7 minutes, and again washed with distilled water. Afterwards the water in the slices was displaced by 60% ethanol solution for 25 s and then pure ethanol for 10 s. Finally the sections were cleaned by Neoclear, dried and fixed with Neo-mount, after which they were ready to be studied. Microscopic inspection revealed tetrode marks in the stratum pyramidale of the CA2 hippocampal region.

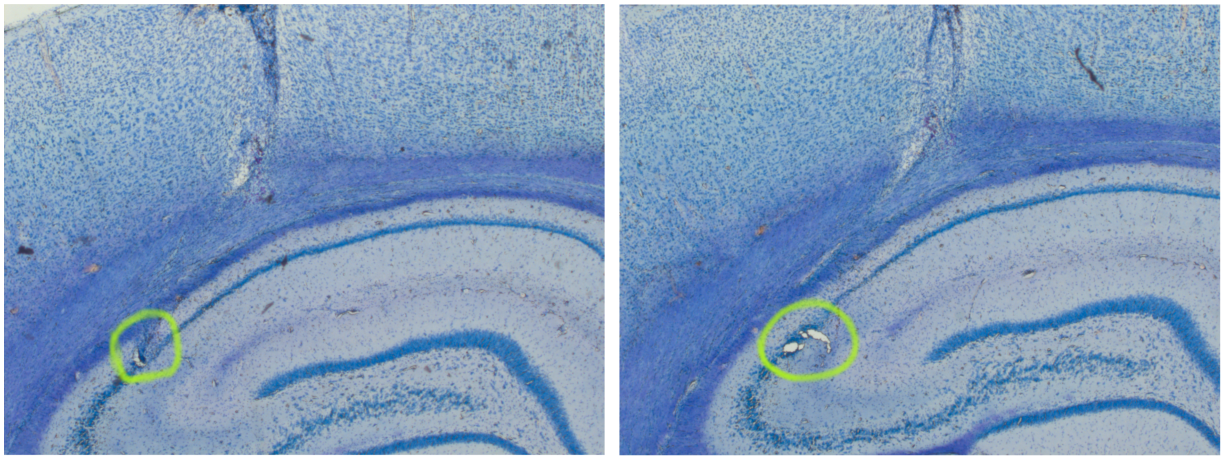

***Figure 12**: the placement of tetrodes was verified by microscopic inspection of the stained 50-µm thin sections of right dorsal hippocampus. The picture shows two examples of sections from one of the experimental animals (Rat-11), exposing visible tetrode marks (highlighted in yellow) in the CA2 stratum pyramidale.*

4. DATA ACQUISITION AND ANALYSIS

4.1 Recording and Spike Detection

Spikes were recorded using a system comprising (a) a headstage pre-amplifier connected straight to the implant, (b) a secondary amplifier with a DAC, and (c) a *system unit*. Raw analogue signal was pre-amplified to prevent interference, then further amplified using the secondary amplifier and finally digitized using the DAC with a sampling rate of 24 kHz. Additional signal processing, such as threshold-sorting and reference subtraction were computed by the *system unit* controlled by proprietary software ("dacqUSB"). The components and the recording software were supplied by *Axona Ltd, Porters Wood, St. Albans, UK*.

Not all extracellular spikes were recorded during experiment. A *threshold* was set just above the noise level to avoid the need for storing excessive amount of

raw data. In a well-prepared implant, the indiscernible spikes resulting in electrophysiological noise were about 40 µV at 10 000x amplification, therefore the threshold was set between 50 - 60 µV. If a spike passed the threshold, a window of 1 ms was recorded, starting 200 µs before and 800 µs after the trigger.

A reference electrode was set for every recording tetrode in order to minimize artefacts and physiological noise. For single unit detection, the reference was an electrode located as close as possible to the recording tetrode.

Digital signal for single-unit detection was "highpass" filtered using Butterworth filter for 300 Hz - 7 kHz. Optionally, if noise levels were high and could not had been referenced out, the signal was filtered for 500 Hz - 7 kHz. As the fastest component of the filtered signal was 7 kHz, a DAC sampling rate of 24 kHz was more than sufficient to satisfy the *Nyquist rate* (Nyquist, 1928).

4.2 Clustering and Unit Identification

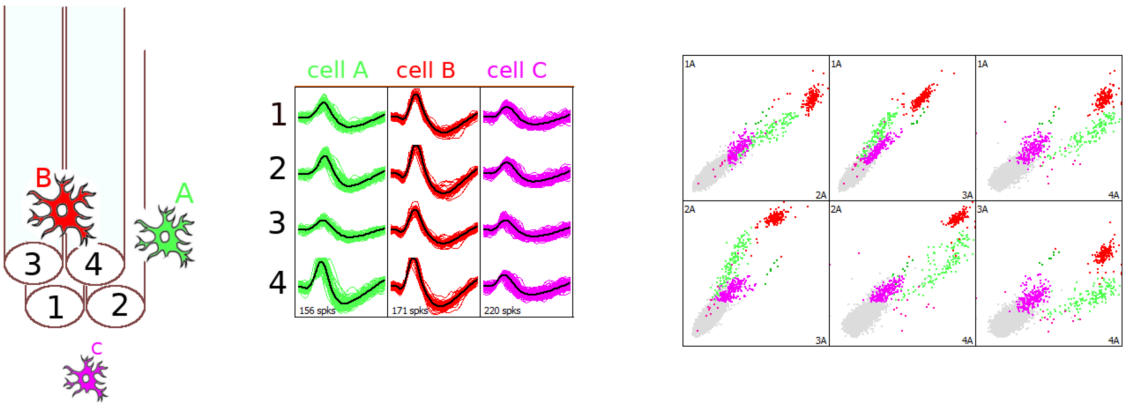

***Figure 13**: Identification of single units on a tetrode by clustering. **Left**, illustration of a tetrode tip and 3 neurons in different distances from the four electrodes. **Centre**, waveforms of the 3 neurons on different electrodes. **Right**, every spike recorded by an electrode plotted against every other spike recorded by other electrodes in a tetrode. On X and Y axes are the relative magnitudes of each spike's amplitude. For example, cell A had the highest amplitude on electrode 4 and lowest on electrode 3 because it was closest to wire 4 while being furthest from wire 3. On a plot showing wires 3 and 4, cell A forms a cluster in the lower right corner. On the other hand, cell B was even closer to wire 4 while also further from wire 3. Hence, on a plot showing again wires 3 and 4 cell B forms a cluster in the top right corner.*

Single units on a tetrode were detected by *clustering analysis*. This method takes advantage of the fact that the extracellular spike's amplitude decreases rapidly with distance from the neuron (Henze et al., 2000). If a neuron is, for example, located closer to one electrode of a tetrode, the recorded amplitude will be the highest on that electrode and lower on other electrodes (see fig. 13). Hence, it very unlikely for two neurons to exhibit the same amplitude on all electrodes of a tetrode. After recording, the amplitudes of all spikes on all electrodes were plotted against each other (see fig. 13, right) resulting in *clusters*. Optionally, if clusters

did not separate well, other parameters (e.g. *time of peak* or *principle component analysis*) were used to separate clusters. All data were clustered manually, automatic clustering algorithms (Rossant et al., 2016) were never used.

Attributing Clusters to Single Units.

In an ideal case, a single cluster belonged to a *single unit* - the putative neuron recorded by the tetrode. In reality, this was not always the case. For example, a pyramidal neurons which has the properties of a *complex-spike cell* may discharge a spike train consisting of a large-amplitude spike followed closely (<5 ms) by several lower-amplitude spikes (Ranck, 1973; Fox and Ranck, 1981) (also see 2.1 Distinct Types of Neurons in the Hippocampus.). Such a spike train would sometimes result in multiple clusters which actually belonged to a single unit. To decide whether two cluster with similar waveforms corresponded to a single neuron, cross-correlation was employed.

Cross-correlation visualises the temporal relationship between two time series. Here, the two time series were the spikes of *cluster A* and spikes of *cluster B.* A cross-correlogram is a histogram of *all* possible time intervals between *any* spike of cluster *A* and *any* spike of cluster *B.* When creating a cross-correlogram, a certain spike of cluster *A* is chosen. Then, the time intervals between this spike and *all* spikes of cluster *B* are calculated. This process is repeated for every spike of cluster *A.* The calculated time intervals are plotted as bins on the x-axis; the number of occurrences of a given interval is plotted on the y-axis. Finally, the cross-correlogram shows the probabilities of *cluster B* spikes occurring at a certain time after spikes of *cluster A.*

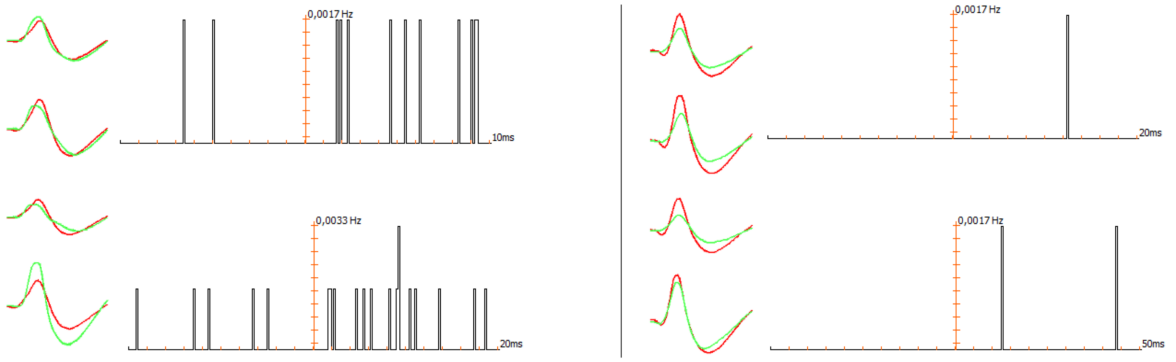

Figure 14*: using cross-correlation to decide between similar clusters.* ***Left,*** *the red and green clusters displayed similar waveforms and were overlapping on the amplitude plot (not shown). To decide whether they belong to a single cell (unit), the two clusters were cross-correlated. Top row cross-correlogram shows 10 ms, bottom row cross-correlogram shows 20 ms. Spikes of the green cluster often occurred 2 - 3 ms after spikes of the red cluster, suggesting that both clusters might belong to a single complex-spike neuron.* ***Right****, another two similar clusters from a different tetrode and a different experimental session. Here, top-row cross-correlogram showh 10 ms resolution, bottom-row cross-correlogram depicts 50ms. In this case the spikes of the green cluster did not occur < 5 ms after the red cluster, hence the clusters were treated as separate units. In both instances, the firing rates across clusters were comparable (152 - 252 spikes per cluster).*

Here, cross-correlation was used to decide whether two alike clusters belong to a single unit. If two cluster displayed similar waveforms, were close together on the amplitude plot and their cross-correlogram showed several peaks < 5 ms from zero, the clusters were attributed to the same neuron (see fig.14, left). Otherwise the clusters were treated as separate units (fig.14, right).

Creating Autocorrelograms.

Autocorrelation is a method similar to cross-correlation in that it depicts the temporal relationship between two time series, only that both of the time series are actually spikes of a single cluster or neuron. Autocorrelograms are histograms of all possible time intervals between spikes of a single neuron. When creating an autocorrelogram, each bin on the x-axis refers to a time interval between *any* two spikes in the dataset. The y-axis describes how much a specific time interval was present relative to other time intervals. In other words, an autocorrelogram shows the probability of a cell firing consecutive spikes XY milliseconds after each other.

In my thesis, autocorrelation was utilised as one of the tools to discriminate complex-spike cells and interneurons (Fox and Ranck, 1975). Interneurons which have the properties of theta cells retain a consistently high firing rate, giving rise to characteristic autocorrelograms (Mizumori et al., 1990; Csicsvari et al., 1999).

4.3 Spatial and Other Analyses.

To assess the spatial organisation of neurons, several computational approaches were taken.

FRMs, Spatial Coherence and Spatial Information.

First, FRMs were created and spatial informations was calculated. As was discussed in the theoretical background, FRMs are made by dividing the spike map (a map of the cell's spikes in the arena) by the time map (a map of the rat's dwell times in the arena) (Muller et al., 1987). During the experiment, the rat's location in the maze was tracked using a small infrared diode on the headstage. The respective maps were represented by three 32*32 numeric matrices. Then, every element in the spike map matrix was divided by the corresponding element in the time map matrix, thus creating a rate map matrix. The rate map matrix was visualized by a colour-coded plot, ranging from the coldest colour (purple - highest firing rate in dataset) to the warmest colour (yellow - zero firing rate).

Spatial coherence and spatial information values were computed as shown in chapter 2.2. (Measuring Spatial Coding of Neurons.). In addition to that, spatial information (as computed in eq. 1) was divided by the mean firing rate, and was therefore expressed in bits per spike (Skaggs et al., 1992). All computations were done in Matlab using custom-made code (E. Kelemen).

Correlations and Normalisation of Data.

In order to compare the spatial organisation of neurons across various sessions, correlations between corresponding firing rate maps were calculated. To obtain correlation between two FRMs, the firing rate in each pixel of one FRM was correlated with the firing rate in each pixel of the other FRM using Pearson's correlation.

In some sections of this thesis, correlation is used to assess the linearity of a relationship between two sets of data. This was also done using Pearson's correlation. In one instance in this thesis, a FRM was rotated. This, as well as the FRM correlations were performed in Matlab with the aid of custom code (E. Kelemen).

Some data, such as spatial coherence and firing rates were often normalised. The mean and standard deviation were calculated for a singe neuron across sessions. The normalisation was done by z-scoring the raw values with respect to the standard deviation.

5. SPECIFIC AIMS

The main goal of this thesis was to study the processing of social information in the rat's CA2 hippocampal region. Specifically, I had six major questions.

One, **what is the behaviour of the experimental rats within the maze**? As my experiment relied on the social interaction of three rats, it was necessary to assess their behaviour. Hence, I asked: do the experimental rats explore the stimulus rats sufficiently? Do the experimental rats navigate through the maze enough for the dwell maps to be created?

Two, **how many of the recorded units were pyramidal cells?** As was explained in the theoretical background, the pyramidal neurons, one of two major kinds of units in the rat's hippocampus, have different electrophysiological as well as computational properties. Since I was interested in pyramidal cells, it was necessary to differentiate them from the interneurons.

Three, **is the activity of neurons in the CA2 organised within the experimental maze?** In recent years, evidence was presented in favour of the CA2 pyramidal neurons encoding spatial information. Researchers have even identified place cells in the CA2. The spatial activity of CA2 neurons, though, has never been studied in the experimental configuration I employed, i.e. a four-arm plus-like maze with stimuli at the ends of the arms. Hence I inquired: will CA2 neurons display discharge preferentially in some portions of the maze? Will the activity of CA2 neurons be organised when social stimuli are added and shuffled around the maze?

Four, **do individual neurons respond to social stimulation?** The CA2 hippocampal region has been proven significant for the processing of social memory, and there are few electrophysiological studies which corroborate that. Therefore, I asked: will the spatial organisation of neurons in my experiment change with the addition of social stimuli? Will the activity of CA2 neurons be organised differently during exposure to social context?

Five, **do neurons respond to the change of social context?** Evidence has shown that the activity of neuronal networks in the CA2 reflects a change of socio-spatial context. Hence, I inquired: will the neurons in my experiment respond to the shuffling of social stimuli? Will the firing fields of neurons prefer a particular stimulus rat, an inanimate object, or will they respond to the context as a whole?

Six, **does the activity of neurons change in sleep**? It has recently been shown that social information may be reactivated in sleep following a behavioural task with social stimuli. Therefore, I asked: will the activity of neurons in my experiment differ in sleep? Will it increase, or decrease when compared to pre-sleep? Will there be any relation to the neurons' activity during wake sessions?

6. RESULTS

The Experimental Rats Shared Some Common Behavioural Characteristics when Navigating in the Maze.

Before inspecting any electrophysiological data, I looked at the experimental rat's behaviour on the four-arm plus-like maze (fig. 15). Judging by the speed diagrams, even during the empty session the experimental rats were more likely to rest in the terminal parts of the arms and usually just ran through the rest of the maze. The distribution of velocities was also similar for all experimental rats and constant during all awake sessions (fig. 16). The experimental rats moved predominantly at speeds between 3-5 cm/s, which I attribute to the rats jolting their head while being stationary (Kay et al., 2016).

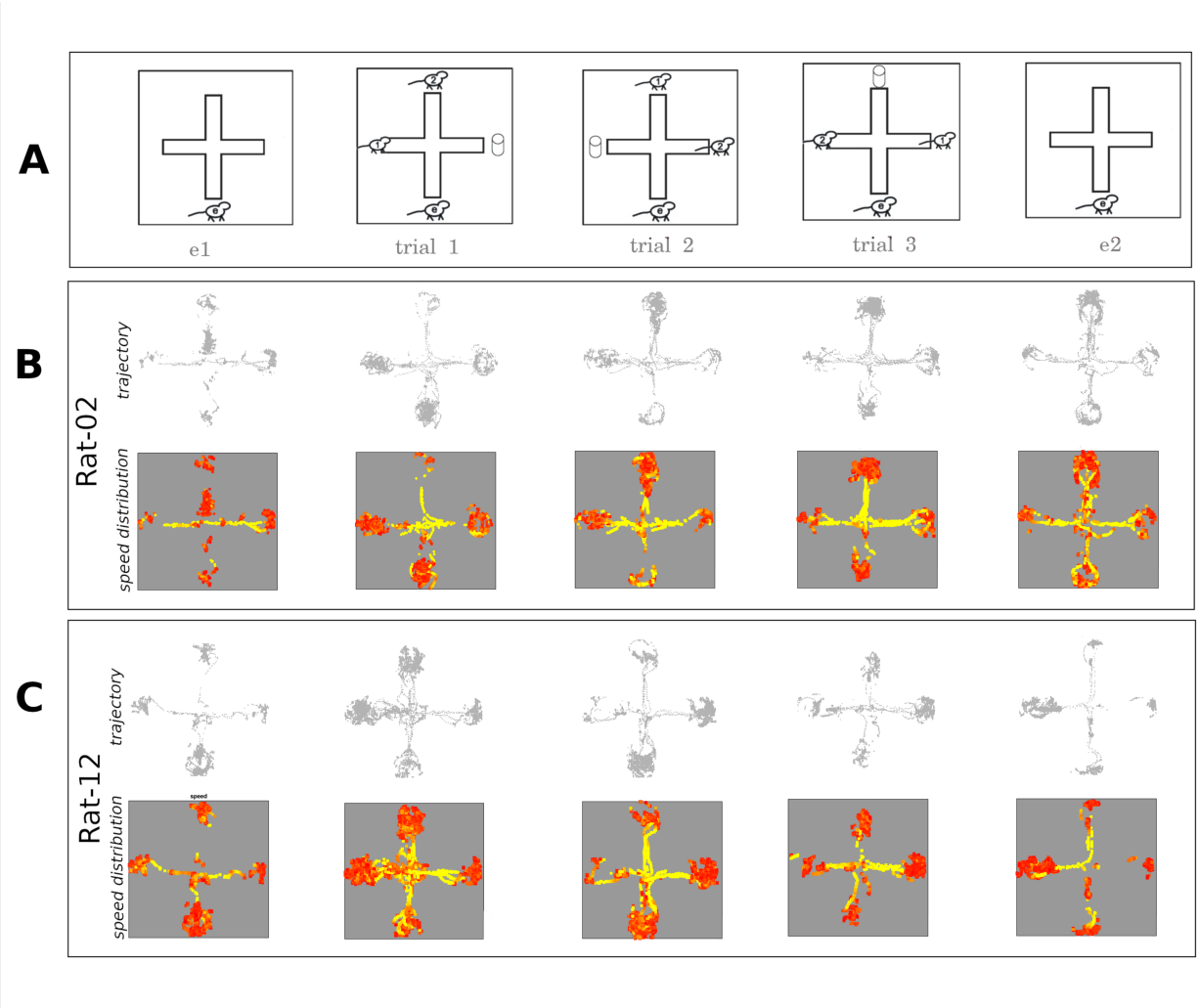

***Figure 15**: some characteristics of the experimental rats' behaviour on the maze. **A**, illustrations of wake sessions as they were included during the experiment. "E" inside the doodle of a rat means it was the experimental rat, "1" and "2" denote stimulus rats 1 and 2. **B**, trajectory diagrams (top row) and speed diagrams (bottom row) for Rat-02 in each session. Every dot in both diagrams represents a recorded position of the experimental rat. In the speed distribution diagram the dots are colour-coded: yellow indicates the rat was running at high speed at that position while dark red means it was stationary. **C**, trajectory diagrams (top row) and speed diagrams (bottom row) for Rat-12 in each session.*

The experimental rats did explore the stimulus rats and the object when those were added into the maze. Rat-02 did not spend as much time exploring stimulus rat 2 as it did exploring the other stimulus rats and the object. Nevertheless, it seems that the behaviour of both experimental rats was homogenous enough to provide sufficient dwell times for the creation of FRMs.

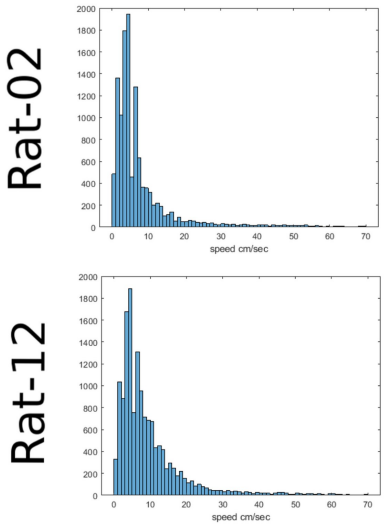

***Figure 16**: the distribution of velocities was similar across sessions and experimental rats. The figure show examples of speed histograms from one awake session from experimental Rat-02 (top) and Rat-12 (bottom). Histograms of other sessions were almost identical (not shown). speed in cm/s is displayed on the x axis, each bin is 1 cm/s wide.*

The Majority of Single Units were Pyramidal Neurons.

I have recorded 25 single units from the rat's dorsal CA2, out of which 23 appeared to be pyramidal neurons and 2 appeared to be interneurons. The two kinds of units were differentiated on the basis average firing rates and autocorrelograms, secondarily also on the basis of spatial information.

The pyramidal neurons displayed lower average firing rates in wake as well as in sleep; the highest

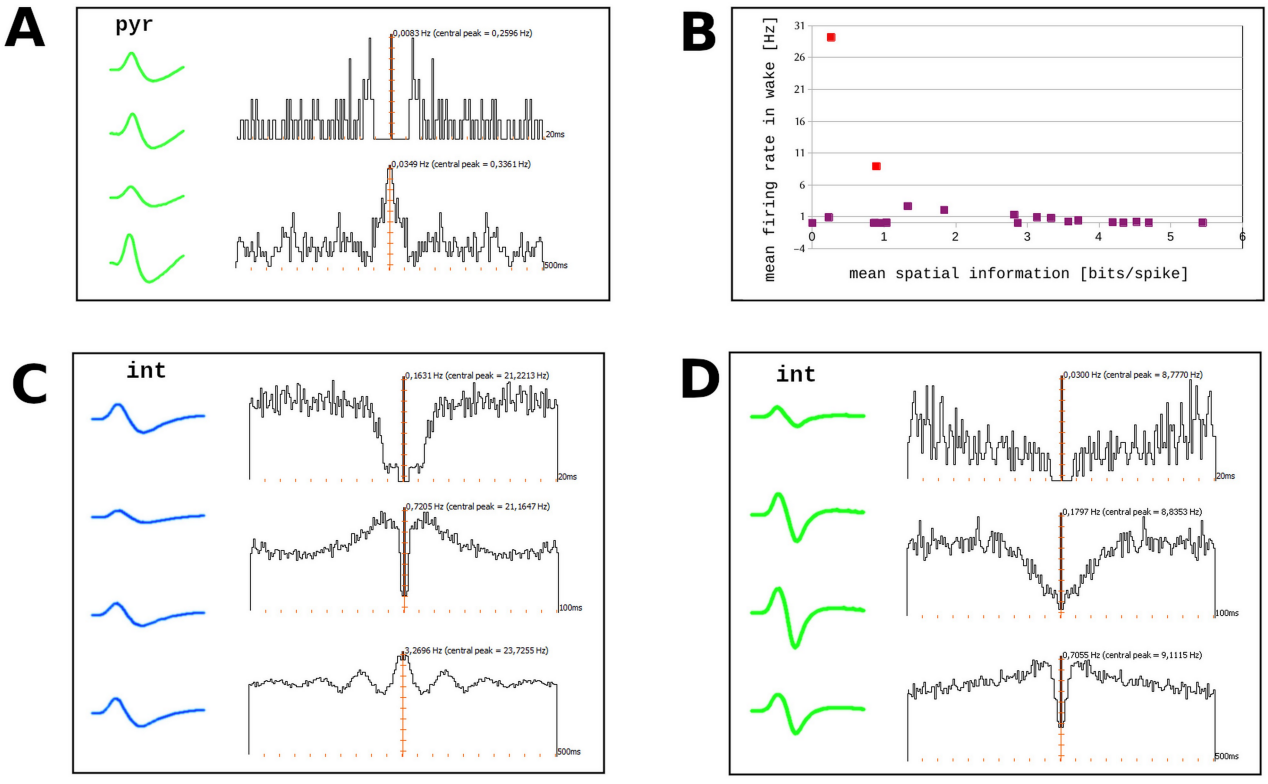

***Figure 17**: the different properties of pyramidal cells and interneurons. **A**, waveform (left) and two autocorrelograms (right) of one of the putative pyramidal neurons. The autocorrelograms are for 20 (top) and 500 (bottom) ms intervals. Note the 0 probability of discharge until cca 2 ms, which then increases only to decrease again after 6 ms. On a side note, example of a pyramidal cell shows some theta modulation. **B**, a plot of mean spatial information (x axis) vs mean firing rate across wake sessions (y axis) for all recorded units. Note the two units which had low spatial information (<1 bit/spike) and high firing rate (>6 Hz) (highlighted in red) . **C** and **D**, the waveform (left) and three autocorrelograms (right) of the two putative interneurons. The autocorrelograms are for 20, 100 and 500 ms intervals, top to bottom. Note that the probability of discharge increases after 5 ms and remains high. A strong theta modulation is apparent from the bottom-most autocorrelogram.*

recorded mean firing rate was 2.56 ± 1.42 Hz in wake and 4.10 Hz ± 5.21 Hz during pre-sleep and sleep. The pyramidal cells were often complex-spike cells, as it was apparent from their autocorrelograms (fig. 17A).

The interneurons were discerned by their high average firing rates, both during the awake sessions (29.18 ± 13.20 Hz and 8.91 ± 1.10 Hz) and during sleep sessions (41.01 ± 3.74 Hz and 8.26 ± 0.44 Hz). Apart from the firing rate, the interneurons displayed characteristic autocorrelograms and low spatial information values (see fig. 17B, C and D). When mean firing rate during wake sessions was plotted against spatial information, the two interneurons clearly separated from the putative pyramidal cells (see fig. 17B).

Of interest, some pyramidal cells as well as interneurons showed theta modulation (fig. 17A and C). It is worth mentioning that the width of the waveforms was not used to assess the types of units.

The Activity of Some Neurons Was Organised Within the Maze.

First, I studied to what extent was the firing of neurons organised in space. To answer that question, I examined the FRMs of individual neurons across all wake sessions. Spatial coherence and spatial information were used to quantify the spatial organisation of neurons. I report that, in agreement with previous studies, (Mankin et al., 2015; Alexander et al., 2016; Oliva et al., 2016, 2020), some pyramidal neurons in the CA2 displayed place cell properties. Cca 25% of the recorded pyramidal neurons exhibited spatially organised activity, displayed high mean spatial coherence (>0.15) and also high mean spatial information (> 3 bits/spike) (fig. 18). This subset of neurons included both stable and unstable cells, i.e. those which were present across at least 4 awake sessions as well as those which were active only transiently in one or two sessions. The discharge of those neurons was often confined to the terminal portions of the maze, even in the empty sessions (fig. 18C). When the mean spatial information was plotted against mean spatial coherence of all units, those neurons appeared in the top right portion (fig. 18A, highlighted in orange).

A few neurons exhibited high mean spatial information (> 4 bits/spike) while having negative mean coherence. Judging from their FRMs, the spatial activity of those neurons was disorganised, as they tended to discharge more spikes at many isolated pixel (fig. 18D). The rest of the recorded neurons had low mean spatial coherence (<0.15) but mean spatial information ranging from 1 to 5.5 bits/spike. Their FRMs were disordered and their activity did not appear to be organised within the maze (fig. 18E).

There were neurons which displayed organised activity in empty sessions as well as in social trials. There were also neurons, however, whose activity was organised only in some sessions, only to become disorganised later. Hence, I inquired about the variability of spatial coherence of neurons It was revealed that there was a positive correlation between the mean spatial coherence and the standard deviation of coherence of neurons across wake sessions (R = 0.6123, fig. 18B). It was often seen that the high mean spatial coherence of place cells was a result of very high coherence in one or two sessions but relatively lower coherence in remaining sessions (data not shown). This variance of spatial coherences across

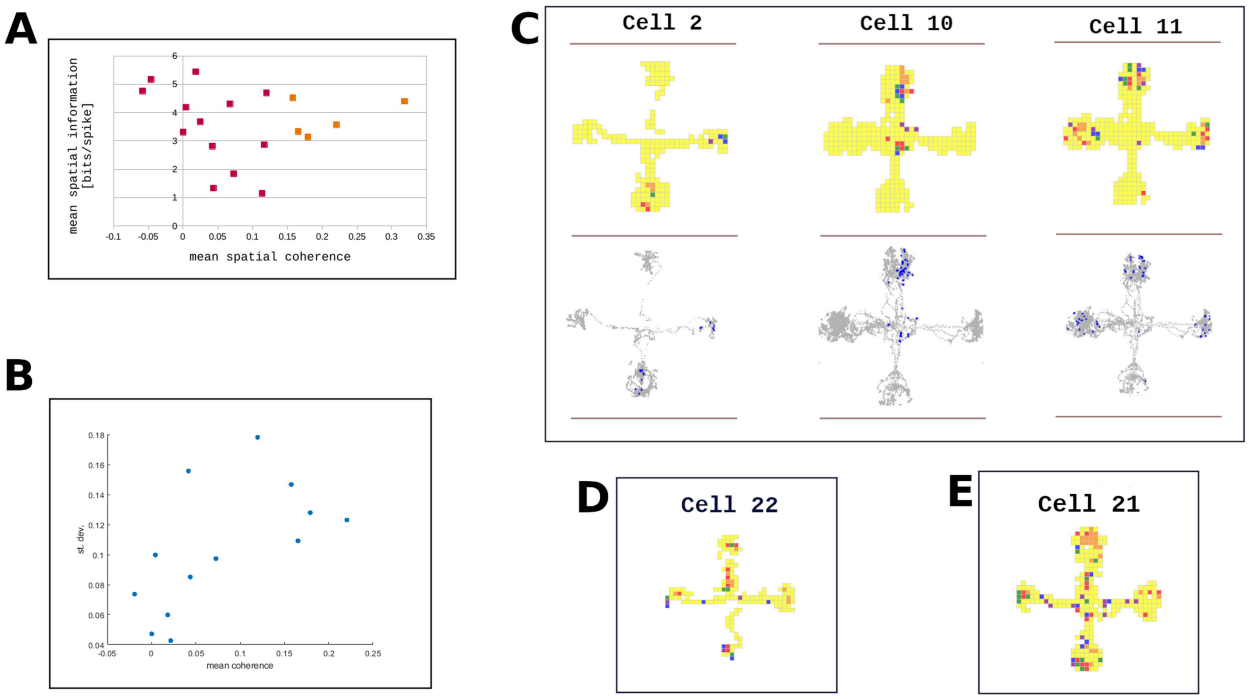

Figure 18: *the activity of some pyramidal neurons was organised within the maze.* ***A****, a plot of mean spatial coherence (x axis) over average spatial information (y axis). The 5 neurons in the top right corner had the most coherent FRMs as well as carried the most spatial information****. B****, mean spatial coherence (x axis) as a function of its standard deviation. Note that the mean coherence of the spatially-organised cells varied greatly across sessions. Plotted only for units which were active for at least 4 awake sessions. Note the neurons with high mean coherence as well as spatial information (highlighted in orange).* ***C****, examples of the FRMs (top) and spike-trajectory diagrams of three neurons which displayed organised firing within the maze. The FRMs are colour-coded, colder colour means higher firing rate - purple denotes the highest and yellow the lowest firing rate in a pixel. A spike-trajectory diagram depicts spikes of a neurons (purple dots) on top of all recorded positions (grey dot).* ***D*** *and* ***E,*** *examples of neurons activity of which was disorganised in space. The neuron in* ***D*** *displayed high mean spatial information but negative mean coherence. The neuron in* ***E*** *had low mean spatial coherence but mean spatial information of 2.8 bits/spike.*

sessions in some neurons will be discussed further. The mean spatial coherence did not seem to be correlated with average firing rate of neurons (R = -0.0362).

To summarise, there were neurons in the CA2 which displayed organised discharge within the maze. Their spatial representations were more coherent and their spikes carried more spatial information. However, there also were neurons whose activity was disorganised and whose spatial representations were disordered.

The Spatial Organisation of Neurons Changed with the Addition of Social Context.

Once it was found that some neurons displayed organised activity in different sessions, I inquired how is this organisation affected by the addition of social context. To answer that question, I correlated the FRMs of neurons between the first empty session (e1) and the first social trial (trial 1). I found that the spatial organisation of virtually all neurons changed between the two sessions (fig. 19A). Their FRMs changed accordingly (fig. 20C-G). Some neurons, however, displayed greater change than others. Cell 10 and cell 20, for example, displayed the highest FRM correlations while cell 5 and cell 21 appear to have changed the most.

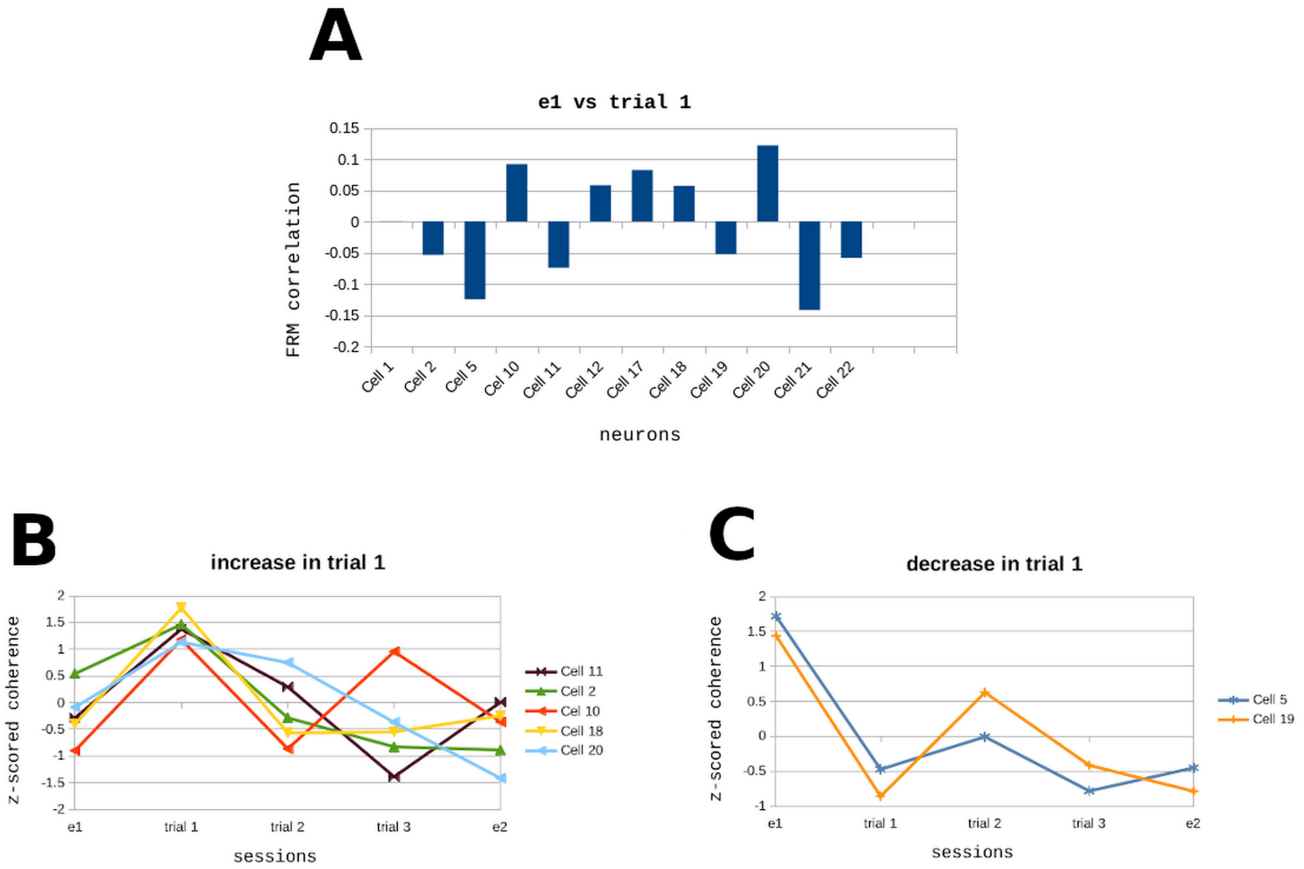

Figure 19: CA2 neurons changed their spatial properties when social context was added. ***A****, FRM correlation values between first empty session (e1) and first social trial (trial 1) for all stable neurons. Note that the correlation was never greater than 0.15.* ***B*** *and* ***C,*** *charts depicting normalised values of spatial coherence (y-axis) across sessions (x-axis).* ***B****, the FRMs of some neurons increased coherence in trial one.* ***C****, the spatial representations of few neurons became significantly less coherent when social context was introduced. Spatial coherence in both* ***B*** *and* ***C*** *was normalised for every cell using the standard deviation of its mean coherence across sessions.*

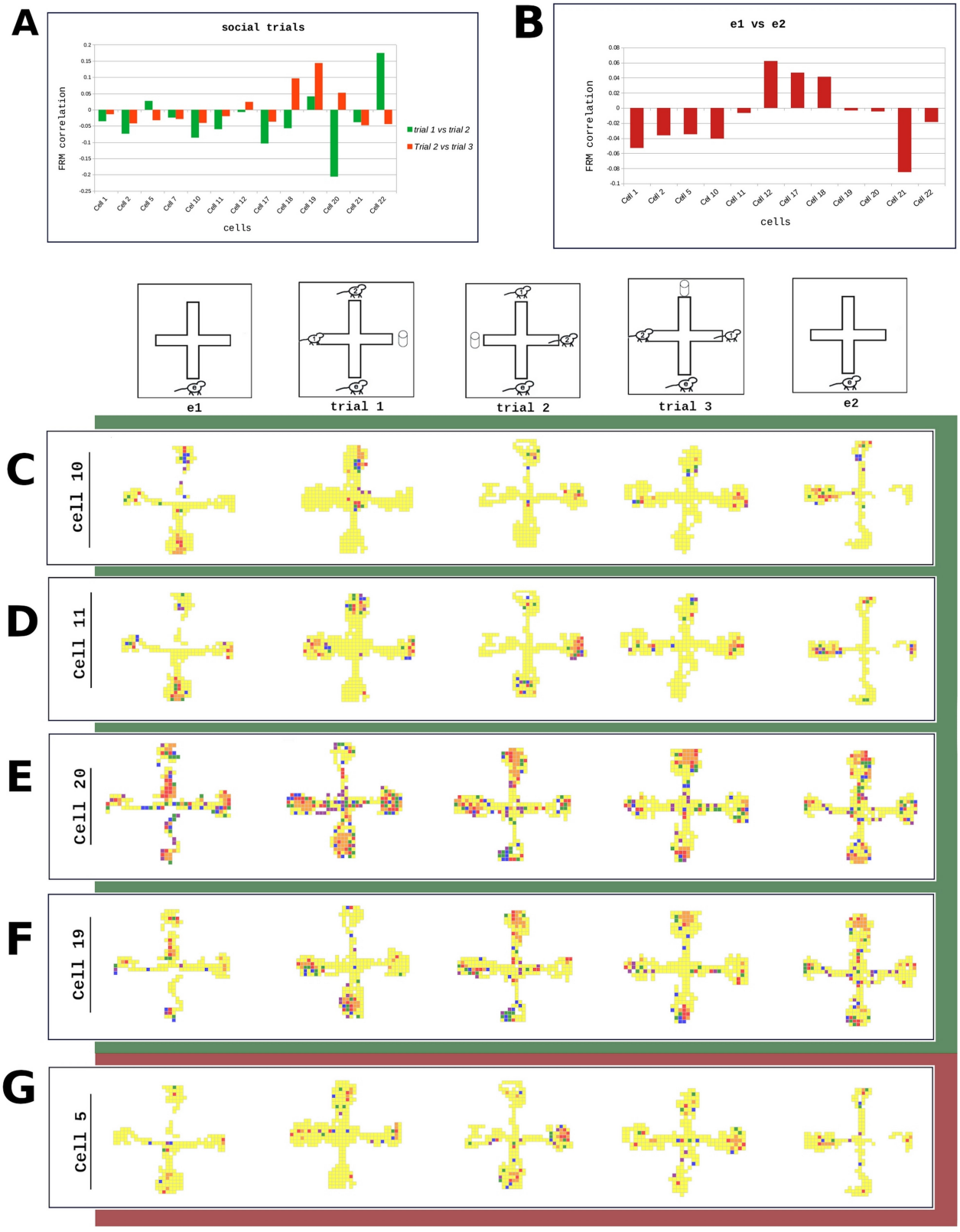

Figure 20: *the spatial activity of CA2 neurons changed within social trials.* ***A****, correlation of FRMs of stable neurons between social trials. Green bars depict correlations between trial 1 and trial 2, orange bars denote correlation between trial 2 and trial 3.* ***B****, correlations of FRMs for stable neurons between first (e1) and last (e2) session. Note that the highest correlation, between FRMs of cell 11, barely exceed the value of 0.06.* ***C - G****, colour-coded FRMs of selected neurons across all sessions. Colder colours mean higher firing rate - purple denotes the highest firing; yellow means that the cell did not discharge at all in the corresponding pixel. Cells in* ***C - F*** *displayed organised firing within the maze (highlighted in green for clarification). Cell in* ***G*** *also remapped between sessions, however it did not display spatially-organised activity (highlighted in red for clarification).*

As was previously mentioned, the better a neuron coded for space, the more variable its spatial coherence was (fig. 18B). Therefore, I also looked at how did spatial coherence change with the addition of social context. I found that the spatial representations of some neurons became more coherent when social stimuli were added in trial 1. 21% of neurons increased their spatial coherence in response to the addition of stimulus rats and object in trial 1 (fig. 19B; see fig. 20C, D and E for examples of FRMs).

Apart from the neurons which increased spatial coherence, I observed a few neurons whose FRMs became less coherent after exposure to social context (fig. 19C, see fig. 20F and G for examples of FRMs). It was later revealed that in both cases, the decrease in coherence in trial 1 was accompanied by an increase in firing rate ($R = -0.65$ for cell 5; $R = -0.56$ for cell 19).

To summarise, there was no spatial correlation of FRMs between session e1 and trial 1. Furthermore, the FRMs of some neurons became more coherent when social stimulation was added in trial 1.

The Spatial Organisation of Neurons Changed within Social Trials.

Next, seeing that neurons in the CA2 have changed their spatial activity when social context was added in trial 1, I inquired whether they will also alter their spatial properties during the social trials, when social stimuli are shuffled. Correlations of FRMs between consecutive social trials were calculated. I report that the FRMs were uncorrelated for all CA2 neurons (fig. 20A, see fig. 20C-E for examples of FRMs).

The correlation coefficients for individual neurons differed. The FRMs of cell 22, for example, were more correlated between trial 1 and trial 2 than the FRMs of other neurons, reaching correlation value of 0.174. This neuron, however, did not display spatially-organised firing. Cell 19 also appeared to have remapped less than other neurons between trial 2 and trial 3 (fig. 20F), with its FRM correlation reaching almost 0.15. Some neurons, on the other hand, were negatively correlated. Cell 20, on the other hand, displayed a FRM correlation value of -0.206 between trial 1 and trial 2 (fig. 20E).

The remaining neurons displayed correlation values slightly above or below zero between both pairs of trials, and their FRMs changed accordingly (fig. 20C, D). Of important note, the FRMs of both spatially-organised (fig. 20C-F) and disorganised (fig. 20G) neurons have remapped between social trials.

Finally, I also inspected the correlation of FRMs between the first and last empty session, e1 and e2. I report that there was no correlation for all neurons (fig. 20B). It should be noted, though, that the highest and lowest correlation values between e1 and e2 barely exceed 0.06 and -0.08, respectively. It appears that all neurons were equally uncorrelated, in contrast to the social trials, in which a few neurons seemed to be correlated more than other between some pair of trials.

To summarise, the FRMs of neurons between consecutive pairs of social trials were not correlated. There was no correlation between the spatial representations of neurons during the first (e1) and last (e2) empty sessions.

The Firing Rates of Neurons Changed in Sleep.

Finally, to study the processing of social information in sleep, I focused on the firing rates of neurons throughout the experiment. When the z-scored firing rates were compared, it became apparent that some neurons have increased while others decreased their firing rates in sleep relative to pre-sleep (fig. 21A). Incidentally, I often observed that neurons were silent during pre-sleep and also during some awake sessions, but became active during the sleep session. Note that those neurons were included in the analysis; their zero firing rate in pre-sleep was often expressed by highly negative z-scored value.

37% of neurons decreased their firing rate in sleep. 63% of neurons, on the other hand, increased their firing rate in sleep relative to pre-sleep. Interestingly enough, it seems that there was also a linear relationship between the firing of a neuron in pre-sleep and it's increase of firing rate in sleep; the less a neuron was firing in pre-sleep, the more it increased its firing rate in sleep ($r = -0.763$).

Next, I inquired whether the neuron's activity in sleep could be explained by their activity during wake. I found that some neurons which decreased firing rate in sleep also decreased firing rate during wake sessions (fig. 21B). I also found, however, some cell's firing rates of which increased during awake sessions but decreased in sleep (fig. 21C).

The majority of neurons which displayed increased activity in sleep also showed elevated firing rates in at least one awake session (fig. 21D). Of interest, half of the cells in this category were ones which were not stable across all sessions. On the other hand, there were a few neurons firing rates of which were elevated in sleep but which displayed relatively low firing rates throughout all awake sessions (fig. 21E).

To summarise, I found that 63% of neurons increased while 37% of neurons decreased their firing rates in sleep relative to pre-sleep. The majority of neurons which increased firing rate in sleep displayed elevated firing rate in at least one awake session.

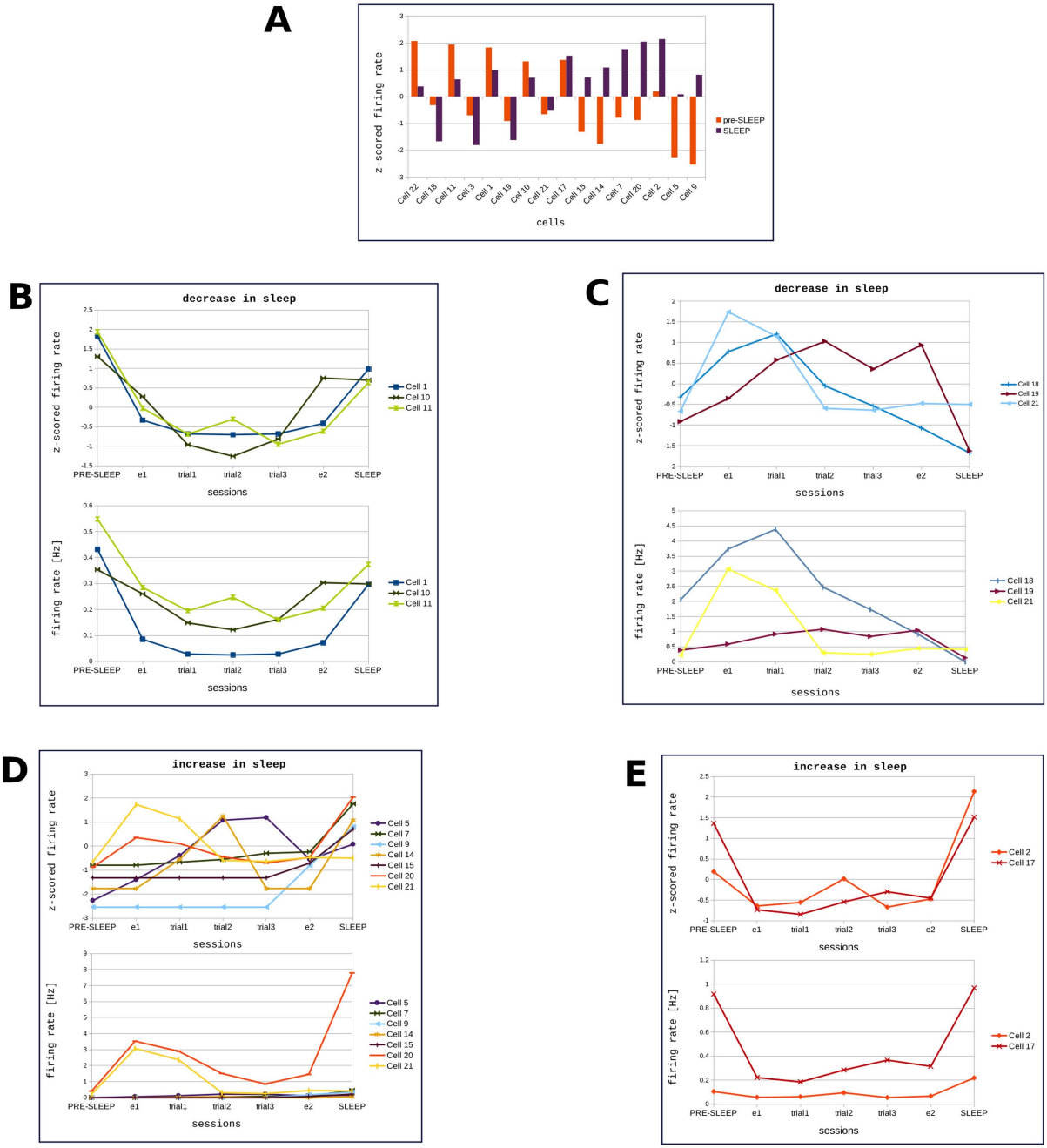

Figure 21*: the activity of neurons changed in sleep.* ***A****, a chart depicting z-scored firing rate of all neurons in pre-sleep session (brown bars) and sleep session (purple bars). Note that some neurons decreased firing rate (on the left) while some increased firing rate in sleep.* ***B*** *and* ***C****, neurons which decreased firing rate in sleep when compared to pre-sleep. The charts depict normalised (top row) and raw (bottom row) firing rates of neurons across all sessions. Note that neurons in* ***B*** *decreased whereas neurons in* ***C*** increased their *firing rates in awake sessions.* ***D*** *and* ***E****, neurons firing rates of which increased in sleep relatively to pre-sleep. Again, the graphs show z-scored (top row) and raw (bottom row) firing rates. Note that the neurons in* ***D*** *increased their firing rates in at least one awake session. 3 neurons in D were silent in pre-sleep and in some awake sessions, but became active later. The z-scoring was done using the standard deviation of a neurons mean firing rate across all sessions.*

7. DISCUSSION

The Ratio of Pyramidal Cells and Interneurons.

Based on anatomical measures, there should be 33 pyramidal cells for every single interneuron in the hippocampus (Aika et al., 1994). However, in electrophysiological recordings, this ratio is usually different, with interneurons accounting for cca 15% of all recorded units (Henze et al., 2000). It is often explained that the interneurons remain active in all experimental conditions whereas individual pyramidal cells are active only in some environments (Henze et al., 2000). The ratio of interneurons and pyramidal cells that I have observed is in agreement with those opinions.

Spatial Properties of CA2 Neurons.

In concordance with previous results (Mankin et al., 2015; Oliva et al., 2016, 2020), I have observed that the activity of some CA2 neurons is organised in space. I showed that 25% of the recorded pyramidal neurons displayed place cell properties in my experiment. In contrast, others have stated that up to 80% of pyramidal neurons in the CA2 are place cells (Oliva et al., 2020). This distinction, though, may be attributed to the differences in the definition of "place cell." It is also worth mentioning that Oliva and her colleagues used smoothened FRMs, which tend to show more consistent firing fields than the unsmoothened FRMs. In my thesis, unsmoothened FRMs were used so that the spatial activity could be visualised as raw as possible, without any bias.

The neurons I recorded generally discharged at many (3-4) locations, whereas others have reported a smaller number of firing fields in the CA2 (Mankin et al., 2012; Oliva et al., 2020). The difference could be due to the fact that I employed a unique experimental design, i.e. two social stimuli and one inanimate object on a plus-like maze. As the number of firing fields changes with the size of the maze (Muller and Kubie, 1989; Fenton et al., 2008), the higher count of firing fields could simply be explained by the fact that my maze was larger. On the other hand, it could also be related to the higher amount of stimuli I used.

The Response of Neurons to Social Context.

I report that, in concordance with previous studies (Alexander et al., 2016; Donegan et al., 2020; Oliva et al., 2020), the spatial organisation of neurons in the CA2 changed when social context was added. The correlation values I have observed were much lower than those of Alexander and her team. They showed that when social context was presented, spatial correlations of CA2 neurons dropped from 0.5 to 0.25 in one experimental condition and from 0.75 to cca 0.5 in the other experimental condition. A few of the neurons I recorded exhibited spatial correlation of 0.15 at best, but overall it appeared that they were uncorrelated between all pairs of sessions. It should be noted, however, that Alexander and her colleagues also recorded neurons which had extremely low spatial correlation values,

but those were counterbalanced by pairs with correlations much higher than mean (Alexander et al., 2016, supplementary). My results may therefore be biased by my relatively smaller dataset. Another possible explanation might be that Alexander and colleagues correlated pixels 2 or 5 cm^2 in area, whereas my pixels were 4 cm^2 large.

The Change of Spatial Organisation of Neurons Across Social Trials.

I have observed that the spatial organisation of CA2 neurons changed when stimuli were shuffled around the maze, as it seems that there was no correlation of spatial representations of neurons between successive pairs of social trials. In this aspect I agree with the work of Donegan and her colleagues (Donegan et al., 2020), who reported that the FRMs of CA2 neurons were not correlated between trials with different social stimuli. Although some of my neurons displayed interesting spatial correlation values in isolated cases, all in all it seems that they were equally uncorrelated between the social trials. Such hypotheses, though, need be tested on larger datasets using appropriate statistical analyses.

Naturally, the change of spatial organisation of neurons in my experiment could be explained by the effect of time (Mankin et al., 2015). It has been observed that the CA2 spatial representations of the same environment become less correlated as soon as after 30 minutes, due to, among other things, an increasing number of firing fields (Mankin et al., 2015). The processing of temporal information, however, was not the initial purpose of this thesis and, as such, the passing of time was not controlled for in my experiment. Was the lack of spatial correlation I have observed due to the shuffling of stimuli? Or would the spatial activity of neurons have changed regardless of those manipulations? It is now becoming clear that a control condition, in which the experimental rat navigates in an empty maze for two consecutive sessions, must be included when work on the experiment is resumed.

Interesting Response of Particular Neurons to the Shuffling of Social Stimuli.

Oliva and her colleagues found neurons in the CA2 which discharged only in the vicinity of a stimulus rat (Oliva et al., 2020). Their cells remapped in such a way that their firing field "followed" a single stimulus rat, while others (Alexander et al., 2016; Donegan et al., 2020) have noted no such thing. My result are not in agreement with the work of Oliva and her team. All neurons which displayed organised discharge within my experiment fired at multiple locations and, as such, it could not have been determined whether the discharge of a neuron followed around a particular stimulus rat. The discrepancy between mine and their observations could be trivially explained by the length of recording sessions (see also section 2.3.). It could also be attributed to the difference in experimental paradigm. For instance, Oliva and her team used a cca 50×50 cm large square arena with two stimulus rats in opposite corners, which is a very different setup from the one I used. Since the amount of firing fields of a neuron increases with the size of the environment (Fenton et al., 2008), it is possible that the size of my

maze (125 cm from one arm to the other) might have caused the high amount of firing fields in my experiment.

Nevertheless, some of my neurons have remapped in an interesting manner between social trials. Cell 10, for example, fired in north and south arm during session e1 (fig. C). When social context was added in trial 1, this neuron abruptly increased its coherence and began firing in the vicinity of stimulus rat s2 as well as in the centre of the maze. When the position of stimuli was shuffled in trial 2, the neuron was active in the north arm, where stimulus rat s1 was situated, but also in the east arm, where stimulus rat s2 was located. Second shuffling of stimuli caused cell 10 to discharge in the north, west and also east arm. In the second empty session, the cell fired mostly in the north and west arm, however its spatial representation was less coherent (fig. 19B and fig. 20C).

The behaviour of this neuron was most interesting. There appeared to be a rule to the seemingly random remapping of cell 10. Not only did it always discharge in the vicinity of stimulus rat s2, it also seems as if this cell was firing at locations where stimulus rat s2 was encountered in previous trials. Could this neuron encode not only present, but also past information about a social stimulus? It would be tempting to hypothesize that. Again, larger datasets as well as more thorough analyses are required to study the issue further.

Equally interesting was the remapping of another neuron (fig. 20D). Cell 11 fired in all terminal parts of the maze in session e1. When stimuli were introduced in trial 1, though, the neuron concentrated its activity to the terminal parts of all arms except south arm. Then, in trial 2, it discharged in all arms except for the west arm. Second shuffling of stimuli caused its spatial activity to "rotate" again, appearing in the west, north and east arm, same as in trial 1. In session e2, the neuron was yet again active in all arms; its FRM was, however, uncorrelated to the one in session e1 ($r = 0.0066$).

Most interestingly, it appears as if the spatially-organised discharge of this neuron rotated by 90° clockwise in trial 2, and again by 90° anticlockwise in trial 3. Correlations of the rotated FRMs were not evidential, though ($r = 0.0941$ for rotated trial 1 and trial 2; $r = -0.0630$ for rotated trial 2 and trial 3). As was the case with the previously mentioned neuron, more data must be gathered and more analyses performed for a hypothesis to be formed.

The Recruitment of Neurons with the Addition of Social Context.

In few isolated cases, I noticed that neurons were silent but became active when a social stimulation was added. My observations concur with the results of others, who have observed increased firing rates in response to social stimulation, that including an increase from zero (Donegan et al., 2020; Oliva et al., 2020). During the probe trials, I detected an interesting neuron which responded not only to the addition, but also to the removal of social stimulation. This cell was not active at all during the first probe trial in an empty maze, but begun firing when a stimulus rat was added onto the large square platform. Interestingly enough, the cell ceased firing when the social stimulus was removed in the next probe trial. Unfortunately, this neuron was not stable throughout pre-sleep session. Could it be

that this neuron responded to the presence and absence of the stimulus rat? Again, a larger dataset is required to make such assumptions.

The Activity of Neurons in Sleep.

I found that more than half of neurons in my experiment increased firing rates in sleep when compared to pre-sleep. Previous studies have shown that spatial information (Lee and Wilson, 2002) and possibly also social information (Oliva et al., 2020) may be reactivated during post-learning sleep as a result of exposure to stimuli during wake.

Indeed, the majority of my neurons which had increased firing rates in sleep also displayed elevated firing rate in at least one of the awake trials. It is interesting that their firing rate in pre-sleep was often the lowest of all sessions. Many such neurons were completely silent in pre-sleep and some wake sessions, only to become active in later sessions. Could those neuron be reactivated in sleep because they encoded information during wake?

On the other hand, there were also neurons which increased firing rate in sleep relative to pre-sleep but showed decrease of firing rate in wake. Interestingly, their firing rates in pre-sleep were higher than in wake. Furthermore, there likewise were neurons which decreased firing rates in sleep, yet displayed elevated firing rates in some wake sessions. Even though some of my neurons displayed intriguing changes in activity, a larger dataset as well as more thorough analyses are needed to form any hypotheses about the reactivation of social memory in sleep.

Conclusion.

I have recorded neuronal activity from the rat's CA2 hippocampal region in order to study the processing of social information in wake and also in sleep. I employed a novel experimental paradigm, in which social stimuli were shuffled on a four-arm plus-like maze between sessions. Specifically, there are four important outcomes of this thesis.

First, I saw that some neurons displayed organised firing within the maze. There were other neurons, though, spatial activity of which was disorganised. **Second**, I found that the spatial organisation of all neurons changed with the addition of social context. Furthermore, the spatial representations of some neurons became more coherent while the representations of other neurons became less coherent when social stimuli were added. **Third**, I observed that the spatially organised as well as the spatially disorganised neurons remapped when the positions of social stimuli were shuffled. No neurons discharged exclusively in the vicinity of a stimulus rat. **Fourth**, a large portion of neurons became more active in sleep when compared to pre-sleep.

The data presented in this thesis should be perceived as preliminary evidence to a research which is just beginning. My work delivers an elementary, yet essential characterisation of CA2 neuronal activity within our experiment. In many aspects, I have only scratched the surface of the issue at hand, especially in

terms of data analysis. The experimental configuration we conceived is novel in that it combines social stimulation with linear track and, as such, is especially fitted for the studying of reactivation of neuronal ensembles in sleep. Hence, when the work on the experiment is resumed, I plan to focus extensively on the recording and analysis of sleep data.

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