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Offline Reactivation of Hippocampal Place Cell Assemblies: Changes in Trajectory Replay Caused by Learning

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Rebecca Varga, B.A.

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Abstract

This interdisciplinary master thesis investigates offline hippocampal assembly replays, their content and their influence on the learning and behavior of rodents. The observed neural patterns of rats, after a day of learning a novel configuration of reward locations in a complex 8-arm maze, can be categorized into four distinct replay types differing in the number of occurrences. Besides the interdisciplinary (neuroscience, comparative biology, mathematics, medical science and AI) literature research for this paper, a unique R library was created in order to analyze and visualize the empirical data. The raw data was collected by Xu and colleagues (2019) via implanting tetrodes in the CA1 region of the animals' hippocampi. On the one hand, the on-literature-based expected correlation of replays and behavior was not supported by this set of data due to the lack of sequential observations of various rats. On the other hand, the analyzed data demonstrate that firing patterns encode trajectories. These trajectories can be replayed in forward and reverse manners during offline periods. Significant unexpected results show that forward replays consistently outnumber reverse replays during center-bound runs, while the opposite is true for reward-bound trajectories.

Table of Contents

Abstract i
Table of Contents ii
I. List of Figures iv
II. List of Abbreviations v
1. Introduction 1
1.1 Research Context 1
1.2 Motivation 2
1.3 Research Questions 2
1.4 Hypothesis 2
1.5 Research Approach 3
1.6 Interdisciplinarity 3
1.7 Structure Overview 4
2. Theoretical Background 5
2.1 The Hippocampal Formation 5
2.1.1 Neuroanatomy of the Rat Hippocampal Formation 6
2.1.1.1 Dentate Gyrus 7
2.1.1.2 Hippocampus Proper 8
2.1.1.3 Presubiculum, Subiculum and Parasubiculum 9
2.1.1.4 Entorhinal Cortex 10
2.1.2 Neurons of the Hippocampal Formation 13
2.1.3 Cross-species Comparison of the Hippocampal Formation 13
2.1.4 Connectivity 15
2.2 Cognitive Network Science 16
2.2.1 Definition 17
2.2.2 Assumptions about Cognition 17
2.2.3 Methods of Research 18
2.2.4 Support from Other Paradigms 20
2.2.4.1 Emergent Systems Approach 20
2.2.4.2 Predictive Coding 22
2.2.5 Limitations 22
2.2.6 Importance for this Research 23

2.3 Line of Research 23
2.3.1 The Early Stages 23
2.3.2 The “Birth” of Place Cells 25
2.3.3 Navigating between Place Cell Studies 26
2.3.3.1 Relations between Place Cells and Fields 27
2.3.3.2 Directionality 28
2.3.3.3 Replays 28
2.3.3.4 Content of Replays 29
2.3.3.5 Influence of AI 30
2.4 State of Research 31
2.5 Expected Results 34
3. Methodology 35
3.1 Design of the Original Experiment 35
3.2 Data 37
3.3 Analysis and Findings 38
3.3.1 New Arms 38
3.3.2 Path 39
3.3.3 Arm Visits 40
3.3.4 Learning Curve 41
3.3.5 Place Field Reactivations 43
3.3.6 Replayed Trajectories 46
3.3.7 Results from the Other Datasets 53
3.3.8 Statistical Testing 57
4. Discussion 62
5. Conclusion 64
6. References 66
7. Appendix: German Abstract 72

I. List of Figures

Figure 1 Rat Hippocampal Formation. Taken from: (Swanson & Hahn, 2020) 5
Figure 2 Structure of the dentate gyrus. Taken from: (Urban & Guillemot, 2014) 7
Figure 3 Cell types of the superficial layers of the LEC and MEC. Taken from: (Canto, et al., 2008) 11
Figure 4 Cell types of the deep layers of LEC and MEC. Taken from: (Canto, et al., 2008) 12
Figure 5 Hippocampal anatomy: a cross-species comparison. Taken from: (Strange, et al., 2014) 14
Figure 6 Intrahippocampal connectivity. Taken from: (O'Mara, 2005) 15
Figure 7 Levels of brain study. Taken from: (Fröhlich, 2016) 16
Figure 8 Dimensions of the radial 8-arm maze. Created based on (Xu, et al., 2019) 35
Figure 9 Radial 8-arm maze data labeling guide. Created based on (Xu, et al., 2019) 36
Figure 10 Distribution of rewards in four consecutive days 38
Figure 11 Path taken by rat 84 in four consecutive days according to the camera recording 39
Figure 12 Number of visits per arm during learning sessions 40
Figure 13 Learning curve of rat 84 in four consecutive days 42
Figure 14 Statistics of probability sums for 1120 locations during replays 44
Figure 15 Heatmaps of the reactivated place fields 45
Figure 16 Replayed arms during the 2nd night 46
Figure 17 Replayed arms during the four consecutive nights 47
Figure 18 Count of trajectory replays based on replay type (Night 1, TS 84) 49
Figure 19 Count of trajectory replays based on replay type after splitting bi-directional replays (Night 1, TS 84) 49
Figure 20 Distribution of replay categories based on direction and type during the four consecutive nights 50
Figure 21 Distribution of replay categories based on direction and type per arm during the four consecutive nights 51
Figure 22 Stacked bar chart of replay categories based on direction and type per arm during the four consecutive nights 52
Figure 23 Availability of datasets 54
Figure 24 Exceptional learning curve of TS 86 on Day 4 55
Figure 25 Heatmaps indicating multi-trajectory replays 56
Figure 26 Ratio of forward to reverse replays 57
Figure 27 Correlation of arm visits during the day and replays at night 58
Figure 28 Correlation of arm visits during the day and reverse replays at night 59
Figure 29 Correlation of arm replays at night and arm visits during the following day 60
Figure 30 Correlation of forward arm replays at night and arm visits during the following day 61

II. List of Abbreviations

AI – Artificial Intelligence

CA – Cornu Ammonis

CNS – Cognitive Network Science

DG – Dentate Gyrus

EEG – Electroencephalography

ENT or EC – Entorhinal Cortex

fMRI- functional Magnetic Resonance Imaging

HM – patient Henry (Gustav) Molaison

HSE – High-Synchrony Event

IS – Interneuron Selective

LEC/MEC – Lateral-/Medial Entorhinal Cortex

LM – Lacunosum-Moleculare

ML – Machine Learning

NSC – Neuronal Stem Cell

PET – Positron Emission Tomography

RL – Reinforcement Learning

RQ – Research Question

SBC – Subiculum

SD – Standard Deviation

SWR – Sharp Wave Ripple

TS – Test Subject

VTE – vicarious trial and error

1. Introduction

1.1 Research Context

Learning, and in particular spatial learning, is an inevitable part of life that allows cognitive systems to survive in ever-changing environments. Many believe that cognition resides in the neural substrate and emerges from the interaction of neurons in the neural network. Furthermore, learning modulates the structure of the network, its connectivity, and firing patterns. Cognitive Network Science focuses on the mesoscopic range of the spatial scale of brain research, analyzes neural networks and views the brain as a complex system with interacting parts. According to this paradigm, the hippocampus (and all other brain areas) is built up of individual interacting neurons, in which the performance of the interacting network is more than the mere sum of the individual performances, resulting in the emergence of spatial cognition and memory formation. Connectivity can be investigated via several methods, such as lesion studies, neuroimaging and electrode recordings.

The hippocampus established itself as a primary point of interest for memory consolidation after several lesion studies indicated that it is essential for forming and retrieving memories. Moreover, the laminar organization and unidirectional connections of the hippocampus provide an attractive research subject. As the hippocampal formation's basic architecture is similar across some species, it allows researchers to conduct invasive studies in rodents (such as the depth/single-cell electrode recording) and extrapolate the results to human cognition. One of these studies resulted in the discovery of place cells (O'Keefe & Dostrovsky, 1971). Place cells fire when an animal is at a particular location in space, thus, the sequential activation of these neurons can encode entire trajectories. Place cells do not only fire during active exploratory behavior or task performance, but trajectories are replayed (during SWRs) both during pausing in an awake state and during sleep (Gupta, et al., 2010). Several roles have been proposed for the replays ranging from the contribution to memory retrieval and consolidation to planning future trajectories (Bhattarai, et al., 2020). During pausing, trajectory replay plays a role in decision making (Xu, et al., 2019). During sleep, trajectory replay contributes to memory consolidation (O'Neill, et al., 2010). What is more, trajectory replay may not only help the recall of recently learned memories (task-specific), but it may also be involved

in wider processes, such as stabilizing place neuronal representations, strengthening the association of hippocampal representation to the cortex or schema learning. However, a unified theory about the role and content of replays has not been developed yet nor agreed upon until now.

1.2 Motivation

The motivation for conducting this research is two-fold, scientific motivation and personal motivation. Firstly, several studies aimed to uncover how place cell firings and trajectory replays in rats contribute to task performance; however, there is a lack of studies investigating how learning affects trajectory replays. To my knowledge, there have not been any direct studies so far of hippocampal reactivation during sleep with a change in the configuration in spatial learning tasks involving complex mazes. Secondly, I am interested in writing this thesis to obtain a deeper insight into the state-of-the-art research on learning and to gain knowledge in the field of neuroscience.

1.3 Research Questions

This thesis aims to find answers to the following research questions (RQs):

1. What is replayed during offline periods?
2. What is the relationship between forward and reverse replays?
3. Does learning modulate subsequent replays?
4. Do replays reflect future performance or behavioral patterns?

1.4 Hypothesis

According to previously conducted research studies, it is expected that existing trajectories accommodating for directionality (center-bound and reward-bound) within the maze are replayed during offline periods (RQ1). Due to the contradiction within the existing literature, the expectation about the ratio of forward-to-reverse replays is that it is irregular, and there

is no pattern (RQ2). As for RQ3 and RQ4, it is expected that the reactivation of place cell assemblies during sleep is modulated by learning performance and predicts subsequent recall and future behavior.

1.5 Research Approach

The research questions are attempted to be answered primarily by utilizing the datasets received from the Xu et al. (2019) study "Assembly Responses of Hippocampal CA1 Place Cells Predict Learned Behavior in Goal-Directed Spatial Tasks on the Radial Eight-Arm Maze", and partially by literature review. An R library is developed in order to provide with a unique tool to analyze this specific set of data. The number of forward and reverse replays will be compared to see whether a pattern emerges to answer RQ2. Visited arms and learning curves during the day will be identified and related to the number of trajectory replays (overall and reverse subset) at night to calculate the correlations between the number of arm visits and the number of arm replays. These correlations will answer the question about how learning modulates offline replays. The overall and forward subset of the replays will also be correlated with the subsequent day's observations to answer the question of whether replays predict future behavior.

1.6 Interdisciplinarity

Cognitive Science is an interdisciplinary field of study comprising several disciplines from neuroscience to philosophy. One aim of this thesis is to draw attention to the interdisciplinary aspect of an ostensibly only neuroscientific thesis. In this paper, a multitude of scientific fields contributes to the thesis outcome. Comparative biology allows the researcher to view the results of rat studies as significant indicators for learning about cognition in general, not only bound to one specific species. Mathematics is essential for understanding the Cognitive Network approach, which is the leading paradigm in this paper. Medical sciences contribute to both the path that led to the current study (such as the operation on patient HM, or the different brain imaging techniques like fMRI or PET scan) and the current research, as brain operation had to be conducted on the test subject rats to implant and lower the tetrodes into

the CA1 part of the hippocampus. Artificial Intelligence also exerts an influence on the development of the topic of the study. AI, and more specifically Reinforcement Learning, shapes the way how scientists think about biological learning, opening new doors for discoveries in mnemonics. Neuroscience is the main scientific discipline of the thesis as neural activity data is analyzed to reach conclusions. Trajectory replays are encoded by the connectivity and pattern of activations of different neuronal cells and assemblies.

1.7 Structure Overview

The theoretical background chapter introduces the reader to the fundamental knowledge about the hippocampal formation, cognitive network science, and some studies conducted on place cell assemblies in order to facilitate the understanding of the data analysis. While the hippocampal formation subchapter dives deep into the neuroanatomy and connectivity of the subregions, the cognitive network science subchapter introduces the assumptions of the paradigm, its limitations and its importance for this research. The line of research subchapter guides the reader through the forest of place cell studies and paves the path for the discussion about state-of-the-art research. Based on this theoretical background, anticipated results are discussed. The next chapter intends to clarify the research methodology, visualize the data and explain the data analysis. The discussion chapter translates these findings and answers the research questions. It also attempts to explain the unexpected results, identify limitations and open questions. Lastly, the conclusion provides an overview of the entire research and thesis findings with possible future research identification.

2. Theoretical Background

In this chapter, the basic theoretical background is introduced on which the master thesis research is built. Firstly, a short introduction is given on the structural properties of the specific brain areas within the hippocampal formation, as it is crucial for the understanding of the connectivity between its subdivisions and place cells. Secondly, cognitive network science is introduced as the discipline that emerged from the study of the structural properties of the brain. In that subchapter, I elaborate on the assumptions that cognitive network scientists hold about cognition, the support these views receive from other cognitive science paradigms, the limitations that have to be faced and the importance of these views for the current master thesis research. Thirdly, the line of research subchapter is paving the path from the birth of place cells to the subchapter of the current state of research concerning hippocampal place cell assemblies. Lastly, preliminary expectations of the results are discussed that are based on the theoretical background stated beforehand in the chapter.

2.1 The Hippocampal Formation

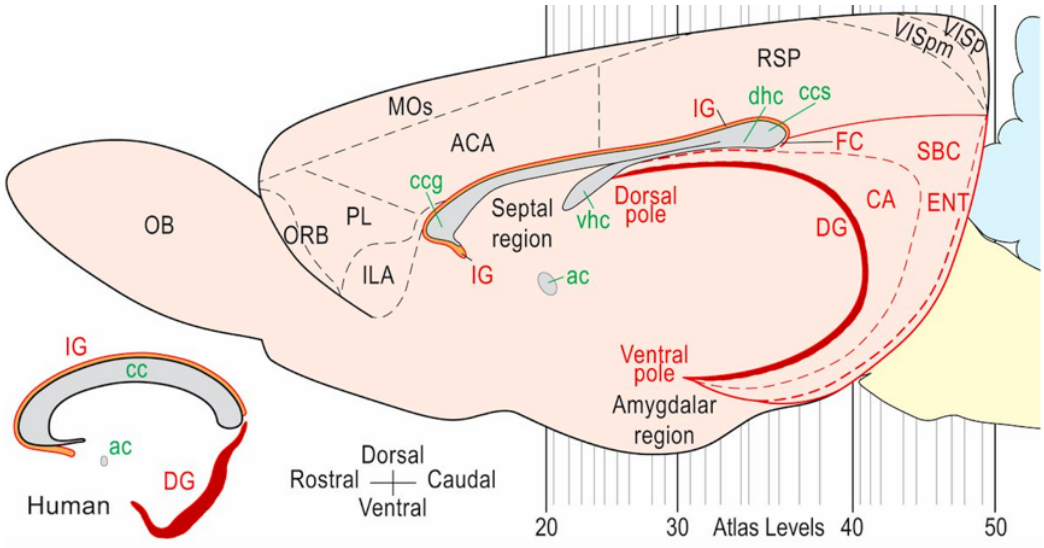

Figure 1 Rat Hippocampal Formation. Taken from: (Swanson & Hahn, 2020)

The hippocampal formation consists of different brain areas, namely the dentate gyrus, the hippocampus, the subiculum, the presubiculum, the parasubiculum and the entorhinal cortex (Anderse, et al., 2007). In *Figure 1* (Swanson & Hahn, 2020), a rat's hippocampus viewed from the side is highlighted with red (Dentate Gyrus = DG, Hippocampus = CA, Subiculum = SBC, and the Entorhinal Cortex = ENT).

According to Anderse and co-authors, studies on the hippocampus are attractive due to the fact that it possesses the following features:

> “
> - A single cell layer and strictly laminated inputs
> - Predominantly unidirectional connections between a series of cortical regions
> - Extrinsic and intrinsic fibers making numerous *en passage* contacts with target neuronal dendrites, running orthogonal to the main dendritic axis
> - Synapses that are highly plastic
> - Tissue that can be used in transplantation studies
> - Neurons that can be successfully grown in culture
> - Acute or cultured slices surviving for prolonged periods in vitro
>
> “ (Anderse, et al., 2007)

Having a single cell layer and predominantly unidirectional connections aids research as the structure and connectivity are simpler than in other brain areas. Extensive research has been carried out in this formation as the understanding of its functions and inner workings can be considered being a first step towards understanding more complicated formations. Another reason for the interest is the highly plastic synapses. Plasticity plays an important part in learning and memory formation, thus researchers, passionate about memory formation, study the hippocampal formation to acquire knowledge about learning. Moreover, the hippocampus is viewed as the principal brain area for spatial cognition, thus it is the primary focus of this research.

2.1.1 Neuroanatomy of the Rat Hippocampal Formation

In this subchapter, some important neuroanatomical discoveries about the hippocampal formation of rats are introduced. I am concerned primarily with rodent brain structure, as the data utilized in this thesis was collected during an experiment on rats. However, similarities between the hippocampal formations of rodents and humans cannot be neglected, thus I will also provide a summary of the comparison in a later chapter. Brain areas will be introduced in the following order: Dentate Gyrus, Hippocampus, Subiculum (including pre- and parasubiculum) and the Entorhinal Cortex.

2.1.1.1 Dentate Gyrus

The dentate gyrus consists of three layers, the molecular (mostly cell-free) layer and the principal cell layer (granule cell layer) make up the fascia dentata, which encloses the polymorphic cell layer (Anderse, et al., 2007).

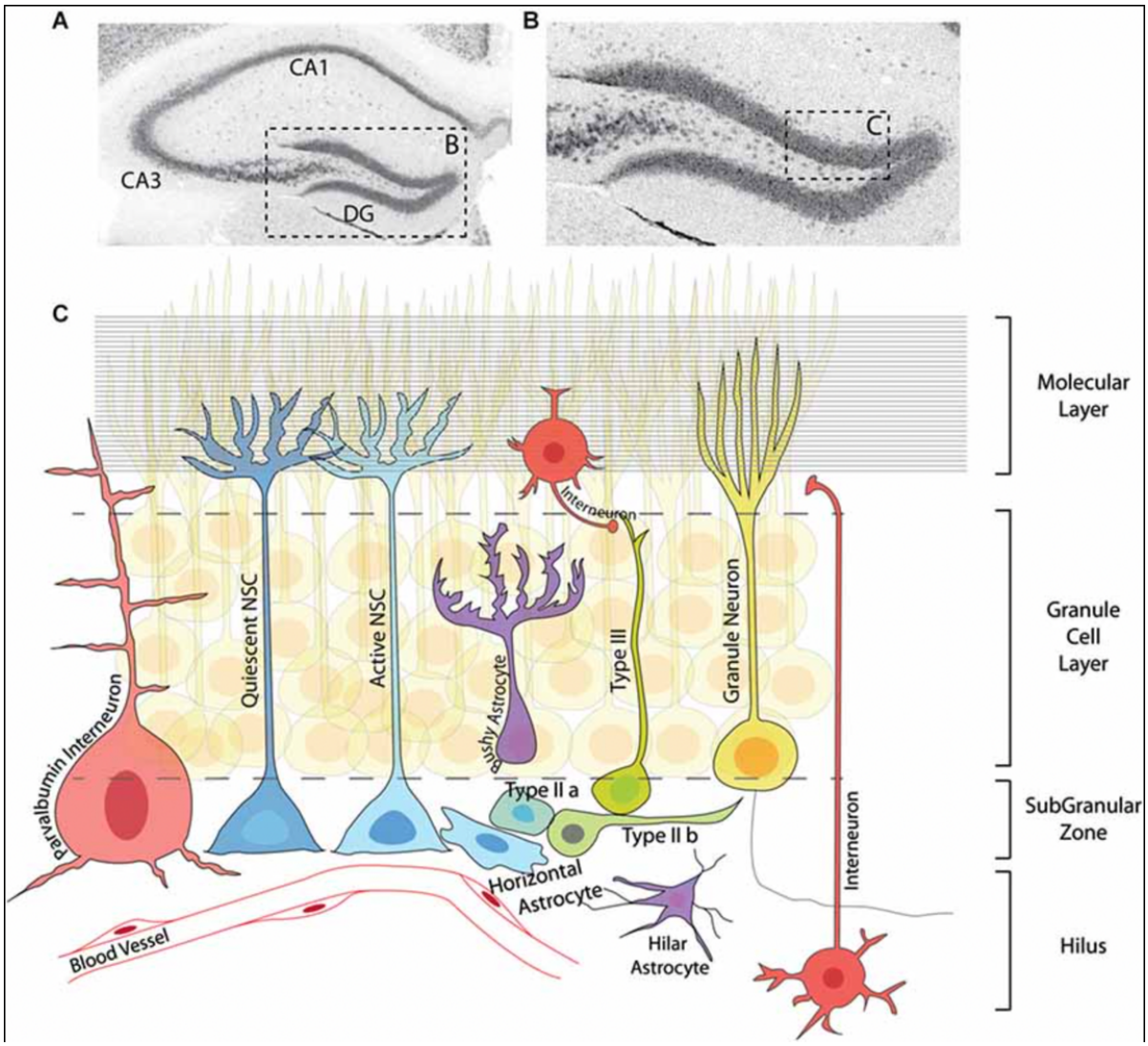

Figure 2 Structure of the dentate gyrus. Taken from: (Urban & Guillemot, 2014)

There are 1.2 * 10^6 granule cells in the rat's principal cell layer (West, et al., 1991); (Rapp & Gallagher, 1996). As *Figure 2* (Urban & Guillemot, 2014) also illustrates, next to the granule cells, there are other types of neurons situated on the border of the granule and polymorphic layers. These can be dentate pyramidal basket cells, astrocytes and neural stem cells (NSCs)

among others (Amaral, et al., 2007); (Urban & Guillemot, 2014). The dendrites of these different types of neurons and the axons coming from the entorhinal cortex are the primary occupants of the molecular layer (Anderse, et al., 2007). The most common cell type of the polymorphic cell layer is the mossy cell (Amaral, et al., 2007).

The entorhinal cortex provides the major input for the dentate gyrus through the perforant path (Ramón y Cajal, 1893); (Anderse, et al., 2007). However, the dentate gyrus receives inputs from other brain areas as well, such as the septal nuclei, the supramammillary area, several hypothalamic nuclei, the pontine nucleus locus coeruleus, the ventral tegmental area, and the raphe nuclei (Loughlin, et al., 1986); (Nyakas, et al., 1987); (Magloczky, et al., 1994). Next to the external connections, the dentate gyrus accommodates several different internal connections, such as the inhibitory basket cell connections to the granule cells and the mossy fiber projections to the polymorphic layer and the commissural zone (Claiborne, et al., 1986); (Frotscher, 1992). The dentate gyrus solely projects to the CA3 region of the hippocampus via the mossy fibers (Swanson, et al., 1978). The 1.2 * 10^6 mossy fibers (granule cells) project to 2.5 * 10^5 CA3 pyramidal cells, with the average of each granule cell projects to 15 pyramidal cells, and each pyramidal cell receives input from 72 granule cells (Anderse, et al., 2007).

2.1.1.2 Hippocampus Proper

The hippocampus proper consists of 3 parts, CA1, CA2, and CA3 (was named after Cornu Ammonis) (Anderse, et al., 2007). Although all three layers contain pyramidal cells, CA1 is denser than the other two. This property of the CA1 region, combined with its ability to receive both excitatory and inhibitory inputs from both intrahippocampal connections (Schaffer, 1892) and extrahippocampal brain areas, such as the entorhinal cortex or the nucleus reuniens (Ramón y Cajal, 1911); (Kemppainen, et al., 2002), attracts scientists to choose CA1 as a primary target for research purposes. This master thesis also builds on data received from tetrodes that were implanted in the CA1 region of rats' hippocampi.

The stratum oriens is relatively free of cells containing the dendrites of the above-mentioned pyramidal cells and other types of interneurons (Anderse, et al., 2007). These interneurons could be pyramidal basket cells, chandelier cells, lacunosum-moleculare cells (LM cells), bistratified cells, and interneuron-selective cells (IS cells) (Lacaille, et al., 1987); (Freund & Buzsaki, 1996).

As the previous section stated, the hippocampal CA3 region receives inputs from the dentate gyrus. The dentate gyrus is not the sole external provider of input to the hippocampus. The perforant path originating from the entorhinal cortex heading to the dentate gyrus perforates and additionally projects to the CA3 and CA2 regions of the hippocampus (Anderse, et al., 2007). Next to the perforant path, the entorhinal cortex also has other projections to the CA1 field of the hippocampus. These connections are reciprocated. (Naber, et al., 2001); (Anderse, et al., 2007). The hippocampal formation cannot be seen as an isolated structure, thus projections from other brain areas are important to be mentioned. Some of these connections are with the neocortex, the amygdaloid complex, the septum, the hypothalamus, the supramammillary area, the tuberomammillary nucleus, the nucleus reuniens and the brain stem nuclei (such as the locus coeruleus, and the raphe nuclei) (Magloczky, et al., 1994); (Dolleman-Van der Weel & Witter, 2000). The hippocampus not only receives input from the other parts of the hippocampal formation or other brain areas, but it also has external projections. The CA1 region of the hippocampus projects to the subiculum and the entorhinal cortex (Amaral, et al., 1991); (Naber, et al., 2001).

Although I have mentioned several external inputs to the hippocampus, most of its inputs come from the hippocampus itself. The three main types of connections are the associational connections, the commissural connections, and the Schaffer collateral connections (Ishizuka, et al., 1990); (Anderse, et al., 2007). The associational connections project from the CA3 to the CA3 and also from the CA2 to the CA2. The commissural projections originate from the CA3 and project to all contralateral areas of the hippocampus (Fricke & Cowan, 1978); (Anderse, et al., 2007). The Schaffer collaterals reach from the proximal CA3 region to the distal CA1 region (Ishizuka, et al., 1990); (Anderse, et al., 2007).

2.1.1.3 Presubiculum, Subiculum and Parasubiculum

The principal cell layer of the subiculum is also filled with pyramidal neurons (Anderse, et al., 2007). The subiculum is a major output structure of the hippocampal formation; it has efferent projections to the diencephalon and the brain stem (Swanson & Cowan, 1975); (Kloosterman, et al., 2003); (Anderse, et al., 2007). The most prominent projections are towards the ventral orbitofrontal cortices, the pre- and infralimbic cortices, the retrosplenial

cortex, the amygdaloid complex, the septal complex, the nucleus accumbens and the mammillary nuclei (Thompson & Robertson, 1987); (Wyss & Van Groen, 1992); (Verwer, et al., 1997). Moreover, it also projects externally within the hippocampal formation to the presubiculum, the parasubiculum and the entorhinal cortex (Anderse, et al., 2007).

The presubiculum contains associational connections, which primarily interconnect the dorsoventral levels and commissural connections, which project to the contralateral presubiculum (Haug, 1976); (Van Groen & Wyss, 1990); (Anderse, et al., 2007). Both the pre- and the parasubiculum are reciprocally connected with the anterior thalamic nuclear complex (Robertson & Kaitz, 1981). The major efferent projections from the presubiculum and the parasubiculum within the hippocampal formation target the entorhinal cortex (Caballero-Bleda & Witter, 1993); (Anderse, et al., 2007). Moreover, the parasubiculum projects to the dentate gyrus (Kohler, 1985). Extrahippocampal connections include the input received from the basal forebrain, the supramammillary nucleus, nuclei of the brain stem and the projections to the mammillary nuclei (Pitkanen, et al., 2000); (Anderse, et al., 2007).

2.1.1.4 Entorhinal Cortex

The entorhinal cortex is a unique part of the hippocampal formation that is both the starting point and the endpoint of information processing within the hippocampal formation (Van Hoesen, et al., 1991); (Anderse, et al., 2007). The entorhinal cortex can be divided into smaller regions based on different criteria. In this paper, I mention two types of subdivisions based on the cytoarchitectonic organization (regional organization and laminar organization). Two areas can be recognized on a regional organization basis, the lateral entorhinal cortex (LEC) and the medial entorhinal cortex (MEC) (Canto, et al., 2008). The EC (both LEC and MEC) has a laminar organization with six layers, two acellular layers (I, IV) and four cellular layers (II, III, V, VI) (Canto, et al., 2008).

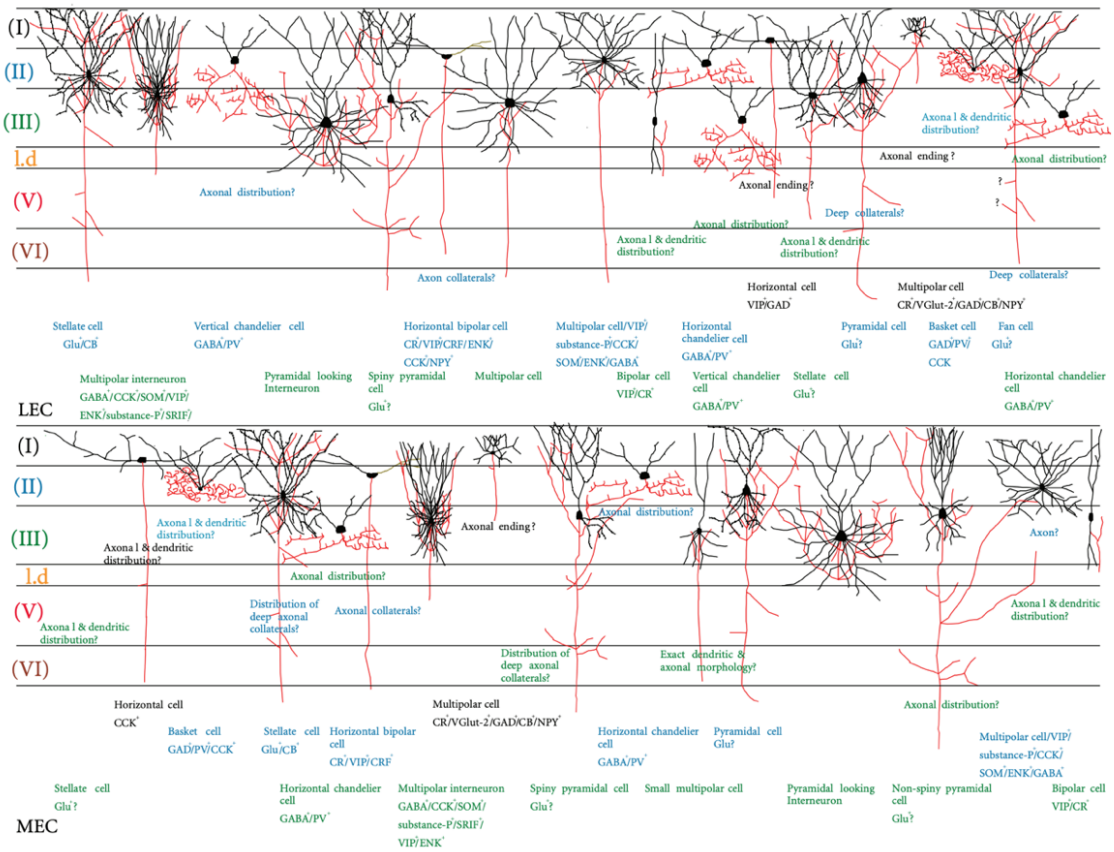

Figure 3 Cell types of the superficial layers of the LEC and MEC. Taken from: (Canto, et al., 2008)

As *Figure 3* (Canto, et al., 2008) shows, layer I (molecular layer) is relatively neuron-free, and it mostly contains the fibers of neurons located in other layers. Layer II (outermost cell layer) is mostly populated with stellate and small 'modified' pyramidal cells (Anderse, et al., 2007); (Canto, et al., 2008). Cells in this layer are relatively larger than cells in the same layer of surrounding brain areas (Canto, et al., 2008). Pyramidal neurons are the predominant cell types in layer III, which is a wide, but loosely arranged layer (Anderse, et al., 2007); (Canto, et al., 2008).

Figure 4 (Canto, et al., 2008) illustrates the deeper layers of the entorhinal cortex. Layer IV (lamina dissecans), similar to layer I, is a "cell-sparse fiber layer" that can be better developed in the MEC. Layer V forms a densely packed layer of small-, medium-, and large-sized pyramidal cells (Canto, et al., 2008). The cell population of layer VI has varying sizes and shapes (Anderse, et al., 2007).

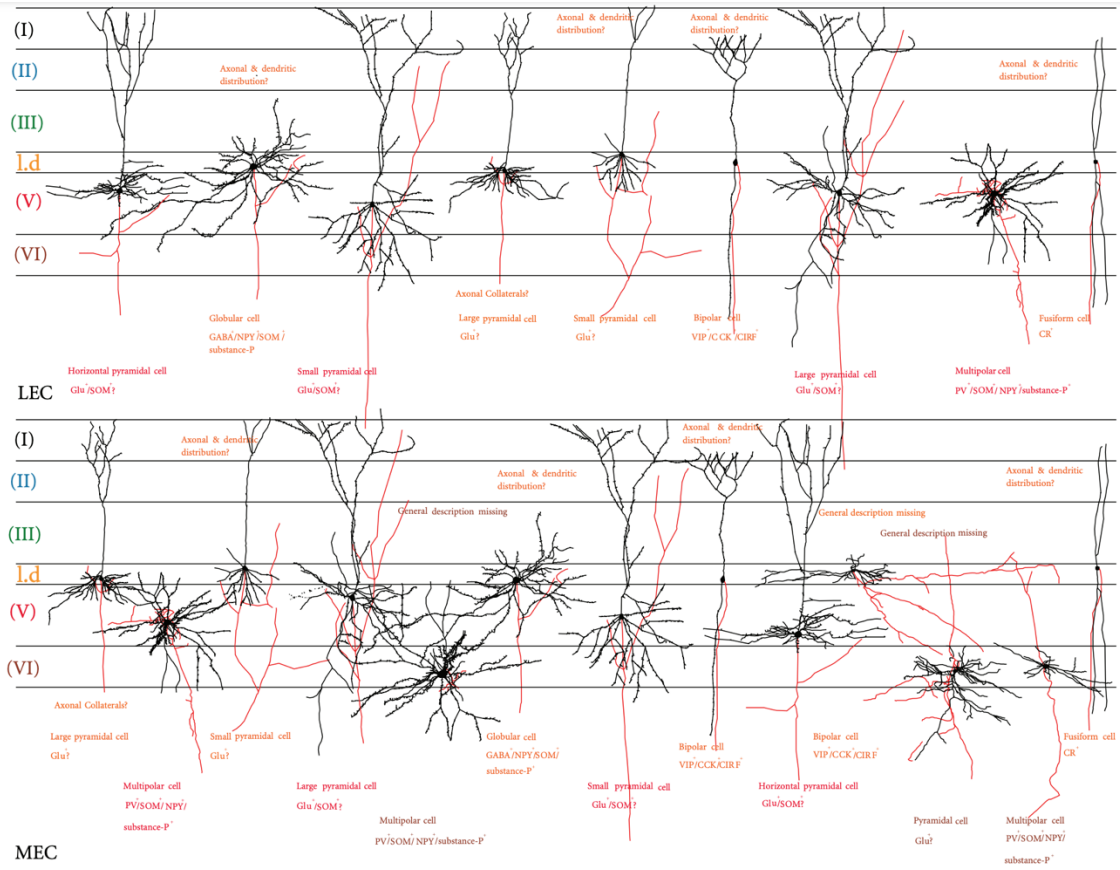

Figure 4 Cell types of the deep layers of LEC and MEC. Taken from: (Canto, et al., 2008)

Layer II projects primarily to the dentate gyrus and the CA3 and also has associational and collateral projections (Klink & Alonso, 1997); (Dolorfo & Amaral, 1998); (Deller, 1998). The perforant and the alvear paths originate from layer III and project to CA1 and the subiculum, while they also provide for collateral projections on the way (Gloveli, et al., 1997); (Dolorfo & Amaral, 1998). Layer Va populated by pyramidal cells primarily collect input from layers I and II and project to the deep white matter and the angular bundle, while their collaterals reach the deep layers of the entorhinal cortex (Hamam, et al., 2002); (Anderse, et al., 2007). Layer VI has both internal and external projections (Anderse, et al., 2007).

The entorhinal cortex has interconnections with neocortical regions (such as the piriform cortex, temporal regions, frontal regions, insular cortex, cingular region, parietal region, occipital region) (Burwell & Amaral, 1998); (Anderse, et al., 2007). Moreover, inputs are received from subcortical regions as well, such as the amygdala, the claustrum and the striatum (Pikkarainen, et al., 1999). Further connections exist with the septum, hypothalamus, thalamus and brain stem (Van der Werf, et al., 2002).

2.1.2 Neurons of the Hippocampal Formation

There are two main classes of hippocampal neurons, the principal cells and the interneurons (Anderse, et al., 2007). The principal cells are the primary output neurons from subregions of the hippocampus that form different pathways. Interneurons are the primary building blocks of intrahippocampal/local circuits and mainly inhibitory; however, they could exert disinhibitory effects on certain principal neurons (Freund & Buzsaki, 1996); (Buckmaster & Soltesz, 1996).

Hippocampal neurons are highly plastic. They demonstrate axonal sprouting and reactive synaptogenesis (Raisman, 1969); (Anderse, et al., 2007). Plastic properties include a remarkably quick and efficient regeneration. Matthews and colleagues (1976) found that the molecular layer of the dentate gyrus showed a maximal reduction of associated boutons five days after a perforant path lesion. However, a substantial recovery was observable after two weeks and the tissue regained its original, normal density of boutons after three weeks of the injury (Matthews, et al., 1976). Moreover, hippocampal neurons are transplantable as shown by Anders Björklund's group. Their experiment proved that catecholamine- or acetylcholine synthesizing neurons in a transplanted tissue were able to extend axons, restore the connections and reinnervate the hippocampus (Bjorklund, et al., 1975); (Bjorklund, et al., 1976).

2.1.3 Cross-species Comparison of the Hippocampal Formation

Basic architecture-wise, human and other animal hippocampi are extremely similar, however, there are differences in size and thickness. As Anderse and colleagues (2007) explain, the hippocampus of a monkey is 10 times larger than a hippocampus of a rat, while the volume of the human hippocampus is 100 times larger than that of the rat. The differences continue with the CA1 region, as it is thicker and more heterogeneous in monkeys and humans than in rats (30 cell-thickness compared to 5 cell-thickness). The entorhinal cortex can be divided into cyto-architectonically distinct subdivisions. Rats have two, monkeys have seven, while humans have 27 (other descriptions recognize only 8) subdivisions (Anderse, et al., 2007). Another example of differences is that rats possess a massive commissural system in the dentate gyrus (Raisman, et al., 1965); (Gottlieb & Cowan, 1973), however, it is almost

absent in dentate gyri of monkeys and humans (Amaral, et al., 1984). Moreover, primate entorhinal cortices have stronger interconnections with the more developed associational areas of the neocortex.

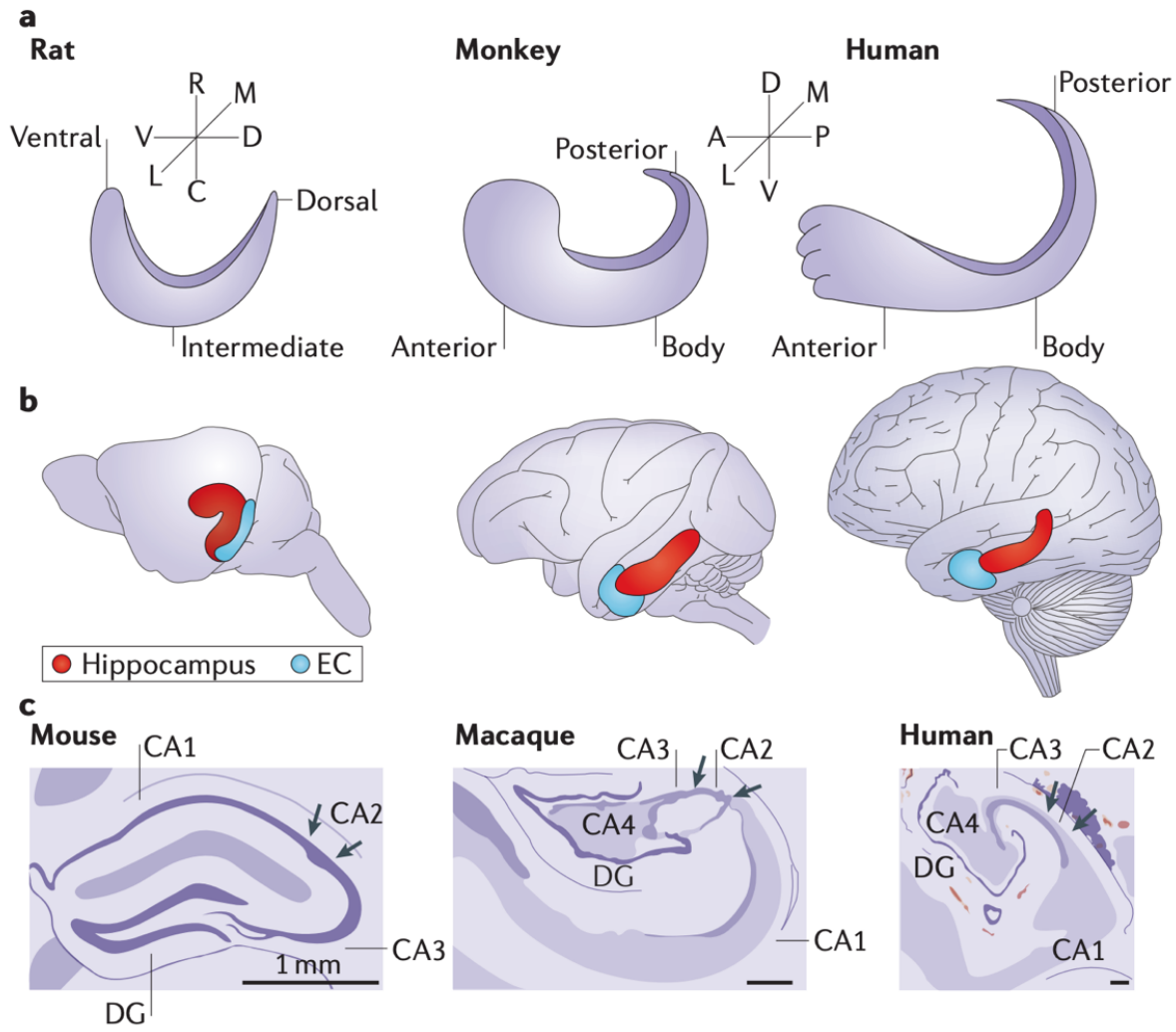

Figure 5 Hippocampal anatomy: a cross-species comparison. Taken from: (Strange, et al., 2014)

Figure 5 (Strange, et al., 2014) illustrates the hippocampal formations of rats, monkeys and humans. As it can be seen in section a.), the basic form of the hippocampal formation in different animals is similar, however, it should be noted that the rat hippocampus has to be rotated by 90-degrees to have the same orientation as in primates. Section b.) visualizes the position of the hippocampal formation within the brain of all three species. The rotation mentioned under section a.) is clearly visible on the illustrations. Section c.) contains the three Nissl cross-section drawings of a mouse, a macaque and a human hippocampal formation, therefore, they provide the viewer with a deeper understanding of its inner structure and subsections.

2.1.4 Connectivity

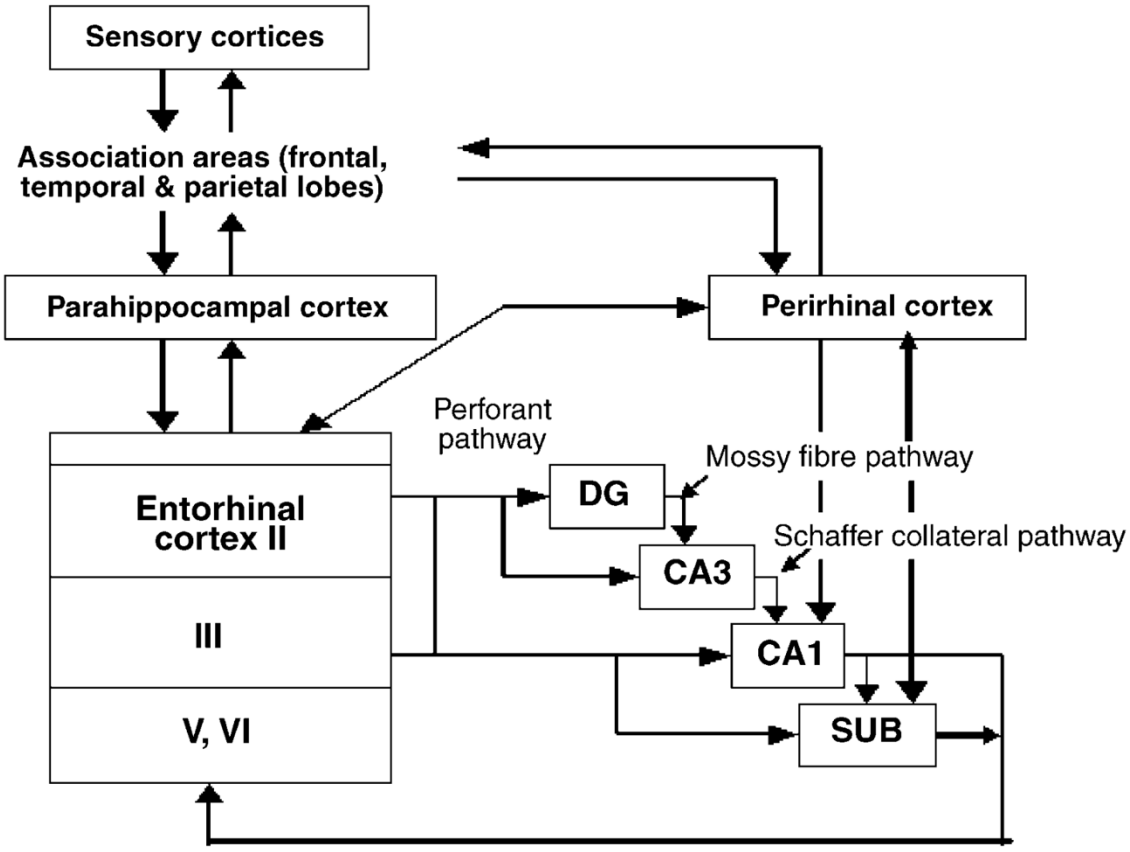

Figure 6 Intrahippocampal connectivity. Taken from: (O'Mara, 2005)

The intrahippocampal connectivity is illustrated in *Figure 6* (O'Mara, 2005). As it can be seen on the diagram, the connections between the cortical areas in the hippocampal formation are predominantly unidirectional. Most of the neocortical input goes through the entorhinal cortex, thus it can be considered as the starting point of the intrinsic hippocampal circuit. From the entorhinal cortex (layer II), axons project to the dentate gyrus. These projections are part of the perforant path, which is the major hippocampal input pathway. Interestingly, the entorhinal cortex does not receive projections back from the dentate gyrus, thus it is a unidirectional pathway. Next to the perforant path, the entorhinal cortex projects to the pre- and parasubiculum. This fiber pathway is called the angular bundle. The temporoammonic alvear pathway is another major route for the entorhinal fibers to reach the CA1 part of the hippocampus. The granule cells of the dentate gyrus project to the pyramidal cells of the CA3 area of the hippocampus. These axons are called mossy fibers. This pathway is also unidirectional as there is no projection from the CA3 to the dentate gyrus. Moreover, CA3

cells project to the CA1 region, with the so-called Schaffer collateral axons. CA1 region provides the major (unidirectional) excitatory input for the subiculum. Furthermore, CA1 also projects to the entorhinal cortex. The subiculum projects to the presubiculum, the parasubiculum and the entorhinal cortex. In the complex fiber system of the alveus, the pyramidal cell layer of the hippocampus and the subiculum forms the origin of some of the alvear fibers going to subcortical regions. (O'Mara, 2005); (Anderse, et al., 2007).

2.2 Cognitive Network Science

Brain activity and behavior can be analyzed on a multitude of levels. As *Figure 7* (Fröhlich, 2016) shows, the analysis can have a wide scope from the microscopic range (nanometers) to the macroscopic range (centimeters) on a spatial scale. Within the microscopic range, the targets of observation are molecules within the brain, synapses, neurons and glial cells. Thus, the microscopic scale is primarily concerned with the smallest building blocks of the brain. On the other end of the spectrum, the macroscopic scope focuses on the biggest building blocks, which are the brain areas. However, there is an important step between neurons and brain areas. The mesoscopic range analyses the networks of neurons.

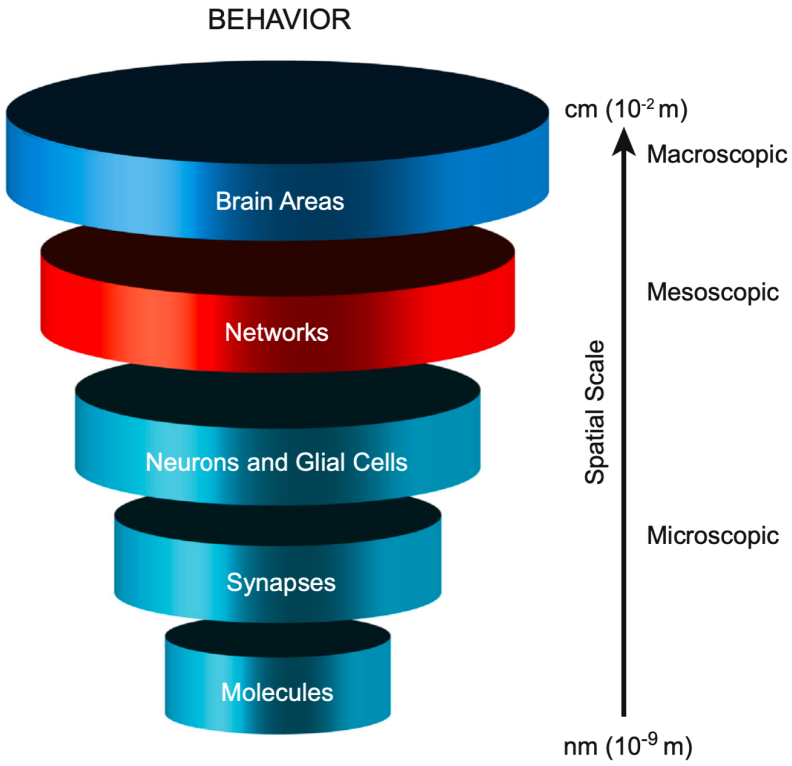

Figure 7 Levels of brain study. Taken from: (Fröhlich, 2016)

2.2.1 Definition

Network science is a scientific discipline that stems from network theory, which is a branch of mathematics. According to the *Encyclopedia of Computational Neuroscience* (2015), network theory involves the analysis of the mathematical abstractions of networks, the graphs (Papo, et al., 2015). The discipline emerged as a result of the establishment of the readily available, large datasets containing information about different types of systems in several fields of study (e.g. social systems, biological systems, technological systems, etc.). The evolution of the discipline facilitated a deeper understanding of the dynamical processes and functioning of the networks in addition to the topological analysis. The emergence of the interdisciplinary field of cognitive network science brought about a conceptual shift in the view of the brain. According to network science, the brain is viewed as a complex system instead of a computer-like entity. As the previous subchapter stated, the Hippocampal Formation possesses several internal and external connections, thus it can be analyzed as a network of neurons. In this scenario, the different brain regions are the nodes, and their relationships are the edges in the graph (Mattar & Bassett, 2020). Mathematically speaking, n nodes and their relationship can be represented in an adjacency matrix with n rows and n columns, where the individual numbers signal the strength of the connection between two specific neurons or brain regions.

2.2.2 Assumptions about Cognition

Pursuant to the complex system view of the brain, “networks are endowed with properties which stem in a nontrivial way from those of their constituent nodes” (Papo, et al., 2015). To apply network science to cognition, one is required to assume that the brain is made up of distinct brain areas and regions (Mattar & Bassett, 2020). The connections between these regions might be either structural or functional. Structural connections are physical connections between neurons and different brain regions, while functional connections are statistical relationships about timely activities. Functional connections can be found during different neuroscientific and clinical studies or examinations. The methods for studying the functions of the hippocampal formation, that are built on similar assumptions, include lesion studies, neuroimaging (PET/fMRI) and invasive electrode recordings. It is important to understand

both the possibilities and the limitations of these methods to be able to fully appreciate and correctly interpret the results of research. These methods will be elaborated on in the following subchapter (*2.2.3 Methods of Research*).

Baronchelli and his colleagues argue that there are several applications of network theory in cognitive science (Baronchelli, et al., 2013). For example, the neural organization can be described with a network framework and cognition is the result of neuronal network activity which is an interaction between different cell assemblies and brain regions (Sporns, et al., 2004). They also reinforce the previously mentioned advantage of network science, that is, researchers can see the brain as a complex system, focusing on the importance and balance of both local processing and global integration (Zamora-Lopez, et al., 2011). The connectome (map of brain connectivity) can provide some crucial insights into brain functioning (Zamora-Lopez, et al., 2011), as different network properties have been found to be associated with pathologies. The study conducted by Barttfeld and colleagues found that decreased clustering, higher path length, and modularity are correlated with autistic spectrum disorder (Barttfeld, et al., 2011). Schizophrenics tend to demonstrate shorter path length and increased assortativity (Bassett, et al., 2008). Secondly, network science can assist with measuring the cognitive complexity that is required for navigating in labyrinths, as relevant points are possibly encoded as a weighted network (Amancio, et al., 2011).

To sum up, the main assumption about cognition is that the brain is a multilayered network of neurons and brain regions; cognition emerges from the interaction of the different neurons and brain regions in that multilayered network.

2.2.3 Methods of Research

This subchapter gives an overview of the different research methodologies that can be used to discover the connectivity in the brain and the network and interaction of neurons and brain regions. Lesion studies, neuroimaging (PET/fMRI) and intrusive electrode recording are the most prevalent methodologies.

Firstly, one method of functional research is the observation and analysis of the behavior of patients with lesions to a specific brain area, in this case to the hippocampal formation (Anderse, et al., 2007). Memory as a function of the hippocampus was brought by the famous study of patient HM, which will be discussed later.

Neuroimaging using PET or fMRI also provides important neural activity data that is connected to the functional activity of different brain areas. According to the *Neuroscience: Exploring the Brain* book, PET operates with the following procedure:

> "a radioactive solution containing atoms that emit positrons (positively charged electrons) is introduced into the bloodstream. Positrons, emitted wherever the blood goes, interact with electrons to produce photons of electromagnetic radiation. The locations of the positron-emitting atoms are found by detectors that pick up the photons" (Bear, et al., 2007).

A neuroscientific application of this method was developed to measure the metabolic activity in the brain. This method could be used to track the connections between neurons. Some of the major limitations of the PET method are its spatial resolution (only 5-10 mm^3, containing thousands of neurons on the image), timely length and the invasive nature of radioactivity. FMRI uses a different procedure, it measures the ratio between the magnetic resonance of oxyhemoglobin and deoxyhemoglobin in the brain. The principle behind this method is that more active brain regions receive more blood and use more oxygen (thus the blood's oxygen donation will be higher). Although fMRI is faster than PET, has a better spatial resolution ($3mm^3$) and is non-invasive, neuroimaging still faces several problems. Most importantly, these studies do not provide causational evidence, only correlational data. In case there is a neural activity during a specific task at that specific brain region that is being imaged, the neural activity could easily be responsible for another task, thus being incidental to the task being observed. Secondly, these techniques lack a baseline activity for the neurons and brain regions (Anderse, et al., 2007).

Another frequently used methodology is depth electrode recording. During this procedure, microelectrodes are placed inside specific brain regions. These electrodes, residing in the extracellular place within neuron vicinity, detect the current flow caused by action potentials. Electrode recordings are capable of recording regions that would not be possible to reach from the outside (like EEG). This technique is used for single-cell recording as it has a high spatial resolution, however, as the electrode is located in the extracellular place, it is not guaranteed that the recording comes from a single neuron (Gazzaniga, et al., 2014). It is more likely that the activity of a small set of neurons will be recorded instead of that of a single cell.

The primary test subjects are rodents and non-human primates out of ethical considerations as the process is vastly invasive.

2.2.4 Support from Other Paradigms

The views of cognitive network science on cognition are shared by other cognitive science paradigms. In the following subchapter, I elaborate on the similarities of views of cognitive network science, emergent systems approach and predictive coding. Within the emergent systems approach, both connectionism and dynamical systems approach are mentioned.

2.2.4.1 Emergent Systems Approach

In contrast to the cognitivist approach, which is based on the main assumption that cognition is a result of symbolic information processing representational systems/result of computations, the emergent systems approach is based on the principles of self-organization, dynamics and emergence (Kelso, 1995); (Clark, 2001); (Vernon, et al., 2007). The computational operations, according to the emergent systems approach,

> "exploit processes of self-organization, self-production, self-maintenance and self-development, through the concurrent interaction of a network of distributed interacting components" (Vernon, et al., 2007),

which directly links to the Cognitive Network Science view of interacting brain regions. The representation framework within the emergent systems approach holds that

> "representations are global system states encoded in the dynamic organization of the system's distributed network components" (Vernon, et al., 2007).

Emergent systems are capable of learning and adaptation by structural re-modeling and self-re-organization (Vernon, et al., 2007). Connectionism and dynamical systems are emergent systems approaches that reinforce specific aspects of cognitive network neuroscience in various ways. The following paragraph will elaborate on connectionism

In the Stanford Encyclopedia of Philosophy (2019) connectionism is defined in the following way. “Connectionism is a movement in cognitive science that hopes to explain intellectual abilities using artificial neural networks” (Buckner & Garson, 2019). The two most defining ideas of connectionism are: firstly, cognition emerges from the interaction (activation propagation) between neuronlike entities, and secondly, these interactions are regulated by weighted connections, similar to synapses in the biological brains (Rogers, 2009). Both principles of connectionism agree with the principles of Cognitive Network Science to some degree. The main difference between the Cognitive Network Neuroscience approach and the Connectionist Systems approach is that connectionist systems do not require entities to be biological neurons or brain regions; entities can be neuron-like artificial nodes. Cognitive Network Neuroscience, on the other hand, mathematically models and maps actual brain areas into a multilayered network. However, both approaches abstract away from the biological substrate, thus limiting themselves from including information about the types of connections/communication between neurons and regions, (such as the exact neurotransmitter used for a certain purpose between specific neurons). Another similarity between the approaches is that they both use multilayered networks to model cognition and the brain (Vernon, et al., 2007). Furthermore, these two paradigms hold that the current state of the network connectivity is “inseparable from its history of transformations” (Varela, 1992); (Vernon, et al., 2007), meaning that at each point in time, the network includes all the adaptation/learning that had taken place so far.

Next to connectionism, the dynamical systems approach also supports some aspects of cognitive network neuroscience. An assumption of the dynamical systems approach that reinforces cognitive network neuroscience is that parts of the system need to be connected, and they must interact (McClelland & Rogers, 2003); (Vernon, et al., 2007). Moreover, both aim to develop simplified models that can represent the high dimensionality of the biological system.

The different approaches operate on different parts of the scale, as connectionist systems address microscopic behaviors and dynamical systems address macroscopic behaviors (Vernon, et al., 2007); (Gibson, 2014). Cognitive network neuroscience forms a bridge between the two ends of the scale operating at the middle addressing the mesoscale.

2.2.4.2 Predictive Coding

The second approach, which is considered as a supporting paradigm for cognitive network neuroscience, is predictive coding. The definition of predictive coding given by Huang and Rao (2011) is as follows: “Predictive coding is a unifying framework for understanding redundancy reduction and efficient coding in the nervous system” (Huang & Rao, 2011). According to this paradigm, the brain is seen as a continuous hypothesis-testing mechanism, that strives to minimize its prediction errors based on the sensory input coming from the world/environment (Hohwy, 2013). Redundancy reduction is possible by the removal of predictable input components through learning statistical regularities of the environment (Huang & Rao, 2011). Thus, neural networks only transmit non-predictable inputs. The base assumption, similar to CNS, is that the computations occur at the neuronal substrate, and information is encoded in the network of neurons. Another similarity between the two paradigms is that both approach cognition from a mathematical standpoint. While cognitive network science focuses on the interaction and network of neurons and their mathematical measurements, predictive coding utilizes the Bayesian statistical framework for error minimization and redundancy reduction purposes.

Predictive coding has been studied in the hippocampus as well. According to Mehta (2001), there are observable, rapid anticipatory changes in the receptive fields of the rodent hippocampus, when the rat repeatedly crosses a track (Metha, 2001).

2.2.5 Limitations

Network neuroscience/cognitive network science opens new pathways for analyzing and studying the brain and provides alternative viewpoints on cognition. However, one should be attentive to its limitations as well. Critics might argue that cognitive network science does not define the nodes sufficiently, as it can group various regions due to structural or functional connectivity in one node (Mattar & Bassett, 2020). At the same time, edges can represent a simplified version of interaction between nodes, thus losing important information about the nature of the connection.

Vitevitch identifies some additional limitations, one of which is the mathematical complexity behind working with multilayered networks (Vitevitch, 2020). As multilayered network

science in mathematics is a relatively new field, several discoveries are still needed to be developed in order to provide a sound mathematical background for the analysis of such networks with several layers.

Furthermore, network neuroscience faces the challenge of analyzing networks that are continuously changing (Vitevitch, 2020). The structural and functional connectivity of our brains is subject to change. These changes occur due to numerous factors, listing some without aspiring for completeness: learning, acquiring new experiences, sensing the external environment, damaging parts of the brain in accidents and decay due to aging.

2.2.6 Importance for this Research

Cognitive Network Science is essential for understanding this thesis, as locations are not encoded in the brain by the firing of a single place cell, but by the pattern/configuration of firing across multiple place cells. Cognitive Network Science is the discipline chosen for this thesis, as it allows the researcher to view the brain as one complex system in its entirety that is interconnected, thus collecting an additional benefit that network science can provide for complex systems research and analysis. It builds on the idea that the collective performance of the interaction between individual parts is greater than the sum of the parts.

2.3 Line of Research

The purpose of this chapter is to set the scientific stage for the discovery of place cells, introduce the reader to the groundbreaking O'Keefe and Dostrovsky study which started the sequence of research studies about place cells. Moreover, the chapter aims to provide an overview of the fundamental research and navigate between studies focusing on place cells, grid cells, cognitive maps and neural replays among others that have paved the path from the discovery of place cells to the current research of this thesis.

2.3.1 The Early Stages

The hippocampus has been a point of interest since ancient times since the first dissections took place in ancient Egypt. Several different theories have arisen since then about the

hippocampal function. Until the 1930s, the hippocampal formation has been studied as a part of the olfactory system (Rose, 1935); (Anderse, et al., 2007). However, in 1947, Brodal argued, that "No decisive evidence that the hippocampus is concerned in olfaction appears to have been brought forward" (Brodal, 1947). He also argued, that anosmic mammals (e.g. dolphins) also possess sizable hippocampal formations, thus olfaction cannot be the sole function of the hippocampus. Brodal's argumentation brought about a change in the study of hippocampal functions.

Parallelly, Papez hypothesized in 1937 that the hippocampal formation plays a role in emotions and that it is an important part of the Papez circuit. According to his hypothesis,

> "The central emotive process of cortical origin may then be conceived as being built up in the hippocampal formation and as being transferred to the mammillary body and thence through the anterior thalamic nuclei to the cortex of the gyrus cinguli" (Papez, 1937).

An important step towards the hippocampal function as a cognitive map was the influential work of Tolman in 1948 (Tolman, 1948). He proposed that there is a possibility that the brain simulates the possible outcomes of actions with the help of a mental map of the environment. The observations that supported his theory were vicarious trial and error (VTE) (Muenzinger, 1938) and latent learning (Blodgett, 1929). As Redish explains, VTE is a "pause-and-look" behavior observed in rats when they face a choice of path in a maze, implicating that they are thinking about the future (Redish, 2016).

From 1950 to 1957, William Scoville and Branda Milner carried out several brain operations (more than 300) to alleviate people from diverse neurological and psychiatric problems; then they made observations about the brain-lesioned patients (Scoville & Milner, 1957). One of their most famous patients, H.M., changed the landscape of studies of the functions of the hippocampal formation. H.M. was treated because of his severe epileptic seizures. "On September 1, 1953, bilateral medial temporal-lobe resection was carried out, extending posteriorly for a distance of 8 cm." (Scoville & Milner, 1957). The surgery successfully reduced his seizures, however, it also resulted in persistent global amnesia (both anterograde and retrograde amnesia). Anterograde amnesia prevents him from remembering any material/episodes that he has experienced since the operation. Retrograde amnesia prevents him from recalling information that he has experienced some period of time before the surgery. There

was complete retrograde amnesia for the period of 19 months before the operation and partial retrograde amnesia for the three years before his operation (Scoville & Milner, 1957). During the operation, a large part of the hippocampal formation and the surrounding cortical regions have been removed, thus the new hypothesis arose that the hippocampal formation is involved in memory. Soon after different animal studies have been launched to test the hypothesis of hippocampal involvement in memory. Interestingly, the early studies on primates failed to provide a model of medial temporal lobe amnesia (Orbach, et al., 1960); (Correll & Scoville, 1967). Studies on rats also fell short of providing convincing evidence of memory deficit caused by a lesion to the hippocampus. However, the rats with damaged hippocampi showed hyperactivity in novel environments, a deficit of habituation and a severe deficit of navigation in complex mazes (Kaada, et al., 1961); (Kimble, 1963); (Kveim, et al., 1964).

2.3.2 The "Birth" of Place Cells

In the article published in 1971 in '*Brain Research*', O'Keefe and Dostrovsky elaborate on the staggering discovery that they have made during an experiment involving rats. Previously, it has been observed that rats with damaged hippocampi are hyperactive in novel environments and seem to lack the ability of spatial learning and navigation. During their experiment, O'Keefe and Dostrovsky have found evidence in support of the theory of cognitive (spatial) map as a function of the hippocampus (O'Keefe & Dostrovsky, 1971). During the experiment, the scientists lowered electrodes into the hippocampal areas (CA1 and 4) and the dentate gyrus of rats to record neural activity occurring in those areas. Altogether 76 units were obtained which have been classified according to the neural activity in response to stimuli. Out of these 76 units, 14 units were 'arousal' units, 21 were 'movement' units, 2 units were categorized as 'expectation' units, and 31 had inconsistent firing patterns, thus are not classified in any category (O'Keefe & Dostrovsky, 1971). The remaining 8 units are the most important for aiding the birth of the spatial map theory. As O'Keefe and Dostrovsky state: "These 8 units responded solely or maximally when the rat was situated in a particular part of the testing platform facing in a particular direction" (O'Keefe & Dostrovsky, 1971). The firing patterns indicated that place (as an abstract concept) is signaled instead of a sensory stimulus. Firstly,

they could not find any sensory stimuli that consistently controlled a cell's firing pattern. Secondly, with experience, place cells fired in the correct location even in the dark; the firing pattern is not dependent on the motivation/reward (O'Keefe & Dostrovsky, 1971). Their findings indicate that the rest of the brain is provided with a spatial reference map by the hippocampus. According to this model, during the movement of the animal or an intention of movement in space, cells specifying the subsequent spatial position and orientation would be activated by cells specifying the previous/current state (O'Keefe & Dostrovsky, 1971). Thus, the sequence of neural activities can encode trajectories. In case the expected ('anticipated') and the actual ('experienced') activation pattern did not match, an exploration is triggered, which attempts to incorporate the new pattern into the already existing map (O'Keefe & Dostrovsky, 1971). Note that this method of comparing anticipated and experienced activation patterns and incorporating the new, unpredicted pattern in the existing map is in line with the predictive coding paradigm's base assumption. It suggests that navigation also follows the prediction error minimization principle. The researchers argue that the defective spatial movement/learning behavior in rats with damaged hippocampi is a result of the missing spatial reference map (O'Keefe & Dostrovsky, 1971).

2.3.3 Navigating between Place Cell Studies

After the introduction of place cells to the scientific community, several neuroscientists-initiated research projects had the objective of gaining more knowledge about spatial coding. These projects shed light, on the one hand, on the basic properties (such as the size and shape) of place fields. Place fields are the locations where particular place cells fire. They also shed light, on the other hand, on the relationship between these place fields and their place cells. A multitude of research has been carried out focusing on the directionality of the place cells in different environments. Discoveries have been made about hippocampal replays, their functions and their content, which are elaborated on in this subchapter. The readers are also introduced to the interaction between neuroscience and AI, followed by pointing out the benefits of this interaction on the research on forward and reverse hippocampal replays.

2.3.3.1 Relations between Place Cells and Fields

Hetherington and Shapiro (1997) found that place fields tend to cluster along the edges of rectangular boxes (Hetherington & Shapiro, 1997). The size of place cells tends to increase from the dorsal to the ventral hippocampus (Jung, et al., 1994); (Maurer, et al., 2005). Place cells can, but do not necessarily, have place fields in different environments (O'Keefe & Conway, 1978); (Kubie & Ranck Jr., 1983). Many cells act like silent cells in different environments and only encode a place field in one or a few (Thompson & Best, 1989). This is important to note, as the data of this thesis was collected in one specific 8-arm maze, thus only those place cells have to be examined that have place fields in that maze. Moreover, studies show that experience contributes to the differentiation of place cell firing, which is called remapping (Bostock, et al., 1991); (Lever, et al., 2002); (Wills, et al., 2005). As rats in this experiment have considerable experience with the specific 8-arm maze, the special encoding is differentiated from other environments.

Multiple studies have shown that spatial learning tasks have a dynamical modulatory effect on hippocampal place cell firing patterns (Markus, et al., 1995); (Wood, et al., 2000); (Frank, et al., 2000); (Hok, et al., 2007); these changes are directly correlated with task performance (Ferbinteanu & Shapiro, 2003); (Dupret, et al., 2010); (Allen, et al., 2012). As task learning has a modulatory effect on the hippocampus, place cells were shown to disproportionally represent goal locations (Hollup, et al., 2001); (Hok, et al., 2007). Other studies have suggested that the hippocampal formation plays a crucial role in forming long-term memories of relevant places to retrieve rewards from goal locations (Olton & Samuelson, 1976); (Morris, et al., 1982); (Rawlins & Olton, 1982). Once the rat is searching for the reward in a maze, place cell firings are not only influenced by the animal's current location, but also by past and future arm choices which indicates a role in decision-making (Frank, et al., 2000); (Wood, et al., 2000); (Ferbinteanu & Shapiro, 2003); (Spellman, et al., 2015).

Eichenbaum and colleagues (1989) reported that neighboring cells have place fields that are closer in space at a higher rate than would be expected by chance (Eichenbaum, et al., 1989). On the other hand, multiple scientists observed, that there is no topographical relation between hippocampal and environmental locations (O'Keefe, et al., 1998); (Lever, et al., 2002). This observation was reinforced by Redish's research group (2001) as well, which provided an insight into the correlation between the closeness of place cells and their place fields.

They found that neighboring place cells do not have a higher tendency to have neighboring place fields than any other place cell located in the hippocampus (Redish, et al., 2001).

2.3.3.2 Directionality

Muller's study (1994) showed that in open environments the place fields are nondirectional (Muller, et al., 1994). However, it is not the case in enclosed environments, such as radial arm mazes (McNaughton, et al., 1983); (Markus, et al., 1995) and narrow linear tracks (O'Keefe & Recce, 1993); (Gothard, et al., 1996). This finding is important for the current research, as the test subjects are recorded in a closed environment, in a radial 8-arm maze, thus the place fields are highly directional. This will have to be considered during the analysis of the dataset containing the firing pattern recordings. The most important variables that seem to be deciding factors in directionality, according to Markus (1995), are whether the test subject can turn around anywhere in the enclosed space and whether one location can be reached via many directions. If a location is reachable only via a limited number of paths (directions), the place cells tend to become directional (Markus, et al., 1995). In this study, most of the locations have a limited number of paths leading towards them, thus the place cells will probably be directional.

2.3.3.3 Replays

Spatial memory formation is assisted by High-Synchrony Events (HSEs), brief periods of synchronous firing patterns of cell assemblies, that can encode a trajectory in a temporally compressed manner (Lee & Wilson, 2002); (Foster & Wilson, 2006); (Davidson, et al., 2009). These trajectories can be replayed during Sharp-Wave Ripples (SWR).

Wilson and McNaughton discovered the hippocampal activity replays during sleep in 1994 (Wilson & McNaughton, 1994). They recorded around 100 neurons during active exploration and slow-wave sleep as well, both before and after the actual active task. They analyzed the activity of these neurons and found that pairs of neurons that had a correlated activity during the task had higher correlated activity during the subsequent sleep (Wilson & McNaughton, 1994).

Lee and Wilson (2002) observed neural replays in rats during sleep after a day of training of running on a linear track (Lee & Wilson, 2002). The replays had the same sequence of activity patterns as during task execution; however, replays were plenty faster. While the sequence of activity during task execution in awake state took around 2.4 seconds, the replayed sequence during sleep took just 120 milliseconds, thus it was 20 times faster (Lee & Wilson, 2002). In addition to forward sequential replays, researchers found that neural activity patterns are sometimes replayed in reverse order, resulting in a backward replay (Foster & Wilson, 2006). Although backward replays are also time-compressed and 10 times faster than awake activation, they are usually slower than forward replays (Euston, et al., 2007).

Later, it had been observed by Johnson and Redish (2007) that replays do not solely occur during sleep, but they are also present at VTEs during awake state at an intersection and predict the future movement of the animal (Johnson & Redish, 2007). Singer and his colleagues found that even though the SWR predicts whether the animal makes a mistake, the reactivated trajectories do not predict the animal's trajectory choice (Singer, et al., 2013). Contrarily, Wikenheiser and Redish found that the length of the trajectory replay is in correlation with choosing a distant or a near-goal location (Wikenheiser & Redish, 2015). What is more, Pfeiffer and Foster argued that the direction of the replays is in correlation with the direction of the goal location (Pfeiffer & Foster, 2013).

2.3.3.4 Content of Replays

A key research question of this paper is, however, what is replayed, what is the content, and the functions of the hippocampal replays. There is no clear answer for the functions of the replays, however, several roles have been proposed. Replays seem to contribute to memory retrieval and consolidation, connecting recent and remote memories, learning from experience, planning future trajectories and building cognitive and/or value maps (Bhattarai, et al., 2020).

The modification of the hippocampal circuitry in order to consolidate learned spatial relations is supposed to be done by reverse replay, which is strongly biased by reward and novelty (Pfeiffer, 2020). Forward replay seems to retrieve stored representations to guide future behavior (Pfeiffer, 2020). The third type of replays (next to forward and backward replays) is the 'imaginary replays' which were observed by Gupta and colleagues in 2010 (Gupta, et al.,

2010). Imaginary replays occur when a sequence of activity patterns is replayed, that encode a path that is possible in the environment, however, it has never been experienced by the animal in an awake exploratory or task execution state (Gupta, et al., 2010).

Replays during awake and off-line periods show fundamental differences. Disturbing off-line replays results in impaired long-term memory but no influence on place field representation (Girardeau, et al., 2009); (Ego-Stengel & Wilson, 2010); (Kovacs, et al., 2016). Blocking awake replays results in an impaired working memory and place field stability, while it exerts no effect on long-term memory (Jadhav, et al., 2012); (Roux, et al., 2017). However, recent studies have shown that there is an imbalance between replays, and there are significantly more forward replays during sleep (Wikenheiser & Redish, 2013); (Grosmark & Buzsaki, 2016). This contradicts the idea, that both reverse replay and sleep are responsible for memory consolidation while forward replay is responsible for future behavior; and it points to the direction that forward replays might have a double purpose: memory retrieval in the awake state and memory consolidation in off-line states (Carr, et al., 2011); (Lewis & Durrant, 2011). Replays might encode episodic memories representing both 'what' and 'where' instead of simple spatial trajectories (Takahashi, 2015). However, it is unclear whether these replays are conscious or subconscious mnemonic mechanisms. Moreover, episodic memory encoding would not account for the 'imaginary replays'.

2.3.3.5 Influence of AI

The advancement of machine learning (ML) brought about a change of view in neuroscience. Researchers started to analyze their data based on knowledge from Artificial Intelligence. Reinforcement learning became a prominent theoretical background. Scientists examined hippocampal encoding and replays and compared it to how RL models learn. In the following section, some of the research that stems from the teachings of machine learning and in particular reinforcement learning will be elaborated on.

In their paper, Stachenfeld and his colleagues (2017) compare the hippocampus to a predictive map. A central thesis has been the cognitive map as the main hippocampal function, which builds on the notion that cells in the hippocampus, called the place cells, encode a "geometric representation of space" (Stachenfeld, et al., 2017). Stachenfeld and his fellow researchers attempted to extend this view of the hippocampal function and theorized that

the encoding is predictive in addition to spatial. Their idea is that the hippocampus supports the "learning of a predictive map that represents each state in terms of its successor states" (Stachenfeld, et al., 2017). This would account for the findings that two places physically adjacent to each other have dissimilar representations, as they represent the different predicted future states, however, two non-adjacent locations with similar predicted future states have similar firing patterns. The entorhinal grid cells contribute to the efficient workings of the predictive map by reducing its dimensionality. In the words of the researchers "grid fields reflect a low-dimensional eigendecomposition of the SR" (SR being the Successor Representation model) (Stachenfeld, et al., 2017).

Another research examining hippocampal replays through the lens of reinforcement learning was done by Cazé, Khamassi, Aubin, and Girard at Sorbonne University in 2018 (Cazé, et al., 2018). They argue that RL could explain the mechanism behind the reduction of the number of iterations that are necessary during environment exploration (Cazé, et al., 2018). Hippocampal replays have been hypothesized to play an important role in memory consolidation and learning, however, the idea of reinforcement learning could be strengthened by replays as well. Reinforcement learning is described by the researchers in the following way: "the learning processes that seek to maximize expected future rewards through trial-and-error interaction with an environment" (Cazé, et al., 2018).

2.4 State of Research

In this subchapter, I will list and elaborate on some of the cutting-edge research in the field of hippocampal place cell assemblies and replays that have been carried out in the past two years. These findings are essential for my thesis research, as they form the base assumptions about replays and direct the expected results. This subchapter also aims to provide a detailed overview of the research that is the precursor of the thesis as the data utilized for this study was obtained during that experiment.

Bhattarai, Lee, and Jung (2020) conducted a study to reveal the roles of forward and reverse hippocampal replays (Bhattarai, et al., 2020). In this research, rats were trained in a spatial memory task in which they had to take a trajectory out of four possible ones within an 8-shaped maze to obtain a reward. Results show that reward enhances forward and reverse replays as well during awake state, however, the influence is different.

> "Reward enhances the rate of reverse replays, but it increases the fidelity of forward replays for recently traveled as well as other alternative trajectories heading toward a rewarding location" (Bhattarai, et al., 2020)

This suggests that both forward and reverse replays play a crucial role in reinforcing representations of every potential trajectory with a reward. They propose that replays contribute to creating a 'value map' of potential trajectories and their values.

Van der Meer, Kemere and Diba (2020) identify and differentiate first-order and second-order analyses of hippocampal replays. According to their definition, a first-order analysis aims to "determine whether the identity and/or temporal order of cell firing is different from chance" (Van der Meer, et al., 2020). These analyses prove the existence of replays by focusing on a single time point and map. Contrarily, the second-order analysis compares SWR contents between different time points and different templates. This thesis research uses the second-order analysis methodology, as it compares the replays recorded during consecutive nights, having a learning task in between during the day.

Haibing Xu and his colleagues (2019) published an article in the journal *Neuron* with the title '*Assembly Responses of Hippocampal CA1 Place Cells Predict Learned Behavior in Goal-Directed Spatial Tasks on the Radial Eight-Arm Maze'* that discusses "how the combined demand on spatial working and reference memory influences place cell assemblies" (Xu, et al., 2019). I devote more attention and space to this paper, as this research is the precursor for the thesis research. The data, that I use for the thesis, has been collected during Xu and his colleagues' research, and they have provided me with access to it. The experiment was carried out on 10 adult male rats. Sixteen independently movable wire tetrodes were implanted into the deep layers of the neocortex, that were lowered into the CA1 region over a 2-week long period. After the recovery period of the animals, wide-band recordings were taken continuously, and the neuronal activity was digitized. The rats were divided into 3 distinct groups: 4 animals were randomly chosen for the combined task, 3 for the working memory and then reference memory tasks, and 3 animals for the reference memory and then working memory tasks. The rats had to perform the tasks in a radial eight-arm maze (the central area is octagonal, the side arms are 70 cm long, 12 cm wide, and 80 cm high), in which pneumatically controlled doors could prevent the rats to enter any of the side arms when risen. Food pallets were placed at the end of 3 randomly chosen arms. The combined task incorporates both

working and reference memory such that the animals had to learn the position of the 3 food rewards, and they had to keep track of the arms that have been already visited during the trial. Once all 3 rewards were collected, all the doors were raised, and a new trial begin with a time delay of 1 minute. Each of the experimental days contained 3 phases, separated by 30 minutes of rest. The first phase is the pre-probe session, during which the animals were tested on the configuration of the previous day for 5 trials. After the break, a new learning session started, in which the rats had to learn the new locations of the food pallets. The new locations contained 2 novel-goal arms (the food pallet is at a different arm than the day before) and 1 familiar-goal arm (the food pallet is in one of the arms that were rewarded the previous day as well). The learning phase consisted of 30 trials. After the second 30 minutes rest, the post-probe session started, where 5 trials of the newly learned configuration were executed. During the working memory task, the trial also ended once the rat found all 3 food pallets, however, only those 3 doors were open which contained food, and the empty arms were blocked. The reference memory task starts the same way as the combined task, however, once the animal returned from an arm, the corresponding door was raised, preventing the animal from visiting one arm twice. The motivation behind this experiment was

> "to test how different neuronal coding schemes that reflect spatial memory traces interact with each other during combined working and a longer-term memory demand and how SWR-associated replay might be involved in decision making" (Xu, et al., 2019).

The results suggest that newly learned rewards influence the relationship between place cells and place fields, moving firing fields closer to the new rewards. During reference memory tasks, goal-novelty influences task-contingent firing, and the future arm is predicted by the replay at the decision point. During only working memory tasks, previously visited arms are encoded by the replays. This research focused on the analysis of neuronal activity during wake/active periods, however, data was collected continuously. My research focuses on analyzing the data that has been collected during offline periods when the animals were asleep.

2.5 Expected Results

According to the research done in the hippocampal replay area, it is expected that the place fields in the CA1 part of the hippocampus will be highly directional, as the data has been recorded in an enclosed space, to which the rats were exposed for an extensive amount of time during learning. This assumption influences the data analysis, each location within the arms of the radial 8-arm maze will have double representations, depending on whether the rat is on a reward-bound or a center-bound path.

Moreover, replays will have to be further divided into reverse and forward replays, as these two different types of replays are assumed to have different functionalities. Within the existing literature, reverse replays seem to be biased by novelty and reward, and they contribute to memory consolidation, while forward replays guide future behavior. As sleep (offline periods) is allegedly important for memory consolidation, it could be expected that during sleep the prominent type of replay is the reverse replay. However, there is a contradiction within the existing literature, stating that forward replays are significantly more numerous during sleep. Due to this contradiction, the expectation about the ratio of forward-to-reverse replays is that it is irregular, and there is no pattern.

As replays facilitate remembering according to the literature, it is expected that the replays reflect how well the animal performs the task after replays, and/or they might reflect the order or preferences at which the rat visits arms. A further expectation is that the reactivation of place cell assemblies during sleep is modulated by learning performance and predicts subsequent recall.

Due to the advancement of machine learning, in particular reinforcement learning, the neuroscientific literature is influenced by the idea that replays could function as a reinforcer, thus reducing the number of iterations necessary during the exploration of the environment. This leads to the expectation that replays at night influence the number of arms the rat needs to explore the next day before finding all three food pallets.

3. Methodology

This chapter gives an overview of the design of the base experiment, from where I have received the data to provide an insight for understanding the detailed data analysis techniques used to answer the research question. It also elaborates on the different types of datasets, their analysis, such as the new arms, paths, arm visits, learning curves, place field reactivations and the replayed trajectories. Next to the analysis, the findings are discussed simultaneously.

3.1 Design of the Original Experiment

During the experiment, the researchers have used adult male rats (Long Evans, 300-500g, 3-6 months old) (Xu, et al., 2019, p. 133). Sixteen independently movable wire tetrodes were implanted in the rats' brains. After recovery, the radial 8-arm maze was introduced to the animals.

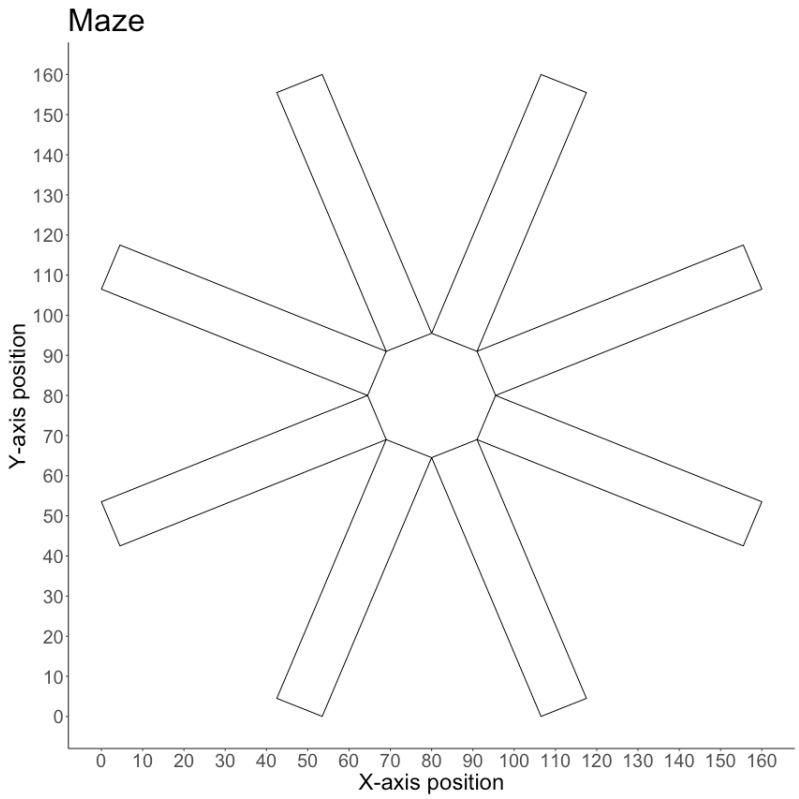

Figure 8 Dimensions of the radial 8-arm maze. Created based on (Xu, et al., 2019)

Figure 8 [created based on (Xu, et al., 2019)] is a graphical illustration of the radial 8-arm maze that has been used during the experiment. The dimensions of the maze are as follows: the octagonal central area has a side length of 12cm, the side arms are 70cm long and the apparatus is 80cm high. There is a pneumatically controlled gate at each of the side-arm entrances, which can be raised separately in order to prevent the rats to enter the arms.

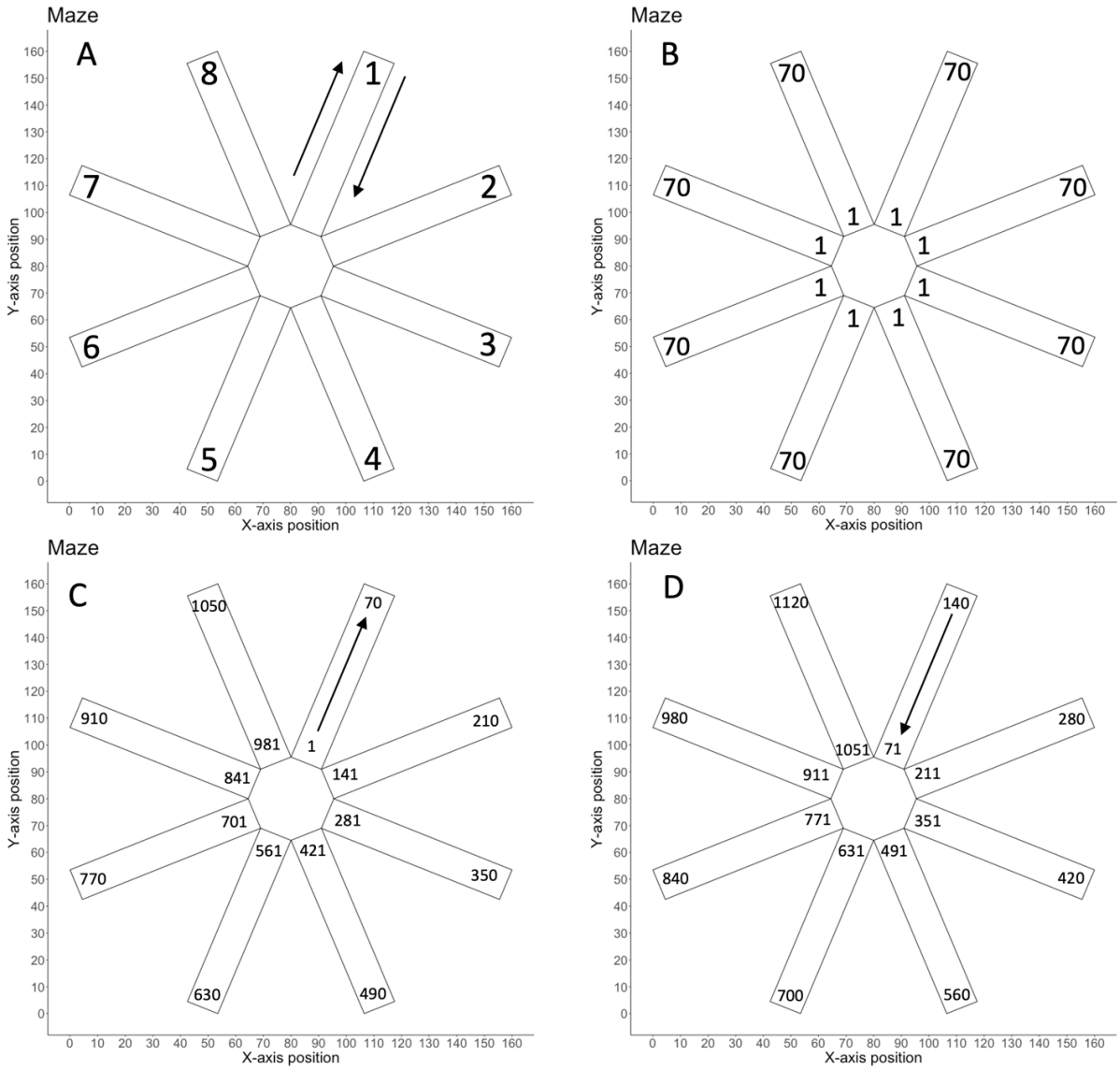

Figure 9 Radial 8-arm maze data labeling guide. Created based on (Xu, et al., 2019)

Figure 9 [created based on (Xu, et al., 2019)]: **A-D**: the explanation for data labeling. **A**: There are 8 arms, 1-8 labeled in the shown way. Important to note that the rat can move inside the arms forth and back. Moving toward the end of the arm is a "reward-driven" motion, as the rewards are located at the end of the selected arms. Moving back to the octagon is labeled as "center-driven" motion. **B**: Each of the arms is divided into 70 segments, 1 is the segment closest to the center, while 70 is the end section of the arm. As the arms are 70cm long, one segment is 1cm long. **C-D**: Reward-driven and center-driven motions are separated, thus the segments must be labeled differently depending on which way the rat was going. As there are 8 arms, 2 directions, and 70 segments per arm, there are 1120 (8*2*70) individual locations.

3.2 Data

This master thesis builds on data received from the research done by Xu and colleagues (Xu, et al., 2019). According to the experiment, several different types of data have been recorded. This subchapter introduces the datasets and their importance for the research.

Firstly, the '*new arms'* dataset shows which three arms were equipped with the rewarding food pallets each day. This aids with the analysis of the difference between novel- and familiar-goal arm replays.

Secondly, there is a '*camera coordinates'* data, which recorded the rats' movement inside the radial 8-arm maze during the learning and task-performance periods. It is crucial for the research, as it served as a baseline for the researchers to identify the place fields of place cell assemblies while the rats were active. Moreover, it is important for this thesis to identify how the active behavior exerts influence on replays during offline periods.

Thirdly, the '*itarm'* dataset contains a summary of the movements of the rats. It includes the trial number of the arms that have been visited and for each arm, the timestamps when the rat entered, reached the end and exited the arm. This dataset provides the basis for the learning curve analysis and how learning affects offline replays.

The fourth dataset is called *'jjbayes'*, which contains the reactivation data. For each of the 1120 locations, it shows a probability that that specific location has been reactivated during the specific SWR that has been recorded during sleep. This database provides the basics for assembling trajectory replays.

Lastly, the *'uu'* dataset is the summary of all offline reactivated trajectories. This serves as the base for the trajectory replay analysis, both for the amount of forward and reverse replays and for the changes occurring due to learning.

It has to be mentioned that the data is coming from multiple rats. Rat number 84 has all of the data collected in four consecutive days; thus, this rat will be the primary subject of analysis for the comparison studies. The data from rat number 63 was collected in two consecutive days; thus, rat 63 will be the secondary subject and the first control. Rat 86 has partial data from four consecutive days, missing the summary of movements ('itarm') from the two middle days. The data from all the other rats are from unique days, therefore, they will be analyzed in differently.

3.3 Analysis and Findings

This subchapter contains the analysis of the different datasets and aims at answering the research questions by discussing the findings of the data analyses. For analytics purposes, a unique R library was built. This library facilitates dataset specific investigation from data cleaning through data visualization to final correlation studies. The codebase and the plots that have been created throughout the process can be accessed at https://github.com/rebeccube/msc_thesis.

3.3.1 New Arms

The radial 8-arm maze contains three reward locations with food pallets and every time the rats are tasked with finding all three. Between consecutive training days, one location stays the same as the day before (familiar-goal location), and the other two change (novel-goal locations).

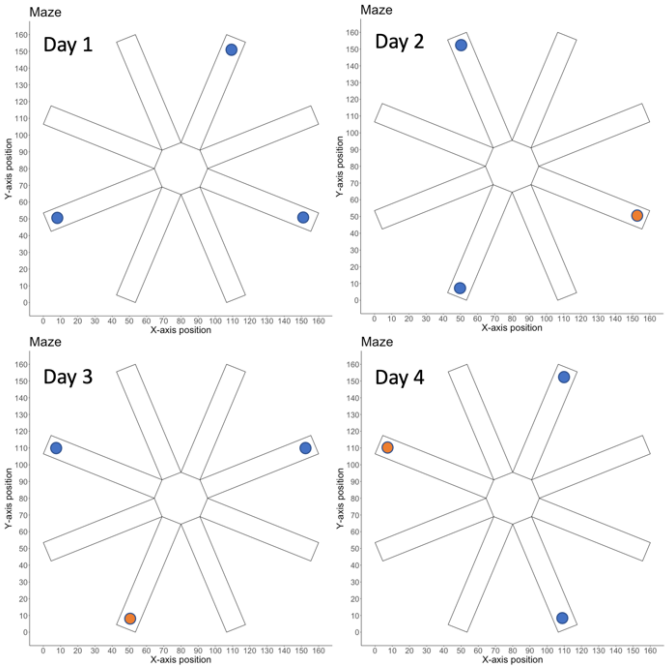

Figure 10 Distribution of rewards in four consecutive days

Day 1	1	3	6
Day 2	3	5	8
Day 3	2	5	7
Day 4	1	4	7

Figure 10 illustrates the reward distribution in four consecutive days for test subject 84. There is no data about the pallet configuration from the day before the first observation point, thus, it does not show the familiar-goal location. On the following days, the pattern of one familiar goal and two novel goals are clearly visible.

It is important to note, that within the 16 datasets, I have found only 4 unique configurations, six times [2,5,7], four times [1,3,6], three times [3,5,8] and another three times [1,4,7]. Upon closer observation, it is visible that all configurations have the same pattern, leaving

twice two arms and once one arm between the reward locations. There is no configuration within the received data where two adjacent arms would contain the reward. The lack of diversity of configurations might exert an effect on learning such that the pattern and not the specific arms are learned.

3.3.2 Path

According to the camera recording, the path taken by the rats in each of the sessions can be visualized. Each session lasts until the rat can find the food without an error for a certain amount of tries consecutively.

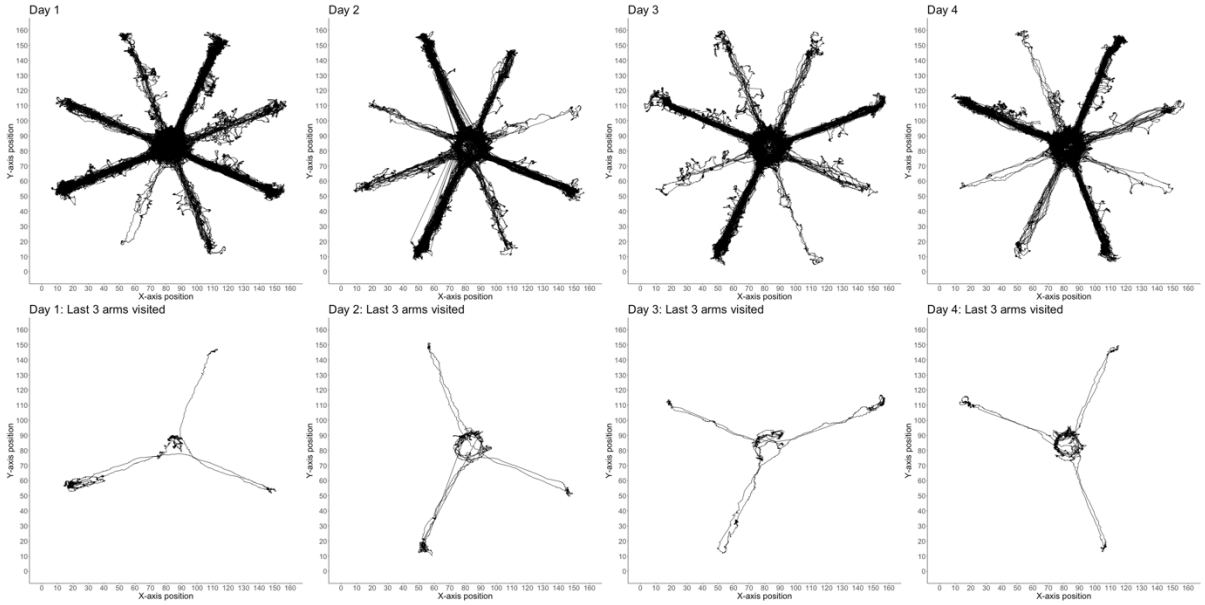

Figure 11 Path taken by rat 84 in four consecutive days according to the camera recording

In *Figure 11*, the upper row shows the overall path that rat 84 took during the four consecutive days, while the lower row maps the last three arms that have been visited each day. On the first day, arms 1, 3, and 6 had the food pellets, thus, arms [1,3,6] were the last 3 arms that have been visited that day. Moreover, those arms are the most frequently visited during the whole day. However, arms [4,7] seem to be frequented, which leads me to believe that the previous day arms 4 and 7 could have possibly been the goal-arms. Extending the assumption with the new arm patterns observation, the familiar goal-arm on day 1 could have been arm 1, leaving 3 and 6 as the novel-goal arms. As a reminder, the goal arms at each day are

as follows: (Assumed Day 0 → [1,4,7];) Day 1 → [1,3,6]; Day 2 → [3,5,8]; Day 3 → [2,5,7]; and Day 4 → [1,4,7].

The paths that the rats have taken were animated and movies were created which can be accessed at: https://github.com/rebeccube/msc_thesis along with the path plots and movies of all the other rats.

3.3.3 Arm Visits

During the learning sessions, each session terminates once the rat found all 3 rewards. As the doors are not lifted once the rat explored an arm, it is possible for the rodent to visit an arm multiple times in a session before finding the third reward. The following analyses uncover information about how many times each arm has been visited during all the training sessions on a given day. The graphs illustrate data collected on test subject 84.

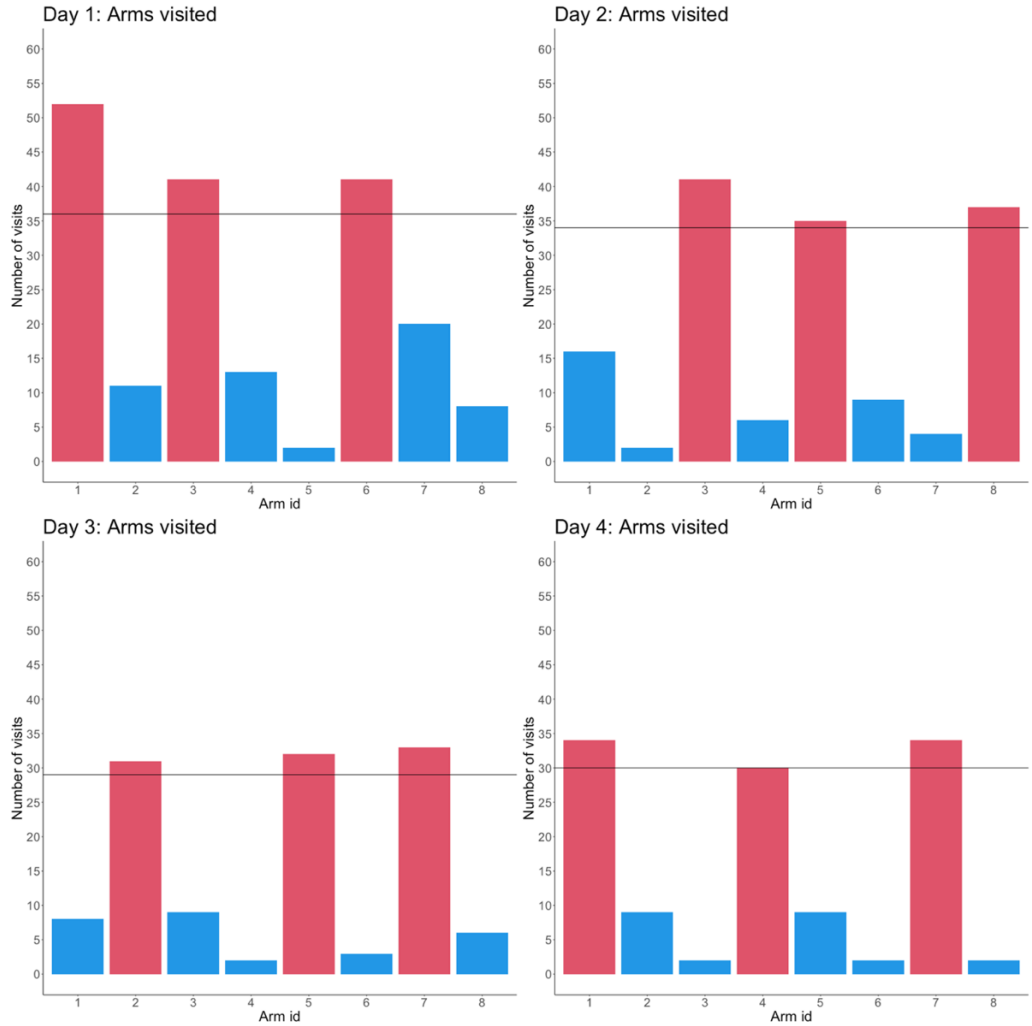

Figure 12 Number of visits per arm during learning sessions

Figure 12 illustrates the combined number of visits per arm per day during the learning sessions for all four consecutive days. On the x-axis, the arm ids can be seen from 1 to 8. The color of the column corresponds to reward (red) vs non-reward (blue) arms. Note that each arm with the color red has to be visited at least once before a training session is over. The y-axis shows the combined number of visits. The black line indicates the number of training sessions completed by the rat in order to learn the new pattern. When a column surpasses the black line, this indicates that the rat visited that arm more times than the number of training sessions, thus it visited the arm multiple times within at least one but probably multiple sessions that day. In case a column is blue and under the black line, it can still happen that that arm was visited multiple times within one session and was not visited at all in another.

There is an observable relationship between the goal-locations of the previous days and the number of arm visits for each given day. As a reminder with the assumption of day 0: Day 0 → [1,4,7]; Day 1 → [1,3,6]; Day 2 → [3,5,8]; Day 3 → [2,5,7]; and Day 4 → [1,4,7]. On day 1, arms 4 and 7 were the most visited non-reward arms and arm 1 was the most visited reward-arm, which reinforces the assumption that the pattern for day 0 was [1,4,7]. On day 2, the most visited reward and non-reward arms respectively are 3, and 1,6, the pattern of the previous day. Similar patterns are observable for the other two days. This points to the direction that the pattern of the previous day influences the behavior of the rat during learning. The question arises, whether the rodent "only" learns the new pattern during the day, or whether it needs to unlearn the pattern from the previous day. Reinforcement learning could provide an insight into this question.

The total number of arms visited during day 1 is 188, during day 2 is 150, during day 3 is 124 and during day 4 it is 122. This indicates a faster speed of learning each day the rat is exposed to the 8-arm maze and is required to learn the new patterns. The next subchapter discusses the speed of learning and the illustrated learning curves.

3.3.4 Learning Curve

From the summary of the movements of the rats, not only the overall arms visited can be seen, but also the learning curve can be identified. The analysis shown in this section is also based upon test subject 84's performance. The learning curves are illustrated in *Figure 13*. On average, there is a decreasing tendency for the number of sessions required during the day

to learn the new pattern. From day 1 to day 4, the trial counts are as follows: 36 → 34 → 29 → 30.

Next to the decreasing pattern, quicker learning can be observed in the latter days. This point has been addressed in the previous subchapter, stating the overall number of arms visited during each training day. From 188 on day 1, it decreased to 122 on day 4. As there are different numbers of trials, we need to be careful about the comparison. This is the reason why the average number of arm visits must be calculated as well. The average number of arms visited during one session per training day equals to: Day 1 = 5.22; Day 2= 4.41; Day 3= 4.28; and Day 4 = 4.07. The tendency of decreasing arm visits and quicker learning still holds in the average realm. It raises the question of why the learning is quicker each day, when the pattern always changes, thus the task changes.

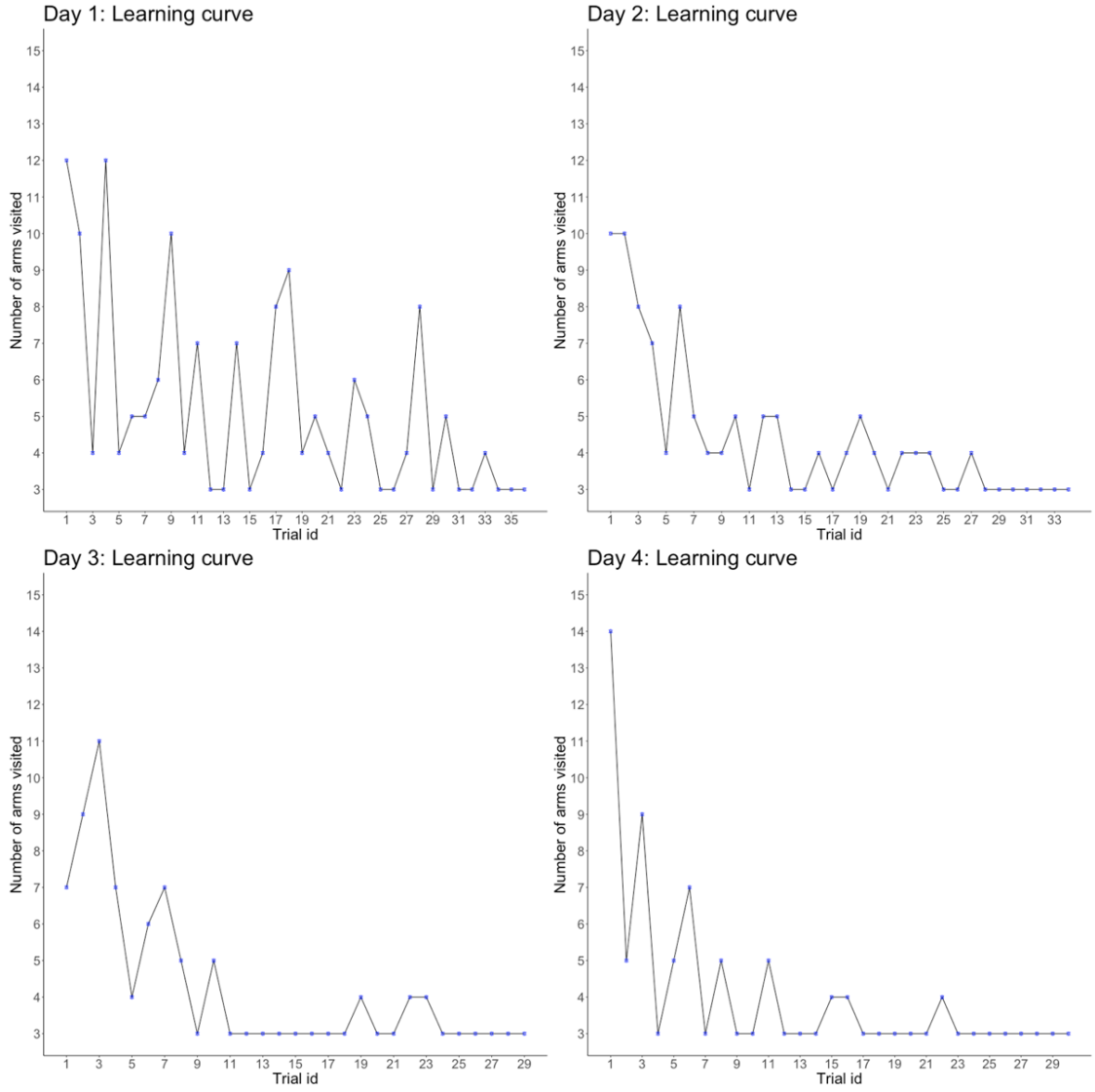

Figure 13 Learning curve of rat 84 in four consecutive days

3.3.5 Place Field Reactivations

The implanted tetrodes recorded the hippocampal place cell assembly activity continuously, thus, there is available data about assembly reactivation at night, while the rats were asleep. Numerous high synchrony events (HSEs), and sharp-wave ripples (SWRs) have been detected and analyzed. SWR and HSE detection, spike sorting, the generation of rate maps, and identifying replays during HSEs were executed by Xu and colleagues (2019) in the following manner (Xu, et al., 2019):

> *Spike Detection and Sorting:* Local field potentials were measured between the 800-900 Hz range, and those above 5 SD of the mean were selected for further analysis and clustering. Clustering was accomplished first by an automatic clustering software, and then they were refined manually with the help of a graphical cluster cutting program. Xu and colleagues were able to identify the 2430 CA1 pyramidal cells' activity. (Xu, et al., 2019)
>
> *SWR Detection:* Local field potentials were filtered out (band-pass filter with 150-250Hz), and then a non-ripple oscillating channel signal (reference signal) was subtracted to remove noise (common-mode noise). The threshold for SWR detection was 7 SD above the baseline. (Xu, et al., 2019)
>
> *HSE Detection:* The multi-unit activity of clustered spikes formed the base for detecting HSEs by representing the synchronous spiking rate over time. The threshold for detecting an HSE was 3 SD above the average. HSEs were rejected based on several factors, such as length ('< 75ms' or '> 750ms'), spike count ('< 5'), cell count ('< 4'), and population activity ('< 10%"). Each of the remaining HSEs was subdivided into frames. A frame is a 20ms window within the HSE, and the consecutive frames have a 10ms overlap. (Xu, et al., 2019)
>
> *Generation of Rate Maps:* rate maps were generated in order to identify the place fields of the place cell assemblies. Place maps were calculated for both reward-bound and center-bound movements, considering the directional nature of place fields in enclosed environments. (Xu, et al., 2019). The arms were divided into 70 parts, as was illustrated in *Figure 9*.
>
> *Replay during HSEs:* Bayesian place prediction was used to calculate the probability for each position within the arms that that specific location (1120 in total, as explained by *Figure 9*) was replayed by the reactivation of the place cell assemblies. They used the following formula to compute the probabilities: $P(x|n) = P(n|x)P(x)/P(n)$, in which $P(x)$ is the probability that the animal is at a given location (assumed uniform distribution), $P(n)$ the probability of a given spike count (assumed Poisson distribution). (Xu, et al., 2019)

After conducting these steps, the researchers were able to assemble a dataset that contains the detailed reactivations of place cell assemblies at night, with the probabilities for each

of the 1120 locations within each frame of the replay ('jjbayes' dataset mentioned earlier in the '*3.2 Data*' subchapter).

The count of identified replays at night ranged from 542 to 1246, in our primary subject, rat 84, the range is smaller from 957 to 1246. (Day 1: 1100; Day 2: 957; Day 3: 1089; and Day 4: 1246). In this case, there is no identifiable increasing or decreasing tendency in the count of replays at night in contrast to the increasing trend of learning speed. This might suggest that there is no correlation between the number of replays at night and the learning speed during the day. However, it could be the case that not all the replays have been captured, thus we do not possess the actual number of replays.

Upon analyzing the 'jjbayes' dataset, it became clear that the sum of the probabilities for the 1120 locations does not add up to 1. In the case of test subject 84's first night replays, the statistics of the sum of the probabilities for each frame are shown in *Figure 14*.

Minimum	0.00000
1st Quartile	0.00002
Median	0.02784
Mean	3.40036
3rd Quartile	2.61635
Maximum	41.59803

Figure 14 Statistics of probability sums for 1120 locations during replays

The minimum sum of the probabilities of the 1120 locations in a frame within a replay was 0, while the maximum summed up to 41.59803. Having 50% of the data below 0.02 and having the third quartile's value below the mean indicates, that the distribution of the sums is highly skewed to the right. After this discovery, I have attempted to normalize the data. In order to have the sum of probabilities equal to 1, I have divided all 1120 probabilities with the sum of probabilities.

After this step, I have prepared heatmaps of the normalized probabilities for each of the frames within each of the replay events. *Figure 15* illustrates the first replay event of test subject 84's first night, after learning the reward pattern of [1,3,6]. The first replay event contains four frames, with 1120 probabilities within each frame. The x-axis shows the location within the arm, 1 to 70 (1 being the start of the arm closest to the central octagon, and 70 being the end of the arm). The subdivision of the arms into 70 segments was discussed earlier in the chapter with *Figure 9* as an illustration for easier understanding. The y-axis lists the 8 arms with the directions (d1 = arm 1 reward-bound direction, d2 = arm 1 center-bound direction, d3 = arm 2 reward-bound direction, etc.). On the diagram, multiple reactivated locations can be identified, and the sequence of their reactivation forms the replayed trajectory. Within

each reactivation event, the most probable replayed trajectory has to be calculated, which will serve as the basis of analysis of the replays. In *Figure 15*, it is easily observable, that d5 and d11 had the most probabilities across the different frames. D5 corresponds to arm 3 reward-bound run, and d11 corresponds to arm 6 reward-bound run. During the day, before the offline period, the rat learned the pattern [1,3,6]. According to the assumption stated in this chapter, it is highly probable that the pattern the day before that was [1,4,7]. This means that arm 1 was the familiar-goal arm, whereas arms 3 and 6 were the novel-goal arms, which can be seen to be replayed during the first reactivation event. This finding points to the possibility that during a replay event, not only one but multiple trajectories can be replayed simultaneously. Moreover, it also suggests that replays are modulated by learning.

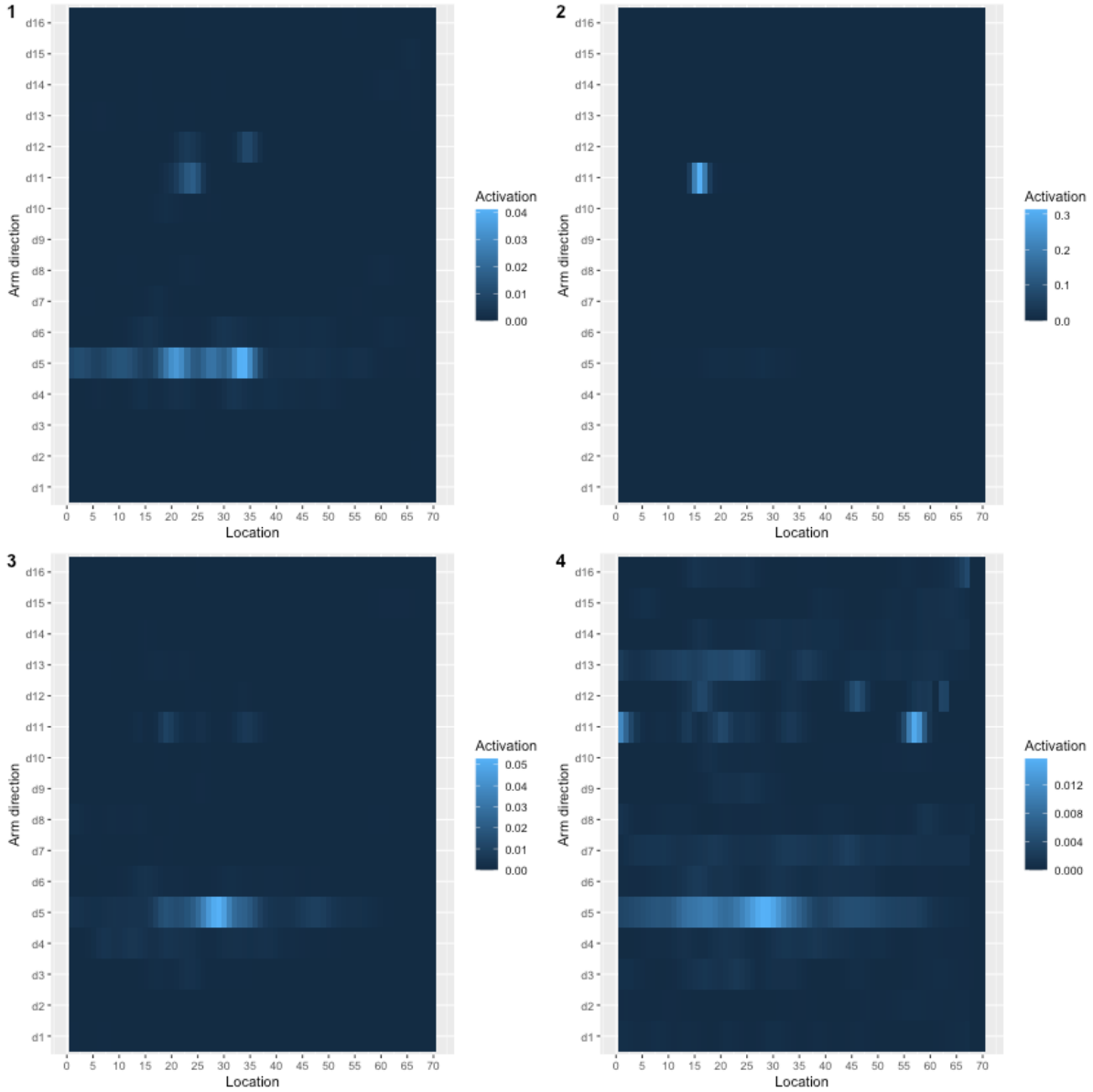

Figure 15 Heatmaps of the reactivated place fields

3.3.6 Replayed Trajectories

The researchers also calculated the most probable path for each replay ('uu' dataset). These most probable paths will be referred to as the replayed trajectories in the following section. It is important to note that the researchers identified only the most probable path, disregarding the possibility for simultaneous multi-trajectory replays. This might provide a skewed representation of the overall number of replays.

Based on the number of replays per arm, an interesting pattern might arise. *Figure 16* illustrates the number of trajectories that have been replayed within an arm during the second night in test subject 84's CA1 hippocampal region.

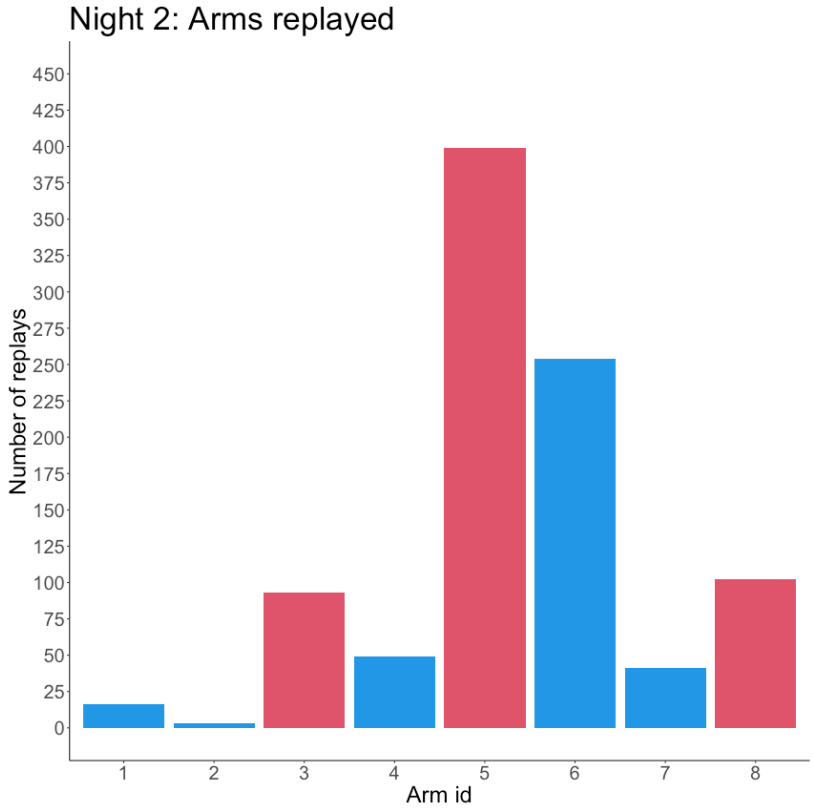

Figure 16 Replayed arms during the 2nd night

The red columns in *Figure 16* represent the goal-arms (pattern [3,5,8]). Out of the three goal-arms, arm 3 is the familiar-goal arm (pattern during the previous day was [1,3,6]), and arms 5 and 8 are the novel-goal arms. Both novel-goal arms are replayed more often than the familiar-goal arm, although there is only a slight difference between arm 8 and arm 3. (Total number of observations: arm 3 = 93; arm 5 = 399; arm 8 = 102). However, as we have seen before in subchapter 3.3.5 Place Field Reactivations, the novel-goal arms may have been replayed simultaneously; taking only the most probable path into account might reduce the

number of replays within an arm. It is also eye-catching that arm number 6 has been replayed the second most frequently, having a more than double count, 254, than that of number 8, a current goal location. As it was stated before, arm number 6 was a goal-arm during the previous day, thus this also points to the fact that replays might have a role in 'unlearning', potentially learning a negative association with a previously positive location, preventing the rat from visiting the arm again and minimizing disappointment. However, this pattern is unfortunately not observable during the other 3 nights. *Figure 17* illustrates the number of trajectories replayed in each arm during the four consecutive nights. Replays in arm 6 give a high count regardless of its status. Moreover, the pattern that goal-arms are replayed more often than the other arms does not seem to be reinforced by the other nights.

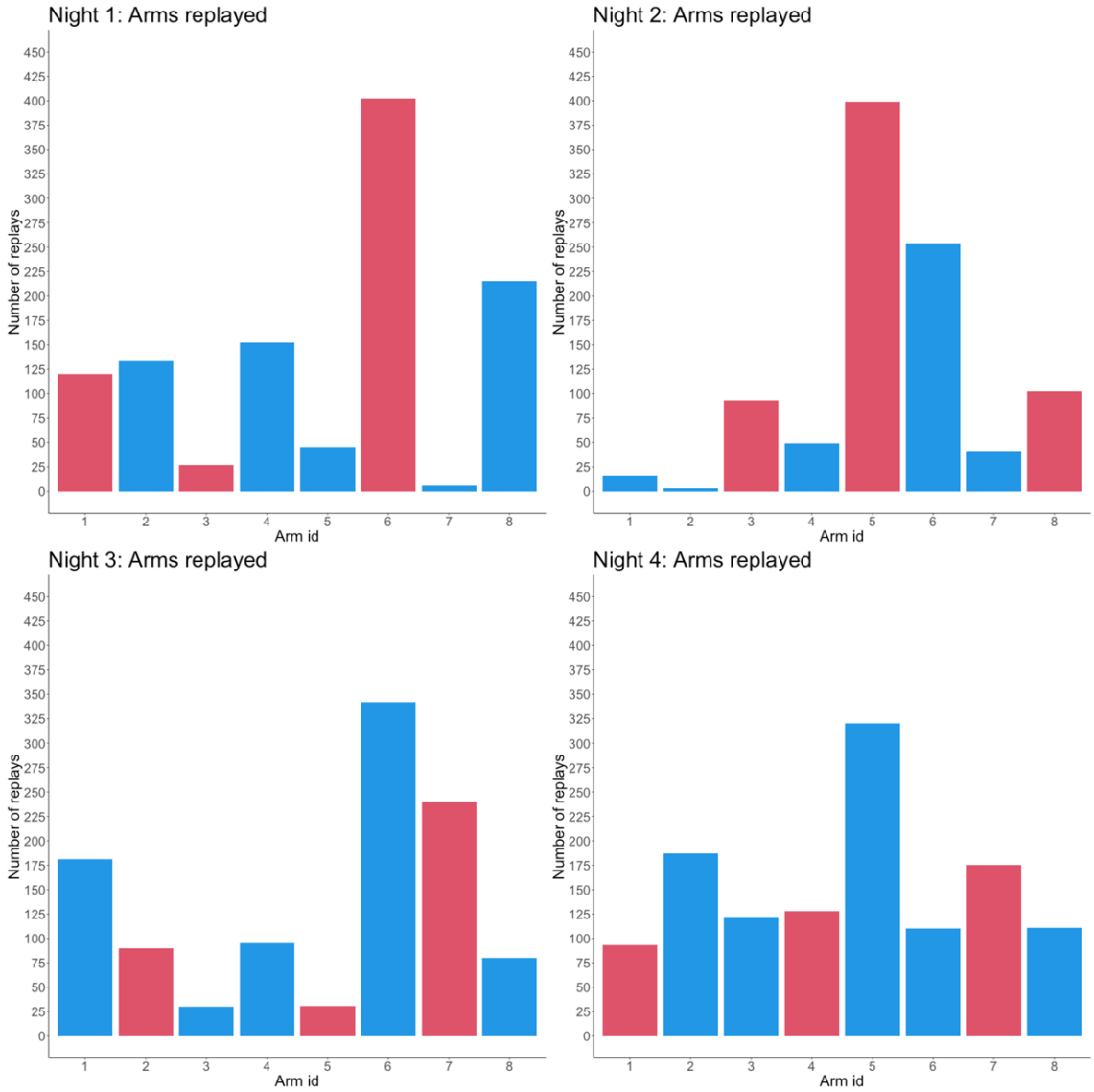

Figure 17 Replayed arms during the four consecutive nights

The next step is to calculate the ratio of reverse and forward replays. There are four different scenarios in our case. As the rats are learning a pattern within a radial 8-arm maze, which is an enclosed environment, the assumption is that the place cells are highly directional, thus we have reward-bound and center-bound runs. Each of these runs can be replayed in a forward and a reverse manner. For the sake of easier understanding, I will provide an example for each of the four cases in the following segment with 4 frames within each replay (please consult *Figure 9* for a graphical illustration):

Reward-bound run:

The rat's hippocampus replays a movement in arm 1 from the center to the end of the arm, locations 1 to 70. Location 1 is closest to the center, while location 70 is the outermost section of the arm.

Forward replay:

Replay starts at location 5, then 12, 25, and at last 31. Thus, the direction is reward-bound, and the replay is forward.

Reverse replay:

Replay starts at location 57, then 48, 29, and at last 13. The direction is reward-bound, but the replay is reversed.

Center-bound run:

The rat's hippocampus replays a movement in arm 1 from the reward location towards the central octagon, locations 140 to 71. Location 71 is closest to the center, while location 140 is the outermost section of the arm. (Note the physical location of 1 and 71 is the same, as well as the location of 70 and 140, and all the pairs in between.)

Forward replay:

Replay starts at 120, then 105, 93, and at last 74. Locations are going from the end to the center; thus, it is a center-bound forward replay.

Reverse replay:

Replay starts at 82, then 100, 107, and at last 132. The direction is center-oriented, but the replay moves farther away from the center and towards the end of the arm. Therefore, the replay is a center-bound reverse replay.

Upon inspecting the 'uu' dataset and categorizing each sequence of reactivations into a trajectory replay type (one of the four mentioned above), I have encountered the following problem. Only a small amount, between 5 to 15 % (depending on the dataset) of the total trajectories could be identified. *Figure 18* contains the actual number of categorized replays found during the initial analysis. Out of 1100 replays during the first night (data from test subject 84), only 94 trajectory replays had a clear direction and replay type. All the other most probable paths had a change in direction, meaning that within an arm the trajectory replays alternated between reward-bound and center-bound directions. As the percentage of uncategorizable replays are extremely high in all four consecutive nights (Night 1: 91.5%; Night 2: 87.1%; Night 3: 93.9%; and Night 4: 84.9%), they had to be dealt with to provide a solid database for further analysis.

Type of Replay	Count
"C": Changing direction	1006
"RR": Reward-bound Reverse Replay	12
"RF": Reward-bound Forward Replay	12
"CF": Center-bound Forward Replay	46
"CR": Center-bound Reverse Replay	24

Figure 18 Count of trajectory replays based on replay type (Night 1, TS 84)

The methodology I used for categorizing those replays that were not straightforward during the first attempt is described below. As all the most probable paths were trajectory replays within a single arm, only in a different direction, I decided to split the replays into two parts. The splitting was based on the direction, whether the certain frame had a place field within a center-bound, or within a reward-bound run. This technique has a twofold effect on the data. On one side, it enables the categorization of multi-direction replays; on the other side, it multiplies the total number of replays that occurred during the night. To illustrate the second point, *Figure 19* shows the new numbers based on categories.

Type of Replay	Count
"RR": Reward-bound Reverse Replay	591
"RF": Reward-bound Forward Replay	439
"CF": Center-bound Forward Replay	661
"CR": Center-bound Reverse Replay	415

Figure 19 Count of trajectory replays based on replay type after splitting bi-directional replays (Night 1, TS 84)

Once the bi-directional replays were successfully split, the total count of replays almost doubled, as was expected. The original expectation of the total number of replays was 1006 * 2 (as there were 1006 bi-directional replays split in two) + 94 already categorized ones, summing up to 2106. Summing the category counts from *Figure 19* (591 + 439 + 661 + 415) results in 2106 replays. The same technique was successfully used for the other datasets containing replay sequences for nights 2, 3, and 4.

Analyzing the newly assembled dataset resulted in some interesting findings that are illustrated in *Figure 20*. There was a consistency between the differences in counts of different types of replays. In case the direction is center-bound, forward replays are more numerous than reverse replays. On the contrary, in case the direction is reward-bound, reverse replays outnumber forward replays.

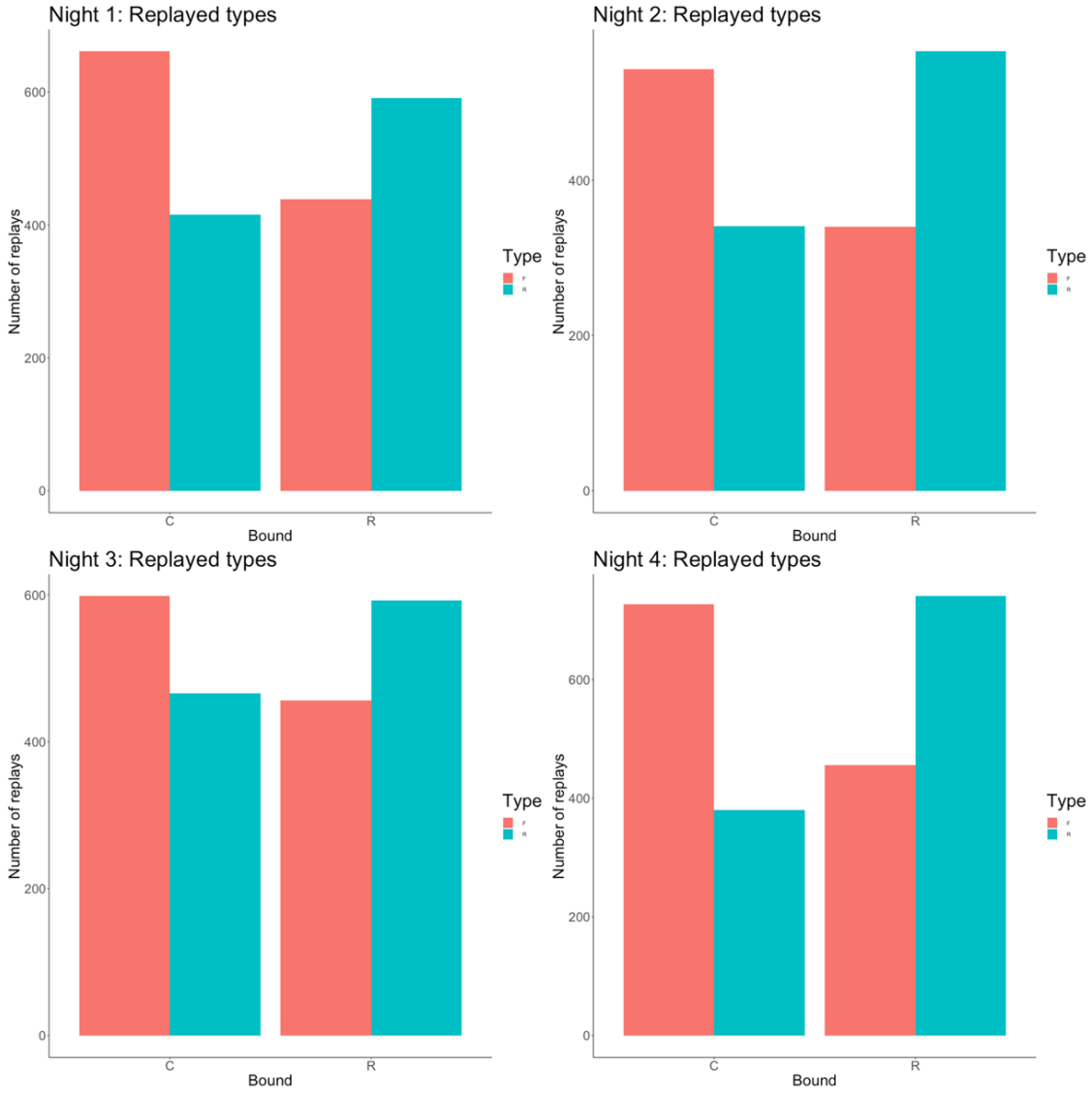

Figure 20 Distribution of replay categories based on direction and type during the four consecutive nights

This is an unexpected phenomenon that could be caused by a multitude of underlying processes. According to the reviewed literature, reverse replays are strongly biased by novelty and contribute to memory consolidation (Pfeiffer, 2020). This could account for the findings that reward-bound runs are replayed in a reversed fashion more often than in a forward fashion. However, there is a contradiction between these findings and the results of other researchers, (Wikenheiser & Redish, 2013); (Grosmark & Buzsaki, 2016), who found a significant imbalance between replays, observing more forward replays than reversed trajectory replays. According to the data analysis, the overall count of forward and reversed replays is relatively balanced, and the imbalance shows only within the directional comparison.

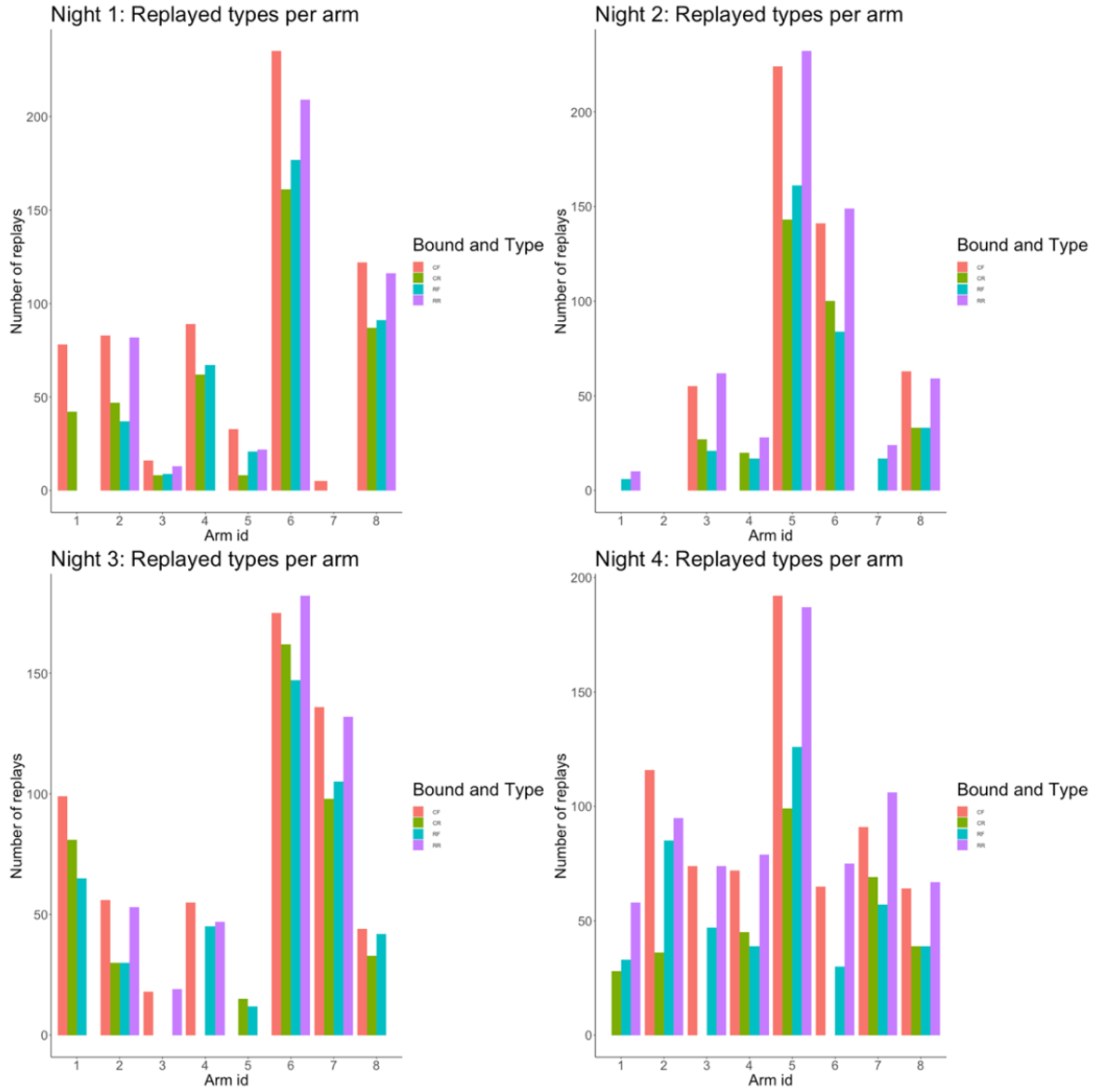

Figure 21 Distribution of replay categories based on direction and type per arm during the four consecutive nights

This pattern is not only consistent within the overall sums across nights but also reinforced by the patterns found in the type distributions per arm across nights, proved by the graphs in *Figure 21*. Within each arm, the forward center-bound replays outnumber the reverse center-bound replays, and the reverse reward-bound replays outnumber the forward reward-bound replays, while the forward and reverse replays seem to be in balance.

Figure 21 and *Figure 22* illustrate the four different types of replays per arm for the four consecutive nights of TS 84's experiment, thus aiding the analysis of how learning could affect replays at night, and how replays influence the behavior the next day. Although *Figures 21* and *22* are based on the same data, *Figure 21* aims at showing the comparison of the counts of the four different types of replays based on an individual arm, while *Figure 22* attempts to draw attention to the replay count differences across arms.

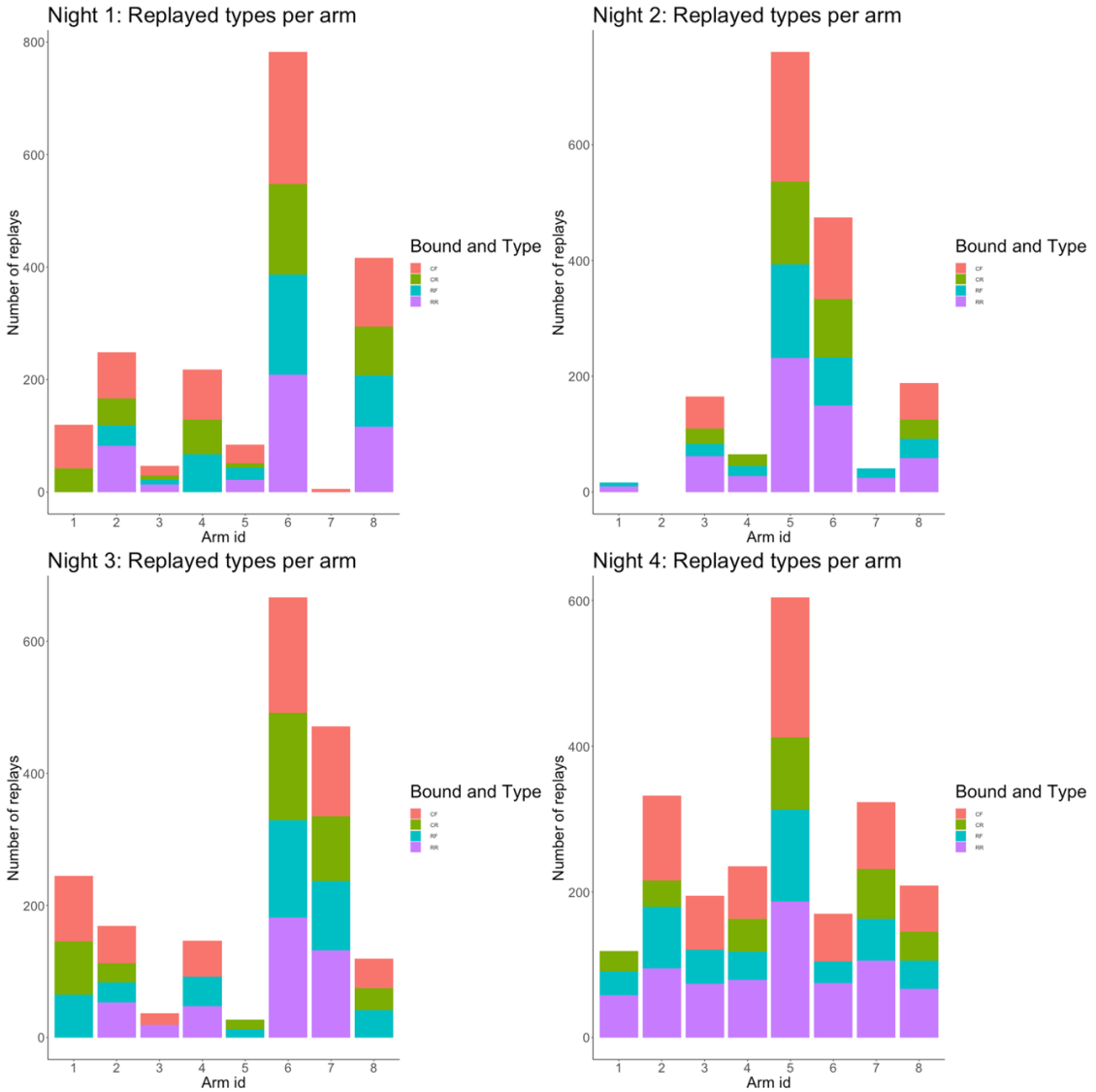

Figure 22 Stacked bar chart of replay categories based on direction and type per arm during the four consecutive nights

According to the reviewed literature, reversed replays are biased by novelty and reward, while forward replays guide future behavior (Pfeiffer, 2020). In our case, the reward location pattern was as follows: Day 0 *'assumed'* [1,4,7]; Day 1 [1,3,6]; Day 2 [3,5,8]; Day 3 [2,7,5]; and Day 4 [1,4,7]. Combining the finding of the literature research and the knowledge about the new arms in addition to the number of visits per arm during learning sessions (*Figure 12*) and the replays (*Figures 21 and 22*) lead to the expectation that the replays at night are both influenced by and exert an influence on learning and behavior. Forward replays should affect the next day's arm choices, while reverse replays should be affected by the new pattern and the learning curve. *Figure 21* shows that during the first three nights the novel arms [3,6]; [5,8]; [2,7] and the first night's familiar arm [1] tend to have slightly more forward than reverse replays. This contradicts the literature-based expectations. While it is true that during night 1, the forward replays of arms 1 and 6 overpowered the reverse replays, and during day 2 the most visited non-goal arms were 1 and 6, which would meet the expectations, this pattern is missing from the other two night-day pairs. This is another contradiction with the assumptions. The third contradiction is the seemingly missing correlation between the number of replays and the goal-arms or arm visits. The correlation studies are discussed in the following subchapter. The reason for these observations might be that not all the replays were detected or during the most probable path search, the possibility of multiple simultaneous replays was disregarded.

3.3.7 Results from the Other Datasets

This chapter introduces the findings with illustrations and diagrams from the datasets of TS 84. However, it is important to include all the other rats in the analysis, as results based on one test subject are not meaningful. Most of the graphs included in the chapter (and the path movies) have been generated for all the available datasets. These, alongside the code written for the analysis, are accessible at https://github.com/rebeccube/msc_thesis.

Unfortunately, not all the datasets were available for each rat. *Figure 23* shows the availability of the datasets and their sequence. (TS 84's four consecutive nights can be found in lines 8-11.) Due to the partial unavailability, the complete analysis could not be reproduced for all rats. For example, the 5th observation for rat 84 (line 12) misses three crucial datasets, thus preventing proper analysis. Rat 86 (line 13-16.) lacks the important '*itarm'* dataset for

both the second and the third day, disrupting the sequence of observations, thus, not allowing for analysis that requires data collection on consecutive days.

Dataset	Rat - Date	Date order	Itarm	Jjbayes	New arms	Uu	Camera coordinates
1.	169-28	1	✓	✓	✓	✓	✓
2.	58-11	1	✓	✓	✓	✓	✓
3.	63-25	1	✓	✓	✓	✓	✓
4.	63-26	2	✓	✓	✓	✓	✓
5.	63-29	5	✓	✓	✓	✓	✓
6.	75-18	1	✓	✓	✓	✓	✓
7.	75-20	3	✓	✓	✓	✓	✓
8.	84-03	1	✓	✓	✓	✓	✓
9.	84-04	2	✓	✓	✓	✓	✓
10.	84-05	3	✓	✓	✓	✓	✓
11.	84-06	4	✓	✓	✓	✓	✓
12.	84-10	8	X	X	✓	X	✓
13.	86-24	1	✓	✓	✓	✓	✓
14.	86-25	2	X	✓	✓	✓	✓
15.	86-26	3	X	✓	✓	✓	✓
16.	86-27	4	✓	✓	✓	✓	✓

Figure 23 Availability of datasets

Even though the other datasets are available, most sets lack the sequential aspect, only allowing for analysis that requires data from one day-night pair disregarding the previous or following days.

The dataset containing the new pattern for the day is available for all sixteen instances and the interesting results that only four distinct patterns/configurations are involved have been discussed earlier in the chapter (*3.3.1 New Arms*). The lack of diversity of patterns might have a modulatory effect on the replays.

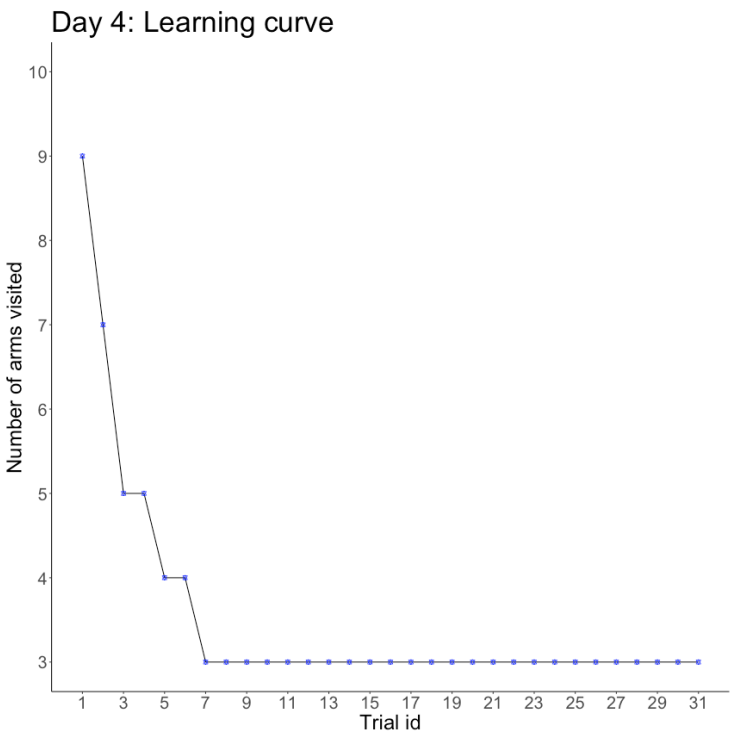

Figure 24 Exceptional learning curve of TS 86 on Day 4

The camera coordinates are available as well for all the instances, which allows for plotting the paths that the rats took during the day and rendering the movies. Upon inspecting the overall paths, the reward configuration and the last path taken during the learning sessions, it becomes clearly visible that the last three arms that any of the rats visited on any given day correspond to the reward configuration, reinforcing the observation that the learning was successful in all cases. Moreover, the learning curves are similar in most of the cases, except in the case of TS 86 during learning day 4, where the learning occurred quicker than average (illustrated by *Figure 24*). The number of visited arms decreased rapidly, the rat learned the correct configuration on the seventh run, and it made no mistakes afterward. All the other learning curves are slower and include a fluctuating performance with mistakes, even after the correct pattern was found for the first time.

Indication for the simultaneous replay at night assumption was found in other heatmaps as well, not only those presented in the chapter beforehand. *Figure 25* shows other replay events at night from various rats that all indicate multi-trajectory replays at night after a day of learning the new pattern. Many more can be found at https://github.com/rebeccube/msc_thesis.

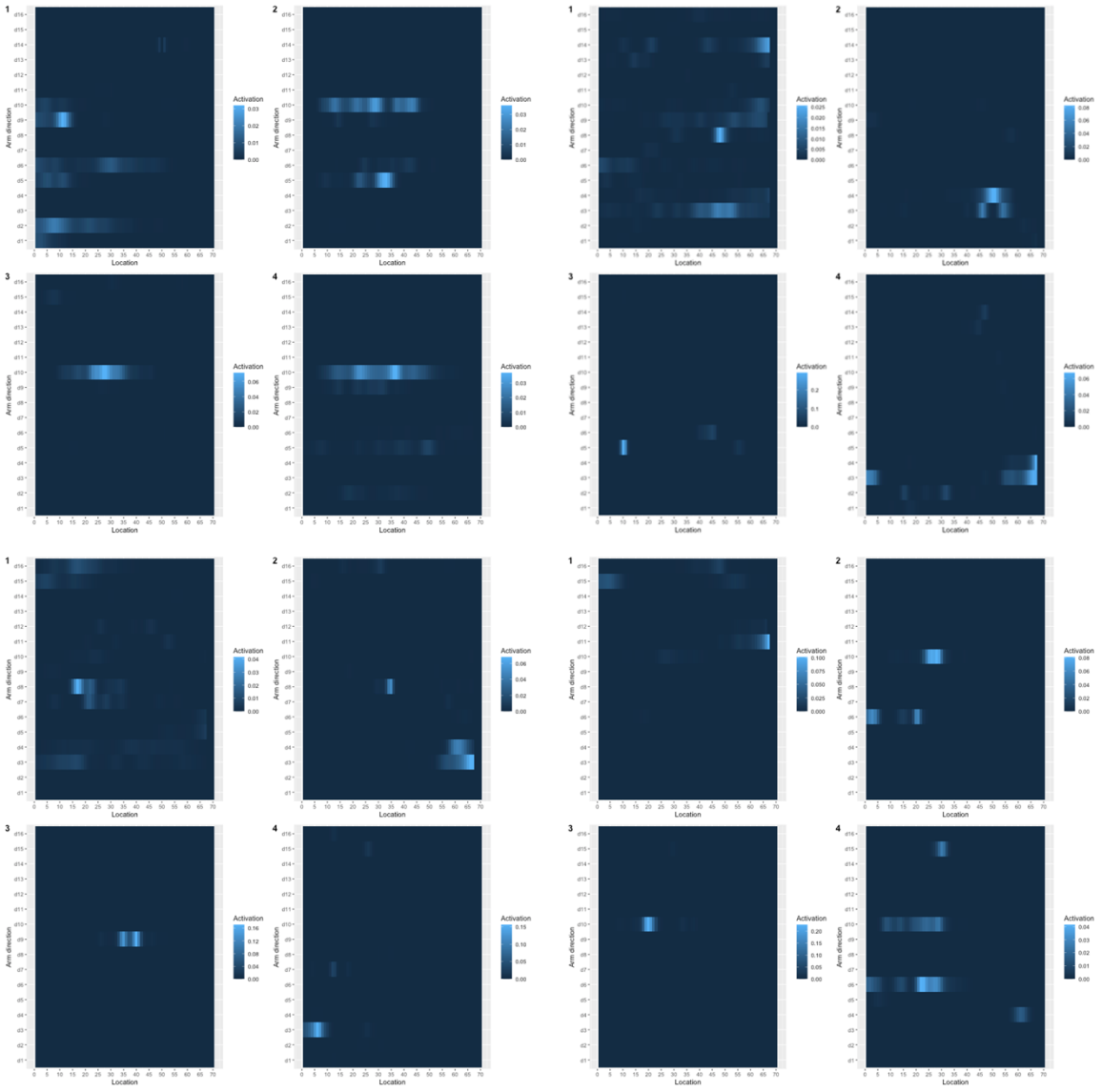

Figure 25 Heatmaps indicating multi-trajectory replays

Plotting the number of replays per arm revealed that reward arms are not replayed more frequently than any others in most of the datasets. This contradicts our previous expectations. However, the observation about the ratio of forward and reverse replays is reinforced by the analysis of the other datasets. Within each dataset, the forward replays of center-bound runs overpowered the reverse replays, while the reverse replays of reward-bound runs outnumbered the forward replays. This observation holds in most cases, even when replays were divided into subsets based on individual arms. The graphs illustrating the distribution of forward and reverse replays within both center-bound and reward-bound replays alongside the diagrams of all four types of replays based on individual arms can be found in the same repository as the other plots.

3.3.8 Statistical Testing

The observations that have been made during the exploratory data analysis and data visualization are highly relevant and interesting, however, not sufficient. It is inevitable to assign statistical values to the observations in order to answer the research questions properly.

One of the research questions aims to find a relationship between forward and reverse replays. *Figure 26* shows the ratio of forward to reverse replays based on the direction of the replayed trajectory. The line with the intercept at 1 and with a slope of 0 shows the equilibrium in which both forward and reverse replays occur with the same frequency. As the values are calculated by dividing the forward replay count by the reverse replay count, any point below the equilibrium line represents a higher reverse count, and any point above the line represents a higher forward count. Plotting the ratios of all rats and nights reveals that 100% of the time center-bound replays had a higher count of forward replays, while reward-bound replays had a higher reverse replay count in each case.

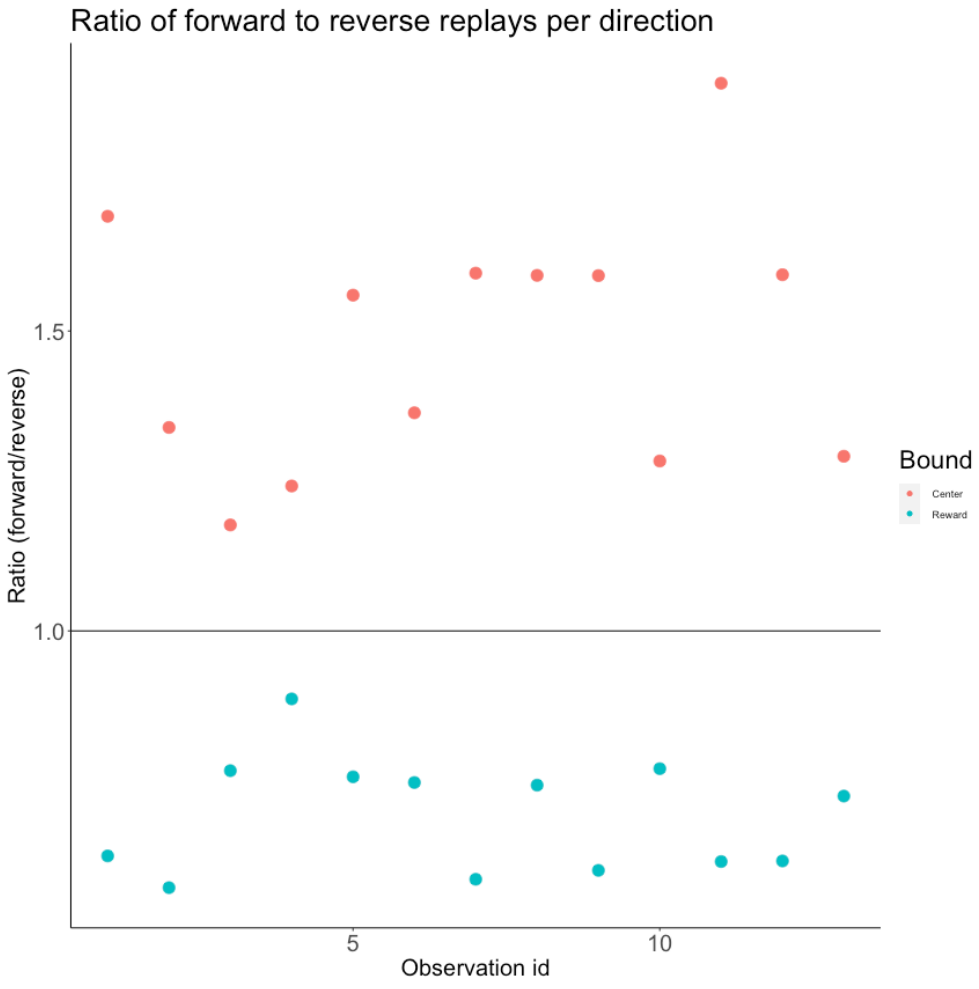

Figure 26 Ratio of forward to reverse replays

Another research question focuses on the influence that learning exerts on replays during the following night. The first correlation study examined the relationship between the number of arm visits during the day per arm and the number of trajectory replays within that arm at night. *Figure 27* shows the correlation with a regression line. Pearson's product-moment correlation revealed that there is a negligible correlation (-0.032), having the 95 percent confidence interval between -0.224 and 0.161 with a p-value of 0.7447. According to these findings, the expected results that learning influences subsequent replays are not reached. There could be multiple explanations for these results which will be discussed later.

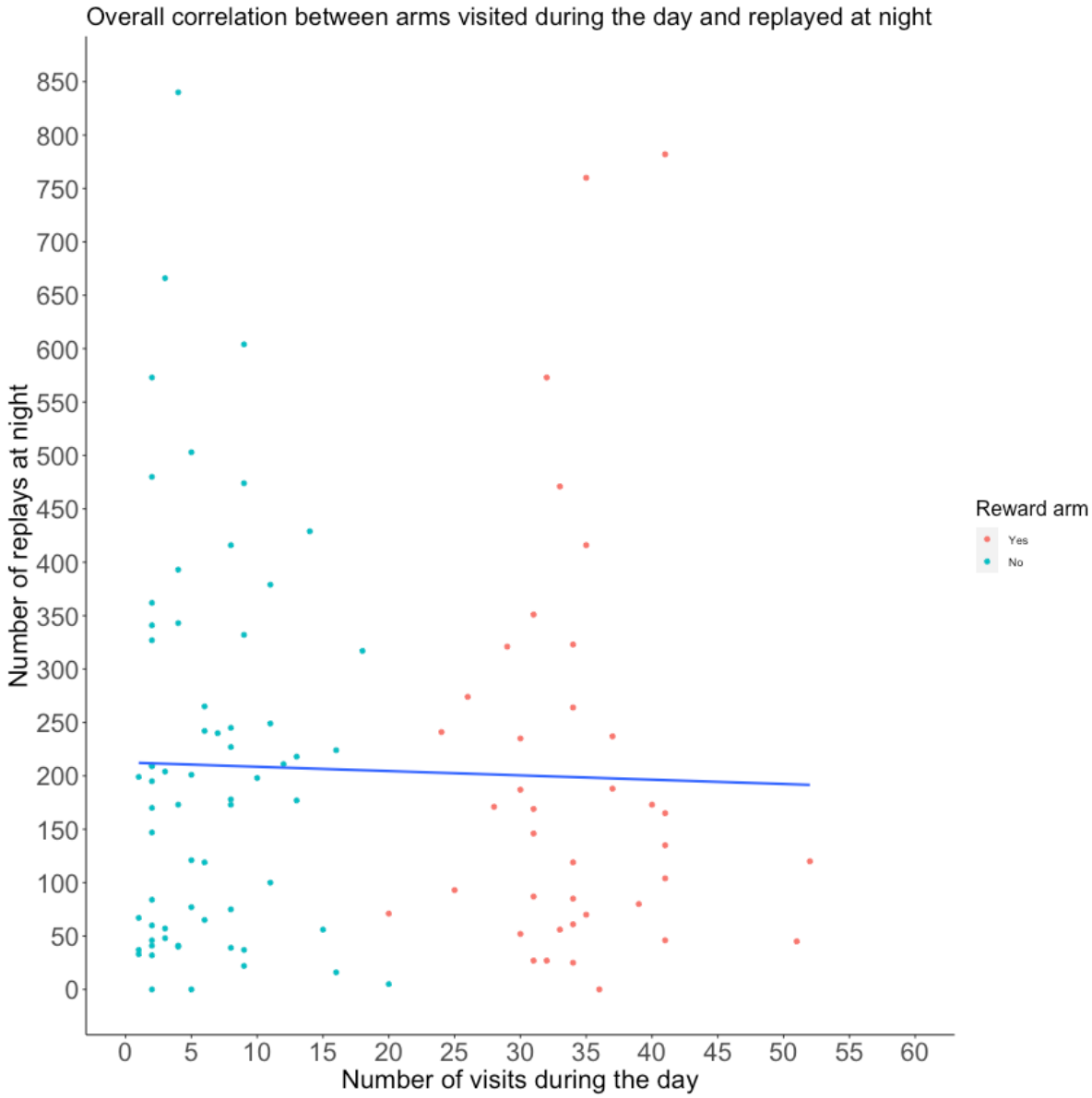

Figure 27 Correlation of arm visits during the day and replays at night

As novelty and learning bias mainly reverse replays according to the literature review, a similar correlation study has been done with the alteration that only reverse replay numbers were examined. *Figure 28* shows the results. Pearson's product-moment correlation revealed that there is no significant correlation (-0.015), having the 95 percent confidence interval between -0.207 and 0.178 with a p-value of 0.8825. Eliminating the forward replays does not improve the results. This is due to the observation mentioned earlier that the forward and the reverse replay seem to be in balance overall.

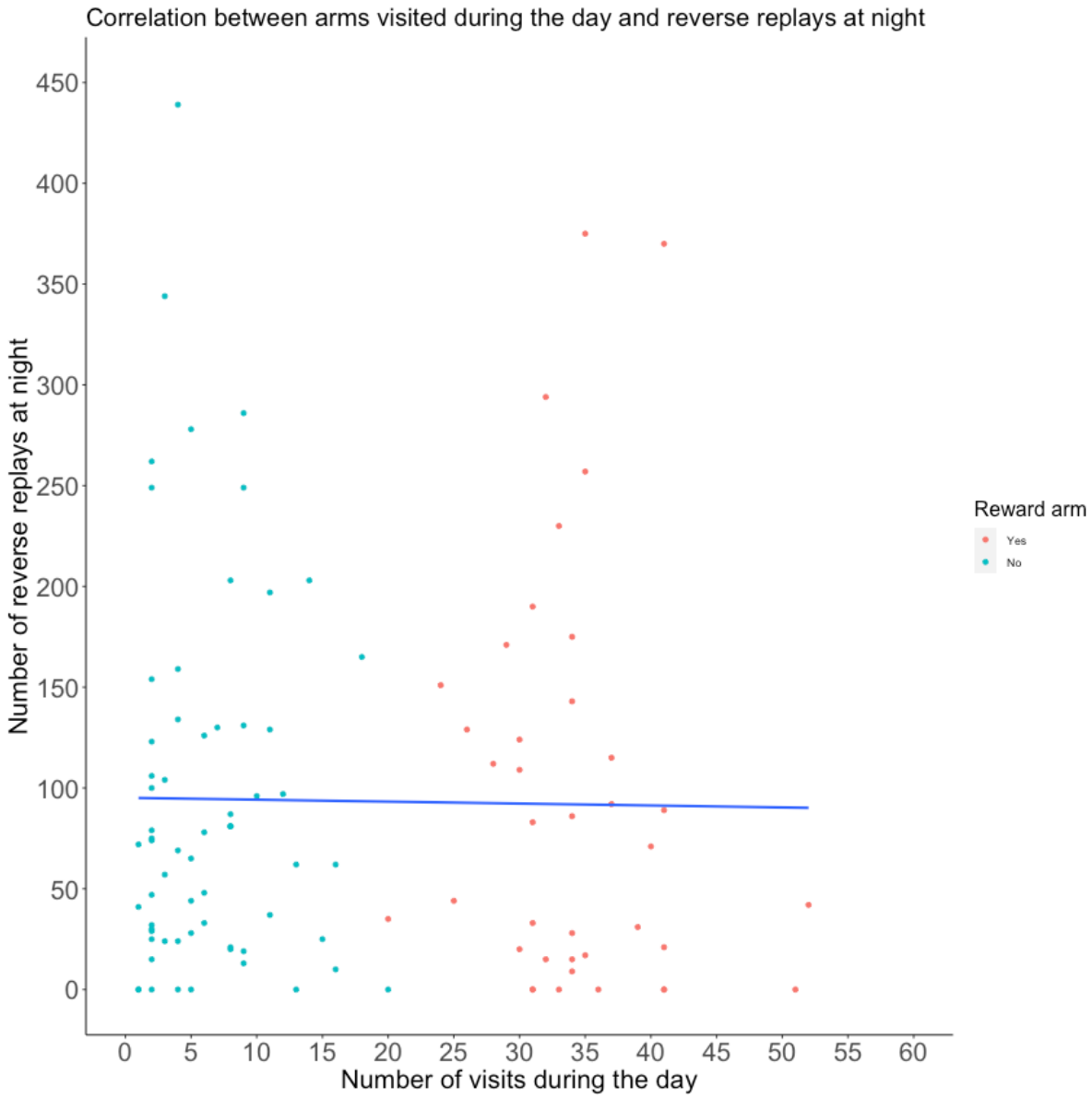

Figure 28 Correlation of arm visits during the day and reverse replays at night

Furthermore, another research question investigates how replays affect behavior the next day. This research question cannot be answered using all datasets, as most of the datasets are not sequential. For the analysis, I used the night replay data from TS 63-1, 84-1, 84-2, and 84-3, alongside the visits during the day of TS 63-2, 84-2, 84-3, and 84-4. First, all the replays, regardless of the type were included. *Figure 29* illustrates the findings. Pearson's product-moment correlation showed an insignificant correlation (0.064), having the 95 percent confidence interval between -0.292 and 0.403 with a p-value of 0.7293. These findings also contradict the expected results.

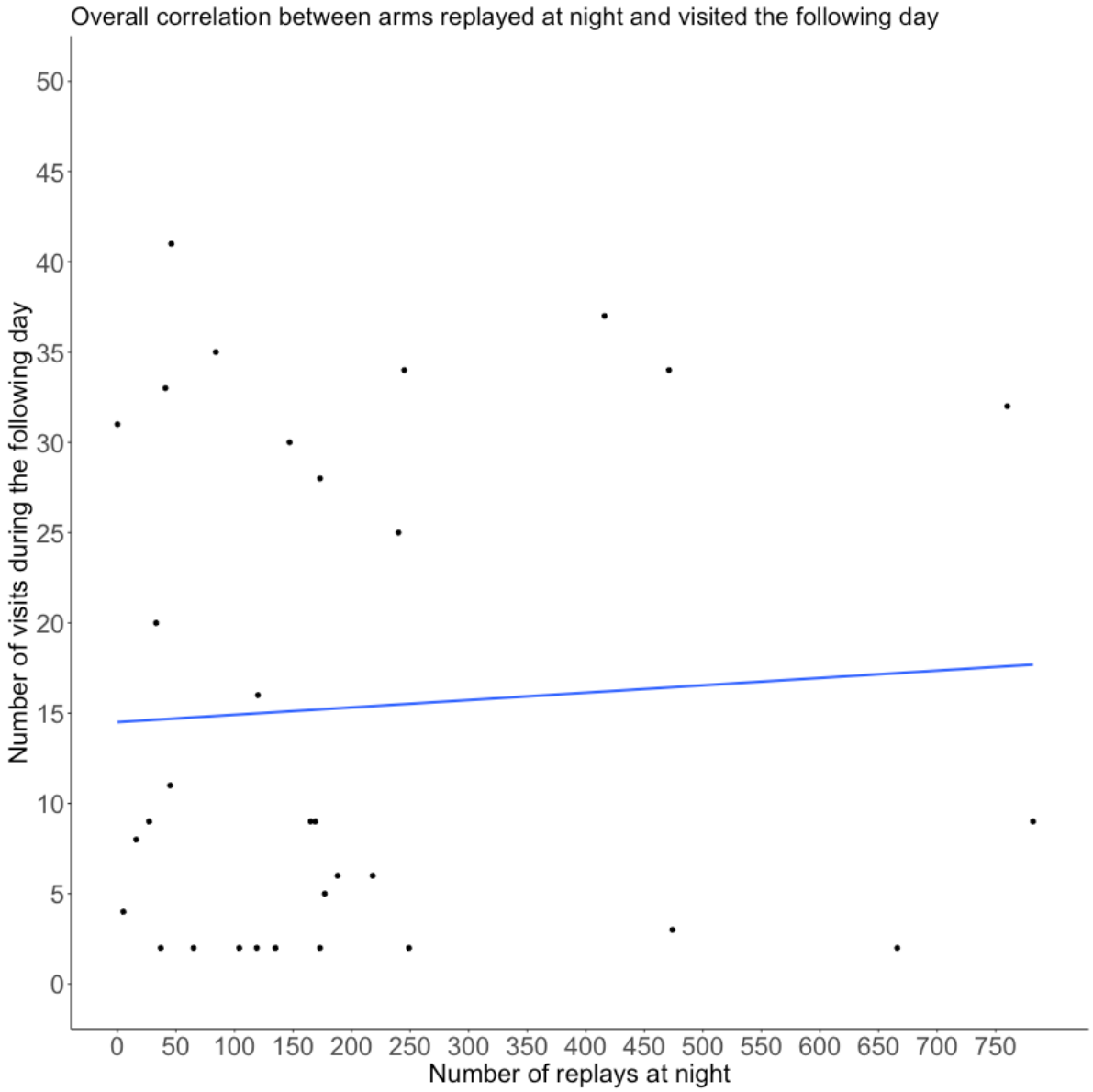

Figure 29 Correlation of arm replays at night and arm visits during the following day

This analysis was extended by only including the forward replays, as those are assumed to guide future behavior. The correlation received by Pearson's product-moment correlation (0.030) is negligible, having the 95 percent interval within -0.322 and 0.374 and a p-value of 0.8723. The illustration can be seen in *Figure 30*.

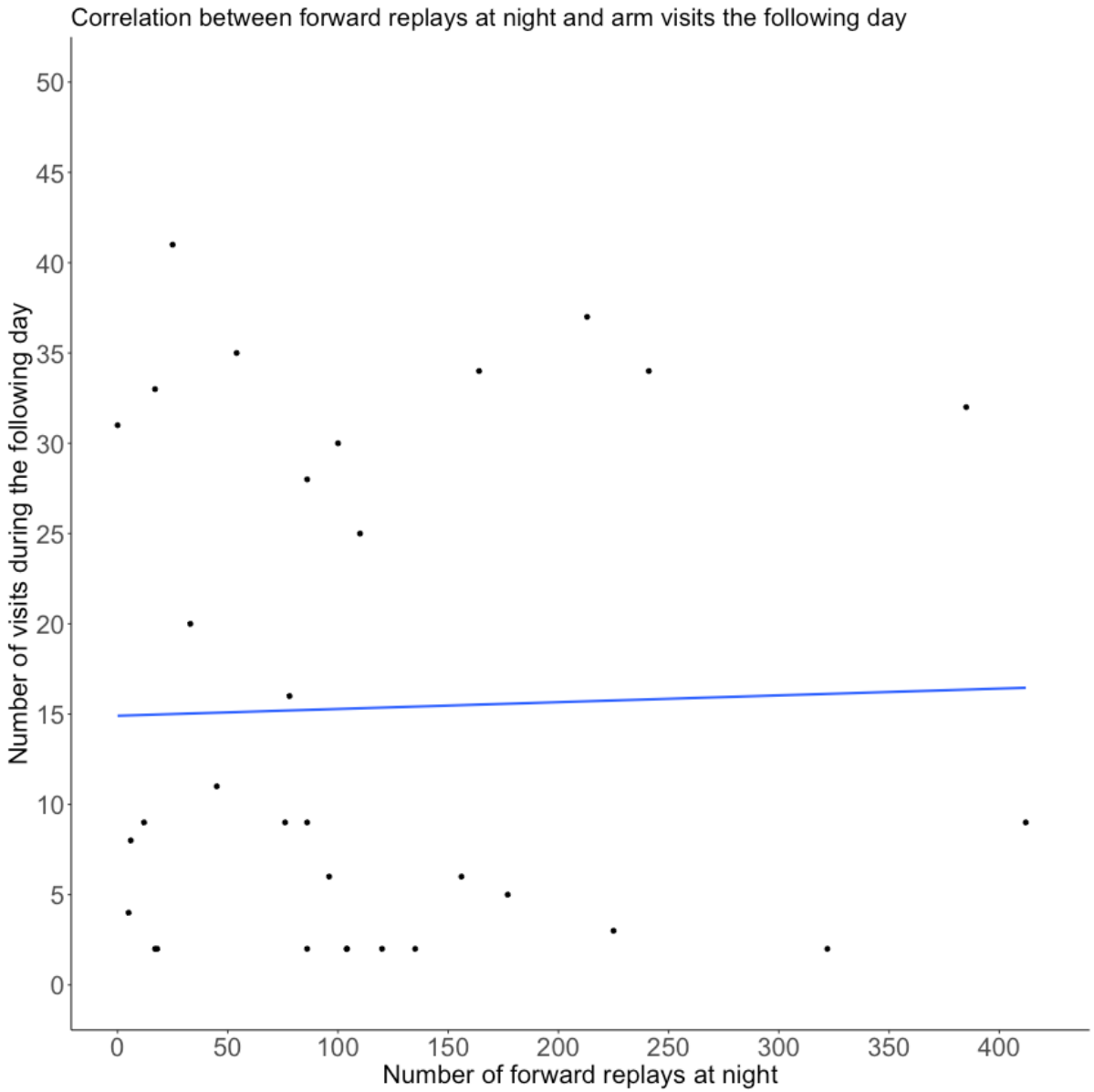

Figure 30 Correlation of forward arm replays at night and arm visits during the following day

4. Discussion

The purpose of this chapter is to interpret the results of the data analysis, explain unexpected results, show the significance of the findings and place the product of the thesis within the scientific literature. Moreover, limitations and open questions are identified.

The new arms dataset revealed that the configurations of the reward locations between different days were conforming, lacking diversity of the pattern. This can be considered a limitation of the basic setup of the study. It also leaves an open question, namely, how would adjacent reward locations and diverse patterns influence learning speed and trajectory replays.

The recordings of the paths taken during the learning sessions proved that the task of learning a pattern of goal-location was successfully accomplished by all test subjects. This result is consistent with the reviewed literature which states that spatial learning is present in rodents and other animals including humans.

The learning curves and the number of visited arms per day indicate that learning accelerates throughout the days. The limitation that this study faces in this aspect originates from the fact that observations from consecutive days are missing in most of the cases. Sequential data exists only with TS 84 (4 consecutive days) and TS 63 (2 consecutive days). Future studies are needed with more sequential data to reinforce these findings.

Place field reactivations were identified during SWRs and HSEs during the offline periods (at night), which corresponds to the reviewed literature. It contributes to answering the first research question about what is replayed. However, the visualized reactivations reveal the possibility that multiple trajectories can be replayed simultaneously during one SWR or HSE. This finding exerts a dual effect on this thesis. Firstly, it forms the basis of the limitations of the correlation studies, as those are based on one identified replayed trajectory disregarding the possibility of multi-trajectory replays. Secondly, it adds an interesting result to the thesis that could inspire future researchers to investigate simultaneous replays.

The analysis of replayed trajectories revealed that place cell assemblies in the 8-arm maze encode directional place fields resulting in four different types of replays, center-bound forward replay, center-bound reverse replay, reward-bound forward replay and reward-bound

reverse replay. This answers the first research question by indicating that directional trajectories are replayed during offline periods. This finding concurs with the expected results.

The analysis of the relationship of forward and reverse replays presented an interesting finding and answered the second research question. A pattern emerged that showed a consistent difference between forward and reverse replay counts. More specifically, the majority of center-bound trajectories were replayed in a forward manner in 100% of the cases. As for reward-bound trajectories, in all cases reverse replays outnumbered their forward counterparts. This pattern was also observable on the arm subset level. This unexpected finding is the most significant result of the thesis. A partial explanation could be provided by existing research which states that reverse replays are more biased by novelty. As the direction is reward-bound, the novelty of the goal or non-goal location could result in a higher reward-bound reverse replay. Another explanation could be that the center-bound runs do not have a novel expect to them, so center-bound replays could be the primary means of future planning that enhances forward replays. However, more extensive research is needed to discover the exact reasons for this phenomenon.

No significant correlation was found between the preferential arms visited during the day and the number of trajectories that have been replayed in that arm during the subsequent night. The correlation study was executed with both overall replay numbers and the number of reverse replays. This contradicts the expected result which assumed that training modulated subsequent replays. The reason for these findings could be based on the limitation that from each replay event only the most probable path was taken disregarding the possibility of multi-trajectory replays, thus, biasing the number of overall replays per arm. The same results were reached for the correlation between the overall and forward replay numbers and the number of visited arms the subsequent day. The limitations are the same with the addition that only a subset of the datasets could be analyzed due to non-sequential observations. The question arose whether mitigation of these limitations would change the results of the correlation studies.

5. Conclusion

This thesis provides a detailed overview of the anatomical structure of the hippocampal formation that is necessary for understanding the research on place cell assemblies. It also introduces the reader to cognitive network science which assumes that our cognition is an emergent phenomenon from the interaction of neural networks, and learning can be described as the modulation of the neural net. Different place cell studies are discussed in detail from the discovery of place cells to the state-of-the-art research. Xu and colleagues' work is elaborated on extensively as that study is the precursor for this master thesis. The data, being analyzed for the purpose of answering the research questions, was collected during their experiment.

Interdisciplinarity is an important aspect of this paper. Comparative biology enables the results to be extrapolated, mathematics aids both with understanding the underlying paradigm of Cognitive Network Science and with analyzing the data. Medical science, next to paving the way to realizing the importance of the hippocampal formation, is inevitable for this thesis, as the data collection could not have happened without implanting tetrodes into rats' brains. AI opens new doors to understanding cognition and shaping the way how scientists think about neural networks. Neuroscience, being the main discipline behind the thesis, provides with the understanding of brain functions, neural firings and their patterns and more specifically a line of research on hippocampal place cell assemblies.

The data analysis, that was carried out by programming a unique R library with multiple functions specific to each of the received datasets, provides answers to the research questions. Firstly, "What is replayed during offline periods?" is answered by identifying four different types of replays (center-bound forward; center-bound reverse; reward-bound forward; and reward-bound reverse replays). The second research question "What is the relationship between forward and reverse replays?" revealed the unexpected significant result that the count of forward and reverse replays consistently differs in the different trajectory directions. The answers to the third and fourth research questions ("Does learning modulate subsequent replays?" and "Do replays reflect future performance or behavioral patterns?") do not concur with the expected results. No correlation was found upon analyzing the dataset.

Several limitations have been identified such as the pattern similarity and lacking adjacent reward locations, minimal sequential data and the single-trajectory assumption during offline replays. All these limitations give rise to potential future research. This thesis could inspire future researchers to conduct in-depth research into the relationship between directionality and replay types based on the significant findings of the number of forward and reverse manners in central-bound and reward-bound trajectory replays. Future research could also focus on the potential multi-trajectory replays during SWRs and HSEs in offline periods.

In a nutshell, thesis objectives are achieved as it provides significant findings that could inspire future research, and it fulfilled personal learning goals by providing a deep insight into neuroscience and learning research.

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7. Appendix: German Abstract

Diese interdisziplinäre Masterarbeit untersucht die hippocampalen Replays, deren Inhalt und Einfluss auf Nagetiere in Ruhephasen. Nach einem Tag des Erlernens von Neukonfigurationen von Belohnungsorten in einem komplexen achtarmigen Labyrinth können Kategorien im neuralen Muster von den beobachteten Ratten hinsichtlich vier verschiedener Replays und deren auftretender Häufigkeit bestimmt werden. Neben der umfassenden interdisziplinären (Neurowissenschaft, Vergleichende Biologie, Mathematik, Medizin, KI) Literaturrecherche für diese Masterarbeit wurde eine einzigartige R-library erstellt, um empirische Daten zu analysieren und zu visualisieren. Die Rohdaten sind von Xu und seinen KollegInnen (2019) erhoben worden, nachdem den Versuchsratten in der CA1 Region des Hippocampus Tetroden implantiert worden sind. Es zeigt sich einerseits, dass die nach der Literaturrecherche zu erwartende Korrelation von Replays und Verhalten nicht anhand dieser Datensets aufgrund mangelnder fortlaufender Beobachtungsdaten mehrerer Tiere unterstützt wird. Andererseits belegen die analysierten Daten, dass neuronale Feuermuster die Bewegungsbahnen der Versuchstiere enkodieren. Diese Bewegungsbahnen können in Ruhephasen vorwärts und rückwärts wiederholt werden. Unerwartete signifikante Ergebnisse der Analysen zeigen, dass Vorwärts-Replays beständig in Anzahl die Rückwärts-Replays übersteigen, wenn das Ziel der Bewegung die Mitte des Labyrinths ist. Wenn das Ziel hingegen die Belohnung ist, übersteigt die Anzahl der Rückwärts-Replays die der Vorwärts-Replays.