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Hippocampal coding of positions of visual objects and prediction of their future interactions

Hipokampální kódování pozic vizuálních objektů a predikce jejich budoucích interakcí

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Abstract

The hippocampus is a crucial brain structure involved in spatial navigation. It contains populations of spatially sensitive cells as the place cells, head-direction cells, grid cells, border cells, or object-vector cells. These neurons together create a cognitive map of the environment that serves for navigation in space. The role of hippocampal cells in the encoding of positions of other objects has also been suggested. Other studies found so-called time cells in the hippocampus that are active during specific delays in a behavioral task and associated them with place cells. While there are recent studies researching the encoding of accessible objects' positions, the encoding of objects in the inaccessible space has lacked research. The neural representation of dynamic situations (that constitute the majority of real-world encounters) has also been only scarcely researched.

We designed a behavioral task to study the learning of static and dynamic spatial visual scenes presented in the inaccessible space and combined it with single-unit electrophysiological recording from the CA1 area of the hippocampus of freely moving rats.

Our results show that rats can discriminate both static and dynamic inaccessible spatial stimuli, and that they prefer dynamic over static stimuli. They can also generalize novel dynamic stimuli using the previously obtained knowledge. We found cells with their firing rate modulated by the presented spatial visual stimuli, both specifically and non-specifically. We propose that static and dynamic spatial situations might be encoded by mutual neuronal mechanisms in the hippocampus.

Key words: hippocampus, spatial cognition, visual discrimination, objects' interaction, single-unit activity

Abstrakt

Hipokampus je klíčová oblast mozku účastnící se prostorové navigace. Obsahuje populace prostorově citlivých buněk jako jsou buňky místa, buňky směru hlavy, mřížkové buňky, hraniční buňky a „objektové vektorové“ buňky. Tyto neurony společně tvoří kognitivní mapu prostředí, která slouží k orientaci v prostoru. Role hipokampálních buněk v kódování pozic okolních objektů byla také navržena. Další studie objevily v hipokampu takzvané buňky času, které jsou aktivní během specifických prodlev v behaviorální úloze, a asociovaly je s buňkami místa. Zatímco existují recentní studie zabývající se neurálním kódováním pozic přístupných objektů, kódování objektů v nepřístupném prostoru zatím nebylo příliš prozkoumáno. Neurální reprezentace dynamických situací (které představují většinu interakcí v reálném světě) je také prozkoumána jen řídce.

Navrhli jsme behaviorální úlohu ke studiu učení statických a dynamických prostorových vizuálních scén prezentovaných v nepřístupném prostoru a zkombinovali ji s jednotkovým elektrofyziologickým nahráváním z CA1 oblasti hipokampu volně se pohybujících potkanů.

Naše výsledky ukazují, že potkani dokáží diskriminovat statické i dynamické nepřístupné prostorové stimuly, a že preferují dynamické stimuly nad statickými. Také umí generalizovat nové dynamické stimuly na základě předchozí zkušenosti. Našli jsme buňky, jejichž rychlost palby byla modulována prezentovanými prostorovými vizuálními stimuly, jak specificky, tak nespecificky. Navrhujeme, že statické a dynamické prostorové situace by mohly být v hipokampu kódovány stejnými neurálními mechanismy.

Klíčová slova: hipokampus, prostorová kognice, vizuální rozlišování, interakce objektů, jednotková aktivita

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List of abbreviations

- AP – anteroposterior
- CA – Cornu Ammonis
- CIR – compact internal representation
- CR – continuous reinforcement
- CSC – complex spiking cell
- D REW – dynamic rewarded stimulus
- D non-REW – dynamic non-rewarded stimulus
- DG – dentate gyrus
- EC – entorhinal cortex
- EEG - electroencephalogram
- FR2 – fixed ratio 2
- GLMM – general linear model with mixed effects
- HD – head direction
- LEC – lateral entorhinal cortex
- LIA – large irregular activity
- MEC – medial entorhinal cortex
- ML – mediolateral
- MWM – Morris water maze
- Non-REW – non-rewarded stimulus
- PFA - paraformaldehyde
- RDK – random dot kinematogram
- REM – rapid eyes movement
- REW – rewarded stimulus
- S REW – static rewarded stimulus
- S non-REW - static non-rewarded stimulus
- SIA – slow irregular activity
- SWS – slow wave sleep
- VR3 – variable ratio 3

Introduction

The hippocampus plays a crucial role in the process of spatial navigation. It contains a population of cells that fire in relation to an animal's position in the environment and are called the place cells (O'Keefe & Dostrovsky, 1971). Other brain areas adjacent to the hippocampus (such as the entorhinal cortex) also contain cells with functions related to spatial navigation – the head-direction cells (Sargolini et al., 2006), the grid cells (Hafting et al., 2005), the border cells (Solstad et al., 2008), and the object-vector cells (Hoydal et al., 2019). These cells together were proposed to create a cognitive map of the environment that is then used by the subject for navigation in space (O'Keefe & Nadel, 1978).

Besides their importance in the encoding of subject's own position in space, the place cells have also been linked to the encoding of positions of other objects (Manns & Eichenbaum, 2009; Deshmukh & Knierim, 2013). The studies investigating this topic have mostly focused on the representation of objects in the accessible space. On top of that, the internal representation of spatial dynamic situations has lacked research. Navigation in dynamic environments is nevertheless a crucial skill animals need to possess to survive in the real world.

Dynamic situations contain two aspects in themselves – space and time. Cells representing sequences during a delay period have also been found in the hippocampus and called the time cells. Time cells have been proposed to be the same neurons as place cells (Eichenbaum, 2014). For this and other reasons listed in the thesis, we assumed that spatial dynamic situations might be encoded by the pyramidal neurons of the hippocampus.

In the practical part of this thesis, I describe the behavioral task we used to study the learning of inaccessible static and dynamic spatial stimuli by rats and the results obtained in the experiment. I also focus on the single-unit electrophysiological recording that we used in combination with the task to determine if the information about the spatial visual scenes is represented in the hippocampus.

THEORETICAL PART

1 Spatial navigation

Navigation in space is one of the crucial skills needed for survival in animals and humans. Both natural predators and prey must be able to move quickly and effectively in different environments to survive. They must navigate their surroundings to successfully find food and water sources, potential partners, nests, etc. Therefore, the question of how this ability is encoded in the animal and human brain has been investigated by scientists since the mid-20th century. During the decades of extensive spatial navigation research, significant progress has been made and numerous brain mechanisms of spatial navigation have been identified, yet much remains to be discovered.

In this chapter, I will review the general knowledge about the navigational strategies used by organisms while placing a particular focus on navigation based on a cognitive map.

1.1 Navigational strategies

A now classical work dealing with spatial navigation is O'Keefe's and Nadel's book *The hippocampus as a cognitive map* (1978). In their book, the authors proposed and characterized two distinct navigational strategies: navigation based on a route and navigation based on a map.

For a proper introduction of these two strategies, it is important to understand how space is internally represented. The internal representation of space can be viewed in the context of two different reference frames: egocentric and allocentric. Within the egocentric reference frame, the observed space is related to the position of the perceiver. In the allocentric representation, the space is represented independently of the observer's position, and the objects in this space are represented in relation to each other (Klatzky, 1998). The egocentric representation forms the basis for the taxon or route-based navigation system and the allocentric representation for the locale or map-based navigation system (O'Keefe & Nadel, 1978).

Route-based navigation is usually considered to be of lower cognitive demands of the two strategies. It is based on a series of stimuli and responses which are utilized in the process of navigation from one place to another. Route-based navigation strategies can be divided into guidances, meaning navigation using environmental landmarks; and orientations, learned sequences of fixed behaviors leading to the desired goal location. In comparison to maps, routes are easier to learn and occasionally represent a quicker way to navigate certain environments, but they are also inflexible and prone to error (O'Keefe & Nadel, 1978).

Map-based navigation is based on an internal representation of the environment. Unlike routes, maps are flexible and highly informational as they offer multiple ways of getting from one place to another. They are also stable since they don't rely on a single environmental cue but rather on sets of cues in relationship to each other (O'Keefe & Nadel, 1978).

Animals use both route-based and map-based strategies in parallel when performing spatial navigation tasks, alternating between them depending on their usefulness in a given environment (McDonald & White, 1994; Hamilton et al., 2004).

1.2 Cognitive map

The term cognitive map was introduced by Tolman, who studied the properties of spatial navigation in rats in maze tasks (specific behavioral tests designed to study spatial cognition in animals, further described in Chapter 3). In opposition to the behaviorist opinion popular at the time that animal maze navigation is purely a series of learned stimuli and responses, he deemed this navigation to be far more complex and stated that rats navigate in mazes using internal field maps of the environment (Tolman, 1948). O'Keefe and Nadel (1978) later revisited this concept and localized the cognitive map to a part of the limbic system – the hippocampus. They did so after the discovery of a population of hippocampal cells active in relation to the animal's position in space (O'Keefe & Dostrovsky, 1971). During the following decades, other cell types with specific spatial functions supporting the cognitive map theory were discovered in the hippocampus and surrounding brain areas.

In recent years, there has been a shift in the understanding of the cognitive map from seeing it solely as means of orientation in the physical space to a more general system that organizes various behaviors across cognition. In this context, the multidimensional cognitive map encodes not only space but also time, social space, and abstract associations independently of their spatial and temporal properties (Schiller et al., 2015). I shall further focus on the temporal aspects of the cognitive map in the last chapter of the theoretical part of this thesis.

2 The hippocampus

The hippocampus is one of the most extensively researched parts of the brain to date. In rodents, the hippocampus is located right beneath the neocortex, assuming a dorsal position on the rostral side, and changing its position to lateroventral on the caudal side of the brain (Fig. 1-A). In humans and other primates, it is located deep in the medial temporal lobe (Schröder et al., 2020; Fig. 1-B).

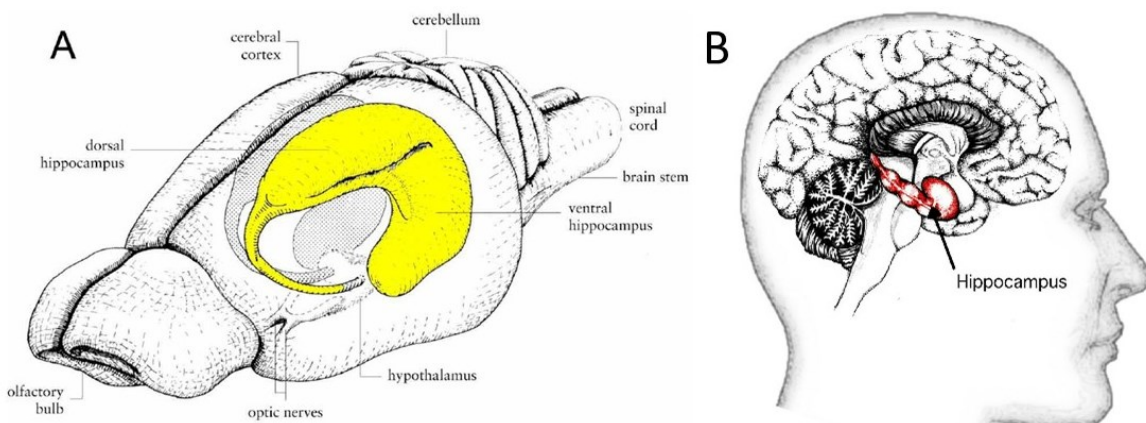


Figure 1: A: Rat hippocampus (Little, 2007; original picture adapted from Cheung and Cardinal, 2005). B: Human hippocampus (Vale, 2005).

The most widely studied functions of the hippocampus are its roles in the consolidation of memory and in spatial cognition. Regarding the latter, the hippocampus plays a crucial role in map-based navigation, as seen in animals with hippocampal lesions that seem unable to use cognitive maps for orientation in the environment and resort to route-based navigation when solving spatial tasks (McDonald & White, 1994; Packard & McGaugh, 1996). Route-based navigation is independent of the hippocampus as shown by lesion and inactivation studies in rodents (McDonald & White, 1994; Lee et al., 2008).

The hippocampus is a part of a larger brain area, called the hippocampal formation, and to better understand its function, it is important to discuss the inputs and outputs of its respective units (Knierim, 2015).

2.1 Anatomy

2.1.1 The trisynaptic circuit

The hippocampal formation is a complex brain area consisting of several interconnected parts: the entorhinal cortex (EC), the dentate gyrus (DG), the hippocampus proper, the subiculum, the presubiculum, and the parasubiculum. The hippocampus proper is further divided into three parts called CA1, CA2, and CA3, the abbreviation standing for Cornu Ammonis (Blackstad, 1956). The individual parts of the hippocampal formation are connected by a series of projections called the trisynaptic circuit (Fig. 2).

The trisynaptic circuit starts with a projection from the entorhinal cortex, a six-layer structure, to the dentate gyrus. This projection is known as the perforant path, and the neurons projecting to the DG reside in the EC layer II. The granule cells of the DG then project to the hippocampal CA3 area via the mossy fiber pathway. The CA3 area sends its axons, called the Schaffer collaterals, to the CA1 area (Andersen et al., 1971; Amaral, 1993). The Schaffer collateral pathway is organized in such a manner that the neurons in the proximal part of CA3 synapse with the neurons in the distal part of CA1 and the distal CA3 neurons with the proximal CA1 neurons (Ishizuka et al., 1990). The CA1 area then projects to the subiculum, and finally, both the CA1 and the subiculum send their projections back to the deep layers (V and VI) of the entorhinal cortex (Amaral, 1993).

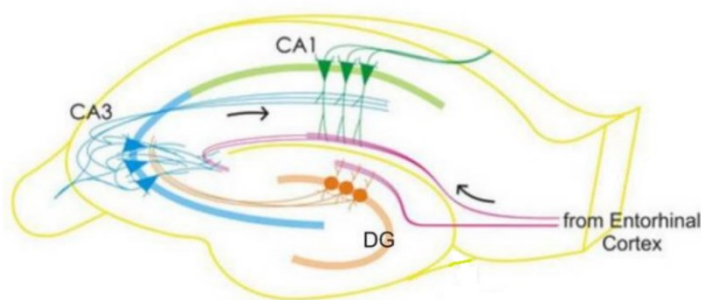


Figure 2: The trisynaptic circuit of the hippocampus. The perforant path is pictured in purple, the mossy fibers and cells of the DG in orange, the cells of the CA3 and the Schaffer collaterals in blue, and the cells of the CA1 in green. Projection to the subiculum is not shown. (Adapted from Kowalski, 2010).

Besides this classical trisynaptic circuit, there are multiple unisynaptic connections between the structures of the hippocampal formation. First, next to its projection to the DG, the perforant path from EC layer II also projects directly to the CA3 area of the hippocampus, and

the EC layer III sends direct projections to the CA1 area and the subiculum (Steward & Scoville, 1976; Witter et al., 1988). Second, the cells of CA3 have synaptic connections not only with the CA1 neurons but also with other CA3 neurons (Ishizuka et al., 1990), and finally, the CA3 projects back to the DG via the dentate hilus mossy cells (Scharfman, 2007).

2.1.2 The longitudinal axis

The hippocampus proper varies in connectivity across its longitudinal axis and therefore, it is typically divided into two functionally distinct parts: the dorsal hippocampus and the ventral hippocampus. The dorsal hippocampus receives primary input from the entorhinal cortex, including its medial spatially sensitive portion, and it plays a crucial role in memory and cognition, precisely in spatial navigation and memory, learning, and context processing. On the contrary, the ventral hippocampus is strongly interconnected with the prefrontal cortex and the amygdala and is mainly responsible for emotional memory processing and emotional and motivational behavior (Fanselow & Dong, 2010; Knierim, 2015).

The names dorsal and ventral refer to the position of these parts in the brain of a rodent. In the human brain, the dorsal hippocampus of the rodent corresponds to the posterior hippocampus, and the ventral hippocampus of the rodent to the anterior hippocampus (Fanselow & Dong, 2010).

2.2 Electrophysiological properties of the hippocampus

The electroencephalogram, or EEG, is a recording of the electrical activity of the brain. It is usually obtained by recording from the scalp in humans during diagnostic examinations. It can also be recorded directly from the brain by intracranially implanted electrodes in experimental animals or in human patients with severe epilepsy, who have implanted electrodes due to presurgical diagnostics (Yuan et al., 2012). The EEG can be divided into distinct patterns mainly based on the frequency and amplitude of the electrical signal.

Regions across the brain differ in the presence of particular electrical patterns and those prevalent in the hippocampus are the theta rhythm, the beta rhythm, the gamma rhythm, and rhythmical ripples occurring together with so-called sharp waves (O'Keefe, 2006; Colgin, 2016). Theta rhythm, sharp waves, and ripples are especially important in relation to spatial navigation and therefore, I only further focus on these types of activity in this chapter.

2.2.1 Theta rhythm

The theta is a slow rhythmical activity with a high amplitude and a frequency from 6 to 12 Hz in the rat (Vanderwolf, 1969; Fig. 3), but the frequency can be even lower (4 Hz) in other animals such as rabbits (Kramis et al., 1975). Theta is usually divided into two types based on its behavioral correlates and pharmacological properties.

The first type is called a-theta, representing arousal or attention-sensitive theta. Its name is descriptive of the behavioral states during which it occurs. Specifically, it can be recorded in situations when an animal is immobile but alert, such as before making a move, but also under urethane anesthesia. A-theta is sensitive to cholinergic antagonists such as atropine and has a lower frequency range of 4-7 Hz (Kramis et al., 1975).

The second type of theta, t-theta, is named after its occurrence during translational movements. These generally include movements changing the position of the animal in space, such as walking, running, jumping, or swimming. T-theta is not influenced by cholinergic input to the hippocampus, as it is not sensitive to anticholinergic drugs (Kramis et al., 1975; Buzsáki et al., 1983), but it is regulated by serotonergic (Sörman et al., 2011) and glutamatergic (Huh et al., 2010; Lazarewicz et al., 2010) inputs. T-theta has a higher frequency range than a-theta, typically 8-12 Hz (Kramis et al., 1975).

In general, the theta rhythm is always present during active movements of an animal through space, and it is also seen during episodes of rapid eyes movement (REM) sleep (Whishaw and Vanderwolf, 1973). The signal is the strongest in the CA1 area and the DG, and it is produced by neuron interactions in these areas and by inputs from the perforant path and the septum. The generation and propagation of the rhythm are controlled by a network of inhibitory interneurons, parvalbumin-positive interneurons being of particular importance (Buzsáki et al., 1983; Amilhon et al., 2015). Recordings from different regions of the hippocampus show that theta is in synchrony across its longitudinal axis. This synchrony is likely to play a role in the processing of new information, including spatial information, and in the formation and consolidation of memory (Long et al., 2015). It also provides a clock for the firing of hippocampal pyramidal neurons, a function that is described in more detail in Chapter 2.3.1 (Skaggs et al., 1996).



Figure 3: Theta rhythm. Recording from the dorsal hippocampus of the rat during wheel running. Theta activity abruptly stops during a change of behavior. (Whishaw and Vanderwolf, 1973).

2.2.2 Sharp waves and ripples

Sharp waves and ripples are electrophysiological patterns occurring in the hippocampus during periods of immobility, for example when an animal sits, drinks, or eats, and during slow-wave sleep (SWS) (Whishaw and Vanderwolf, 1973; Buzsáki et al., 1983). Sharp waves and ripples are always co-occurring and recorded with the highest amplitude in the CA1 area, but the mechanisms and locations of their origin are different (Colgin, 2016). Sharp waves are generated by bursts of pyramidal cells in the CA3 hippocampal area and transmitted to the CA1 (Buzsáki, 1986). One sharp wave typically lasts around 40-100 milliseconds and has a frequency of 0.01-3 Hz (Buzsáki et al., 1983; Fig. 4-A). Ripples are short oscillations in the frequency range between 150 and 250 Hz (Fig. 4-B) generated by interneurons in the CA1 (Ylinen et al., 1995; Jadhav et al., 2012). The sharp wave-ripple pattern is thought to play an important role in the consolidation of memory – including spatial memory – during SWS (O'Neill et al., 2010). Furthermore, its role in spatial navigation, specifically in predicting future trajectories, has also been suggested (Jadhav et al., 2012; Pfeiffer & Foster, 2013; Widloski & Foster, 2022).

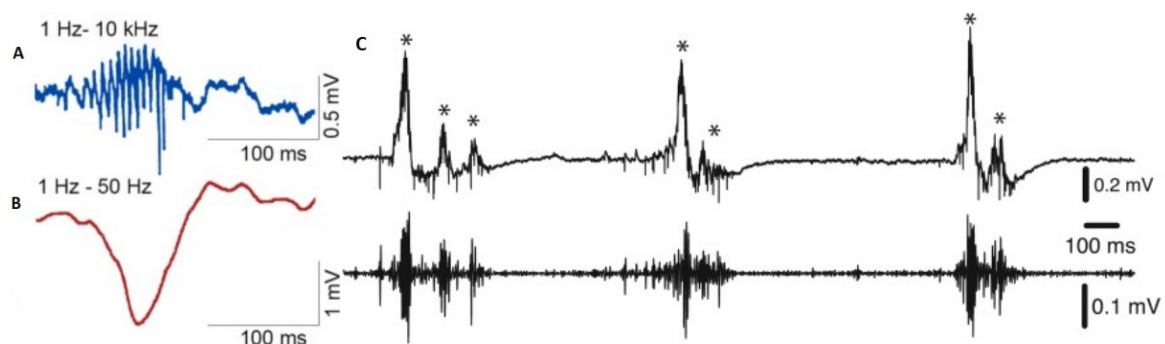


Figure 4: Sharp waves and ripples. A: Ripples recorded from CA1. B: A sharp wave recorded from CA1. C: Sharp wave-ripples recorded from hippocampal slices. The top recording shows the raw signal, on the bottom is the same recording band-pass filtered at 1-50 Hz. (Adapted from Buzsáki, 2015).

2.2.3 Complex-spiking cells

Based on electrophysiological single-cell recordings from the hippocampus, two populations of cells with different characteristics were discovered (Ranck, 1973). The first type is called the complex-spiking cell (CSC), named after its characteristic firing pattern. These cells fire bursts of 2-10 action potentials with an inter-spike interval of a few (typically less than 5) milliseconds. The first spike in a burst usually has the highest amplitude and the amplitudes of subsequent spikes are gradually decreasing (Ranck, 1973; Fox & Ranck, 1981; Fig. 5-A). Hippocampal CSCs are pyramidal neurons, and a big part of their population plays a crucial role in spatial orientation (O'Keefe & Dostrovsky, 1971).

2.2.4 Theta cells

The second type of cells in the hippocampus are the theta cells. Theta cells are inhibitory interneurons with their activity typically phase-locked to the theta rhythm (Fox & Ranck, 1981; Buzsáki & Eidelberg, 1983). They have a higher firing rate than CSCs, all their spikes are of the same amplitude, and they do not occur in bursts (Ranck, 1973; Fig. 5-B). The individual action potentials of interneurons are narrower and have a different shape than those of pyramidal neurons (Csicsvari et al., 1998; Fig. 6).

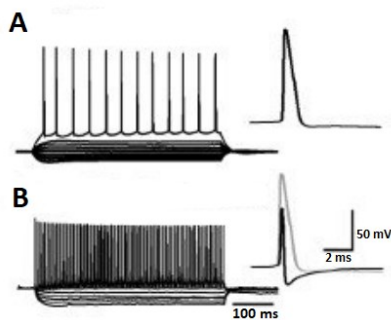


Figure 5: Whole-cell patch-clamp electrophysiological recordings from hippocampal cells. A: A complex-spiking pyramidal cell. B: A theta cell with a much higher firing rate. (Adapted from Booker et al., 2014).

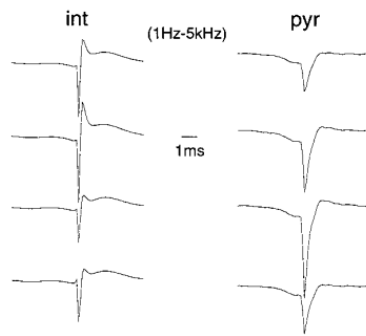


Figure 6: The difference in spike duration and shape between interneurons (int) and pyramidal cells (pyr). Pyramidal cells have significantly broader action potentials. (Csicsvari et al., 1998).

2.3 Hippocampal role in spatial navigation

The first demonstrable evidence for the role of the hippocampus in spatial navigation came from lesion and inactivation experiments done in animals performing discrimination tasks demanding spatial cognition. The results clearly showed the importance of the hippocampus in behavioral tasks based on map-like navigation, as animals with lesioned or inactivated hippocampi performed poorly in these tasks, resorted to alternative strategies, or were not able to solve them at all (Rabe & Haddad, 1969; Means & Douglas, 1970; Olton, 1972; O'Keefe et al., 1975; Olton et al., 1978).

In the 1980s, Richard G.M. Morris invented a novel task to test place navigation. In this task, an animal swims in a pool filled with opaque water and needs to find a hidden platform in order to escape. Morris tested animals with hippocampal lesions in this task and found that not only spatial discrimination but also navigation to a particular place is impaired in these animals (Morris, 1981; Morris et al., 1982). Many other lesion or inactivation experiments performed in the subsequent years supported Morris's findings (Packard et al., 1989; McDonald & White, 1994; Packard & McGaugh, 1996; Lee et al., 2008; Lee et al., 2019).

Another breakthrough in the research of spatial navigation was made with the discovery of its first neural correlate at the level of individual neurons – the place cells.

2.3.1 Place cells

The place cells are complex-spiking cells named after their primary function. They were first described by O'Keefe and Dostrovsky based on single-unit recordings from the dorsal hippocampus of freely moving rats. When recording the activity of CA1 hippocampal cells, they found individual cells that fired only when a rat was at a particular location in the

environment (O'Keefe & Dostrovsky, 1971; Fig. 7). In a subsequent study, O'Keefe rendered more evidence for a spatially sensitive population of hippocampal cells and described many of their characteristics, such as the conditions under which they fire, their firing rates, and patterns (O'Keefe, 1976).

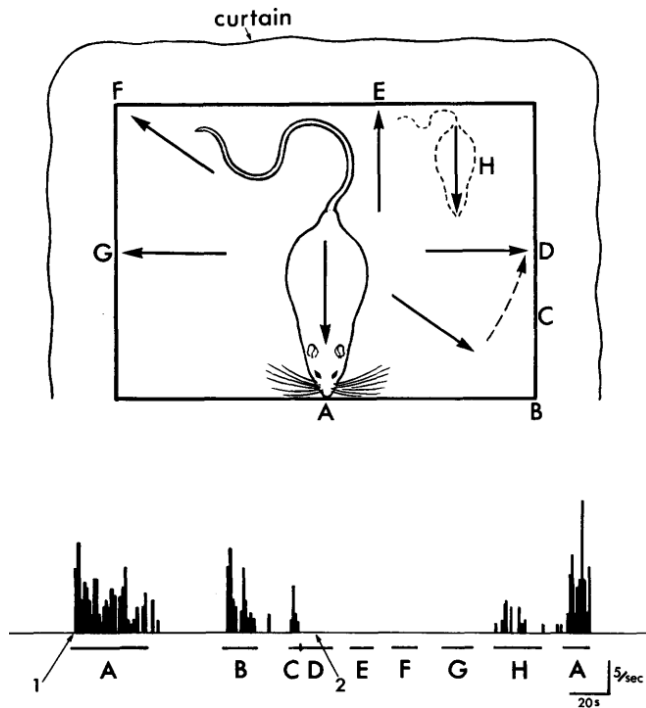


Figure 7: Place cells. The upper picture shows the experimental set-up, letters A-H mark the positions of the animal in the experimental arena. The bottom picture shows the firing of hippocampal CA1 pyramidal cells in given locations. The pictured cell fired the most when the animal was in position A, slightly less when it was in position B, and a little while moving from C to D. (O'Keefe & Dostrovsky, 1971).

During the following years, the goal of scientists investigating spatial navigation was to test whether place cells respond to space and not to some other (sensory, behavioral) aspects. Experiments performed in the dark, in various mazes, or in environments with properties altered by the experimenter (such as removing or switching cues on the walls of the experimental arena) suggested that it is indeed the aspect of space that is encoded by the place cells (O'Keefe & Conway, 1978; Muller and Kubie, 1987; O'Keefe & Speakman, 1987; Quirk et al., 1990).

The portion of the environment in which a place cell fires is called the place field or firing field (O'Keefe, 1976). Different cells have distinct place fields in a single environment, and most place cells are not active across all environments (O'Keefe & Conway, 1978; Thompson & Best, 1989). The space is mapped non-topographically, with neighboring place cells showing no spatial coherence and similarity in their place fields (O'Keefe, 1976; Wilson & McNaughton, 1993). Depending on the size of the environment, a single place cell has either

one or multiple firing fields (O'Keefe, 1976). The sizes of place fields vary along the longitudinal axis of the hippocampus in such a manner that they are the smallest in the dorsal hippocampus and get larger the more ventrally they are recorded (Kjelstrup et al., 2008). Place cells also seem to be far more abundant in the dorsal than in the ventral part of the hippocampus (Jung et al., 1994). These findings deem the dorsal CA1 to be the most spatially sensitive portion of the hippocampus (Jung et al., 1994, Kjelstrup et al., 2008). In certain environments, place cells show a clear sense of directionality. This directionality is observed when an animal is only allowed to move on restricted paths, such as on linear tracks. When running through a linear part of a maze (e.g., one of its arms), the individual place cells of an animal fire in only one direction and not in the other (McNaughton et al., 1983). In contrast, in open spaces such as experimental arenas where an animal can move freely, the firing of place cells is not restricted to movement in any particular direction (Muller et al., 1994).

An important aspect of the function of place cells is a process called remapping. Remapping was for the first time described by Muller and Kubie in the 1980s after changing the properties of experimental environments. It is a process in which a place cell changes its firing between different environments or after the manipulation of a familiar environment (Muller & Kubie, 1987; Fig. 8). Remapping is seen after switching or rotating visual cues, placing objects in the cell's place field, altering the shape and size of the arena, or even changing the color of the arena (Muller & Kubie, 1987; Sharp et al., 1990; Bostock et al., 1991; Colgin et al., 2010). Remapping can have a global character with all recorded cells changing their place fields after the alteration of an environment, but the change can also be partial, with some cells maintaining their firing fields between the changed environments (Knierim & McNaughton, 2001; Moita et al., 2004). Remapping can be seen in both changing the place cells' firing rates and firing locations (Leutgeb et al., 2005; Colgin et al., 2010).

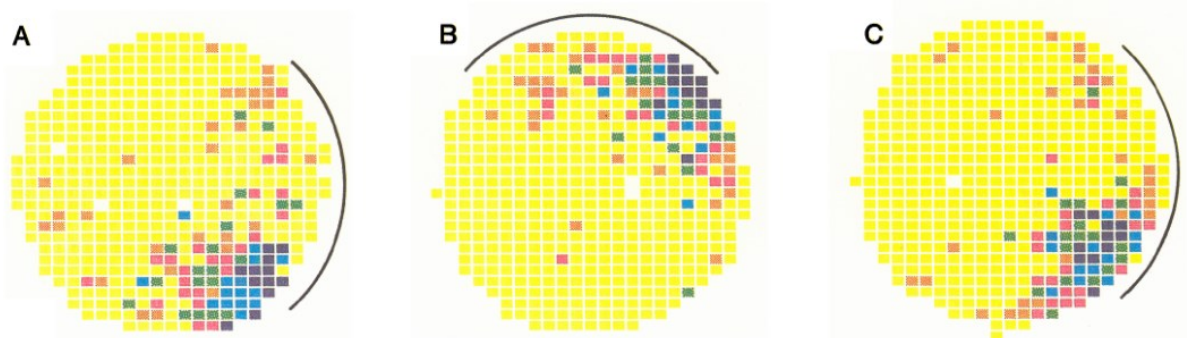


Figure 8: Remapping. Effect of rotating the walls of the arena with a cue card. Picture A shows the firing of a place cell in the original position, picture B shows firing of the same cell after rotating the walls with the cue card

90° counterclockwise, and picture C after rotating the walls back to the original position. Firing rates are depicted as color maps, purple showing the highest and yellow the lowest firing rate. (Muller & Kubie, 1987).

Another important feature of place cell firing that needs to be mentioned is its relationship with the theta rhythm. Place cells recorded as an animal runs through their place fields have been found to fire rhythmically at a slightly faster frequency than that of theta when the animal approaches the center of the place field and at an increasingly slower frequency as the animal leaves the center of the place field. Consequently, individual cells always spike earlier during the successive theta cycles (Fig. 9). This relationship is known as theta phase precession and its implication in spatial navigation is that the spikes at the beginning of the place field an animal runs through occur later in the theta cycle than the spikes at the end of the place field. Theta phase precession is therefore important for self-localization of the animal and for the prediction of future movement, which it determines from the phase of the theta and firing of the respective cells (O'Keefe & Recce, 1993; Skaggs et al., 1996; Huxter et al., 2008; Jeewajee et al., 2014). Theta precession is also believed to play an important role in learning and memory. It has been proposed that by locking the theta rhythm and firing of pyramidal cells together, theta phase precession provides a system for the encoding of novel events as temporal sequences (Yamaguchi, 2003; Feng et al., 2015), including the encoding of the cognitive map (Wagatsuma & Yamaguchi, 2004).

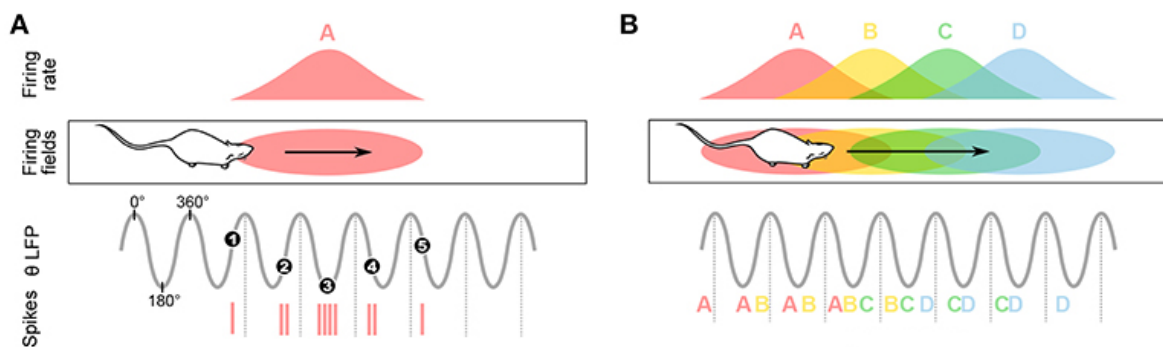


Figure 9: Theta phase precession. A: The top picture shows the place field of a specific place cell. The bottom picture shows the spikes of the place cell as the rat runs through its place field relative to the theta cycle. B: Theta phase precession shown for multiple place cells with overlapping place fields. (Adapted from Drieu & Zugaro, 2019).

The main properties of place cells were discovered in rodents freely running in arenas or mazes and they were linked to the encoding of animals' own positions in space. However, later experiments showed that place cells can encode not only one's own location but also locations of other objects (Komorowski et al., 2009; Manns & Eichenbaum, 2009; Deshmukh & Knierim,

2013; Vandrey et al., 2021) or individuals (Danjo et al., 2018; Omer et al., 2018). The object-sensitive cells were found to encode the animal's position jointly with the position of certain objects in space (Deshmukh & Knierim, 2013; Vandrey et al., 2021). Cells encoding the positions of other individuals of the same species have been found in the hippocampus of rats (Danjo et al., 2018) and bats (Omer et al., 2018) and called the social place-cells. Some of these cells encode only the position of the observed individual while other also simultaneously encode the position of the observer.

Recent findings also propose the existence of a subpopulation of place cells responding to goal locations. These goal-sensitive place cells were first found in bats (Sarel et al., 2017), and subsequently in mice where they were called the reward cells (Gauthier & Tank, 2018).

2.3.2 Head-direction cells

Head direction (HD) cells were first discovered by James Ranck in 1984. In his original study, he recorded HD cells in the dorsal presubiculum (Ranck, 1985), but later they were also found in many other brain regions, including further parts of the hippocampal formation – the parasubiculum (Taube, 1995; Boccara et al., 2010) and the medial entorhinal cortex (MEC) (Sargolini et al., 2006). Head direction cells fire when an animal is facing a specific direction. The firing of these cells can be pictured as a triangular tuning curve that shows the maximum firing rate of each cell in a single direction, with the firing ceasing on each side of this direction. The tuning curve has a range of around 90° for most cells, although it can be as low as 60° and as high as 150° (Taube et al., 1990a; Taube, 2007; Fig. 10).

HD cells also undergo remapping following the rotation of environmental cues or other changes in the environment, but unlike place cells that remap individually, HD cells rotate their tuning curves together in synchrony, keeping their mutual relationship intact (Taube et al., 1990b; Taube & Burton, 1995; Yoganarasimha et al., 2006).

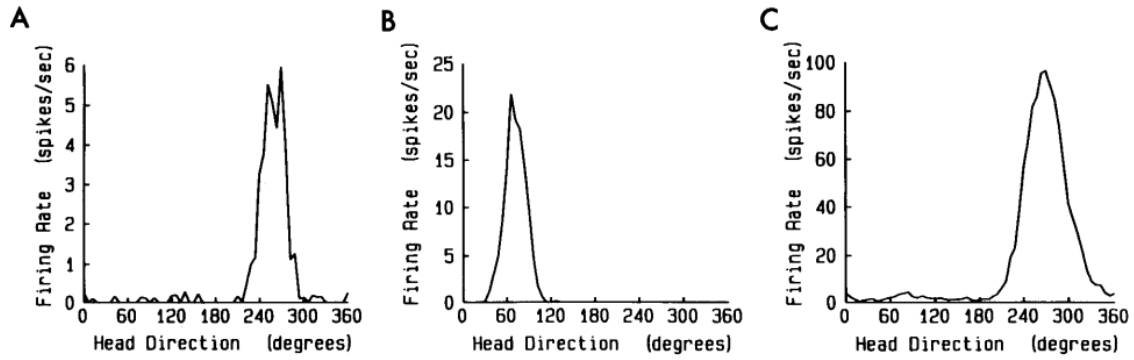


Figure 10: Head direction cells. Examples of the tuning curves of three head-direction cells, differing in preferred head direction and firing rate. (Taube et al., 1990).

2.3.3 Grid cells

Grid cells represent a specific population of cells found in the superficial layers of the MEC (Fyhn et al., 2004; Hafting et al., 2005), as well as in the presubiculum and parasubiculum (Boccaro et al., 2010). Each grid cell has multiple firing fields distributed across the environment. These firing fields cover the entire surface of the environment, creating a triangular or hexagonal grid-like pattern (Fyhn et al., 2004; Hafting et al., 2005; Fig. 11). Grid cells were suggested to represent the brain's metric system, showing different scales of space, and to supply the place cells with information about distance traveled during motion (McNaughton et al., 2006; Solstad et al., 2006). They are also important for the integration of self-motion and vector-based navigation (Fiete et al., 2008; Bush et al., 2015; Banino et al., 2018).

Grid cells, same as HD cells, but unlike place cells, don't change their mutual relationship when remapping in different environments and instead, they show a high coherence among their population (Fyhn et al., 2007; Yoon et al., 2013), which is preserved even during sleep (Gardner et al., 2019; Trettel et al., 2019).

In another aspect, however, the grid cells resemble place cells. Firing fields of grid cells recorded from the dorsal MEC are small in scale, with the scale getting progressively bigger towards the ventral MEC (Hafting et al., 2005; Sargolini et al., 2006). Grid cells along the dorsal-ventral axis of the MEC are organized into several modules varying in scale and grid orientation (Stensola et al., 2012; Khona et al., 2021). Some cell populations in the deeper layers of the MEC have been found to have the characteristics of both HD and grid cells and are called

the conjunctive cells (Sargolini et al., 2006). Moreover, evidence for the modulation of non-conjunctive grid cells by head direction was also reported (Gerlei et al., 2020).

Despite a previous study suggesting that grid cells require hippocampal input for their periodical firing (Bonnevie et al., 2013), a more recent study found that grid cells preserve their coherence even during hippocampal inactivation, and therefore brought evidence for the grid cells' intrinsic generation in the MEC (Almog et al., 2019).

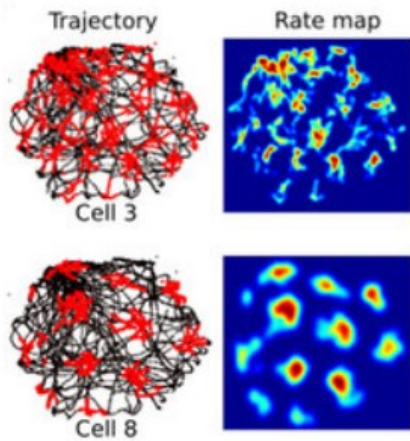


Figure 11: Grid cells. An example of the firing of two grid cells. The left pictures show the trajectory of the rat in black with superimposed firing of the grid cells in red. The pictures on the right show the firing rate map for these cells, with red depicting the highest and blue the lowest firing rate. (Jauffret et al., 2015).

Another recently reported finding is the grid cells' role in the encoding of a goal location. This encoding manifests in restructuring of the entorhinal grid map in the proximity of a goal (Boccaro et al., 2019; Butler et al., 2019). Grid cells were also found in the human neocortex where they are hypothesized to encode the locations of various sensory stimuli and their features and therefore, to play a broader role in learning complex models of objects in space (Constantinescu et al., 2016; Julian et al., 2018; Hawkins et al., 2019).

2.3.4 Border cells

Border cells represent yet another spatially sensitive cell type found in the MEC and the parasubiculum (Savelli et al., 2008; Solstad et al., 2008), but populations of border-sensitive cells have also been found in the hippocampus (Rivard et al., 2004) and the subiculum (Lever et al., 2009). They fire whenever an animal moves along the borders of an environment, such as walls or edges of an elevated platform. Border cells, like HD and grid cells, remap with preserved internal coherence. Some border cells fire while an animal is running around a border in both directions while others only fire in one direction (Solstad et al., 2008).

2.3.5 Object-vector cells

Finally, the most recently discovered type of cells in the hippocampal formation related to spatial representation, are the object-vector cells. They were found in the MEC and respond to the positions of other objects in relation to the animal's own position. The firing fields of these cells are determined by the distance and direction from an object but not by the object's own location or other characteristics (Hoydal et al., 2019). Object-vector cells are different from the object-responsive cells in CA1 (Manns & Eichenbaum, 2009; Deshmukh & Knierim, 2013; Vandrey et al., 2021) in the sense that their vectorial coding seems to be more general, encoding for a broad variety of objects in the environment (Hoydal et al., 2019). They also differ from object-sensitive cells found in the lateral entorhinal cortex (LEC) which only fire when the animal approaches the objects (Deshmukh & Knierim, 2011; Tsao et al., 2013).

3 Behavioral tasks studying spatial cognition

To study spatial cognition in animals (predominantly rodents), many different behavioral tasks have been developed throughout the years. These tasks were designed with the goal to test a specific component of an animal's behavior, the most often tested cognitive abilities being navigation to a goal, the recognition of a certain place or of an object's position, assessing spatial working and long-term memory. The most widely used types of tasks in the research on spatial cognition are so-called mazes.

A popular task used for spatial memory experiments is the T-maze, a three-arm apparatus resembling the letter T (Fig. 12-A). The T-maze is used for the testing of the ability of spontaneous alternation, which is associated with intact spatial memory, and for experiments using forced alternation in the maze, that test reference and working memory (Wenk, 2001; Deacon & Rawlins, 2006; McHugh et al., 2008; Shoji et al., 2012; Davis et al., 2017; Czajkowski et al., 2020).

To investigate working, reference, or long-term spatial memory in laboratory rodents, a more complex task called the radial maze was invented. This maze consists of a central circular area and eight or more arms leading from the center in all directions (Fig. 12-B). For working memory testing, a food reward is placed in all arms of the maze and the animal is supposed to visit all of them without repeating visits to the same arms. For reference memory testing, reward is always placed in the same arm (or a small number of arms) and the experiment consists of

several trials. For long-term memory research, the trials are repeated after a longer delay period. Many different adaptations of this task have been invented over time (Lichtman, 2000; Bird et al., 2003; Egashira et al., 2004; Ranade et al., 2008; Zlomuzica et al., 2009; Schlesiger et al., 2013; Guitart & Roberts, 2015).

In order to test navigation to a certain location and the ability of spatial learning, the previously mentioned Morris water maze (MWM) is often used. In this task, the experimental animal swims in a pool filled with opaque water and searches for a non-visible platform using distal cues around the pool (Fig. 12-C). Spatial learning is assessed over multiple sessions. The main advantage of the MWM is that, in contrast with the previously mentioned “dry” mazes, animals can’t use intramaze odor cues in it. The task has multiple modifications, such as changing the position of the platform to study reversal learning (Morris, 1984; Morford et al., 2002; Vorhees & Williams, 2006; Dong et al., 2013; Barnhart et al., 2015; Portero-Tresserra et al., 2018; Barry & Commins, 2019; Thonnard et al., 2021).

For experiments focusing on the ability to recognize the position of a certain object, a behavioral task called the object-position recognition task was developed. The original version of this task consists of a rectangular arena with a set of objects placed in various locations. The design of the test is based on the notion that rodents spontaneously spend more time exploring a novel object or an object that has changed its position since the previous session compared to the objects that remained unchanged or at the familiar position (Dix & Aggleton, 1999; Sutcliffe et al., 2007; Barbosa et al., 2013; Goh & Manahan-Vaughan, 2013; van Hagen et al., 2015).

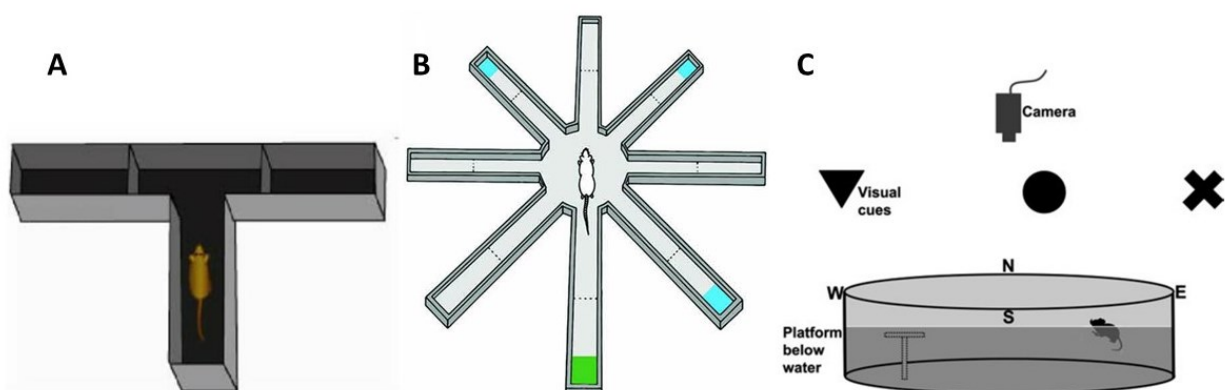


Figure 12: Behavioral apparatuses for spatial cognition tasks. A: T-maze testing spontaneous alternation. (Leising & Blaisdell, 2009). B: Radial maze consisting of eight arms. Green and blue color signal reward in respective arms. (Baker et al., 2015). C: MWM. Animal swims in opaque water and searches for the hidden platform. It can use visual cues around the pool for navigation. (Sherrard, 2011).

With the development of new technologies, many behavioral tasks using computer screens for stimuli presentation and virtual reality simulations were introduced. Noteworthy are computer-based variations of the object-position recognition task that use screens to present visual objects at different locations on the screen, and other screen-utilizing tasks testing spatial working memory and cognitive flexibility (Talpos et al., 2010; Levčik et al., 2013; Hvoslef-Eide et al., 2015; Kim et al., 2015; Kumar et al., 2015; Oomen et al., 2015; Buscher et al., 2017; Kim et al., 2018; Piantadosi et al., 2019). Some advantages of these computer-based tasks are the significantly easier control over the presented stimuli compared to experiments in real environments, and their adaptable design.

Numerous opportunities lie in the use of virtual reality and the simulation of real-world situations. Virtual reality is becoming more and more popular in spatial-navigation experiments with rodents (Pinto et al., 2018). It is often used in combination with head fixation of the tested rodent placed on a polystyrene ball that forms a rotating apparatus allowing the animal to run while being presented with an illusion of a real environment (Dombeck et al., 2010; Schmidt-Hieber & Häusser, 2013; Aronov & Tank, 2014; Chen et al., 2018; Casali et al., 2019; Fig. 13).

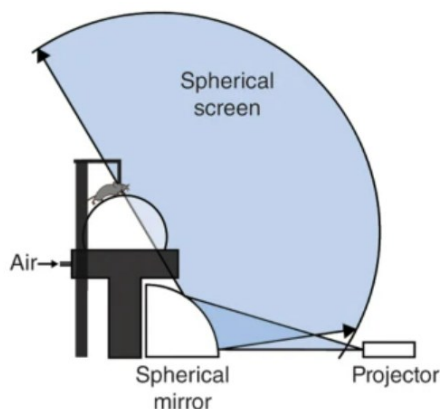


Figure 13: Apparatus for virtual reality. A head-fixed animal is running on a polystyrene ball, while being presented a virtual reality task on a spherical screen (Schmidt-Hieber & Häusser, 2013).

The above-mentioned tasks are often used with some sort of recording or imaging techniques – including single-unit extracellular (Aronov & Tank, 2014; Gianelli et al., 2018), or patch-clamp (intracellular) (Schmidt-Hieber & Häusser, 2013) electrophysiological recordings and in vivo two-photon microscopy (Dombeck et al., 2010; Go et al., 2021). They are also being combined with neuronal manipulation techniques, such as optogenetics (Maisson et al., 2018; Mouchati et al., 2020; Robinson et al., 2020), or chemogenetics (Zhao et al., 2016; Vantomme et al., 2020; Schwabe et al., 2021).

4 Perception of dynamic environments

4.1 Difference between the perception of static and dynamic situations

Spatial navigation in various environments has been studied for decades. For a long time, the main focus has been on studying the animals' behavior in stable – and rather empty – experimental arenas. Unlike experimental settings, however, the real world is highly dynamic and constantly changing. How animals perceive dynamic situations became more widely studied with the blossom of technology and the emergence of virtual environment tasks that made this pursuit easier.

Many different aspects of cognition and behavior can be studied in animals presented with dynamic environments. Special behavioral tasks have been designed to study visual perception of motion (Stirman et al., 2016; Marques et al., 2018), discrimination between self-motion and the motion of objects (Sasaki et al., 2017; Nagy et al., 2018), fear and anxiety (Choi & Kim, 2010), defensive behavior strategies (Vale et al., 2017), and finally, also spatial navigation and the perception of space (Ho et al., 2008; Telensky et al., 2011, Danjo et al., 2018; Omer et al., 2018). To this day, the most frequently used tools in these dynamic tasks are computer screens displaying moving objects, or robots and other artificial moving objects placed directly in the mazes or arenas in which animals forage. Figure 14 shows examples of the experimental settings of some of these tasks.

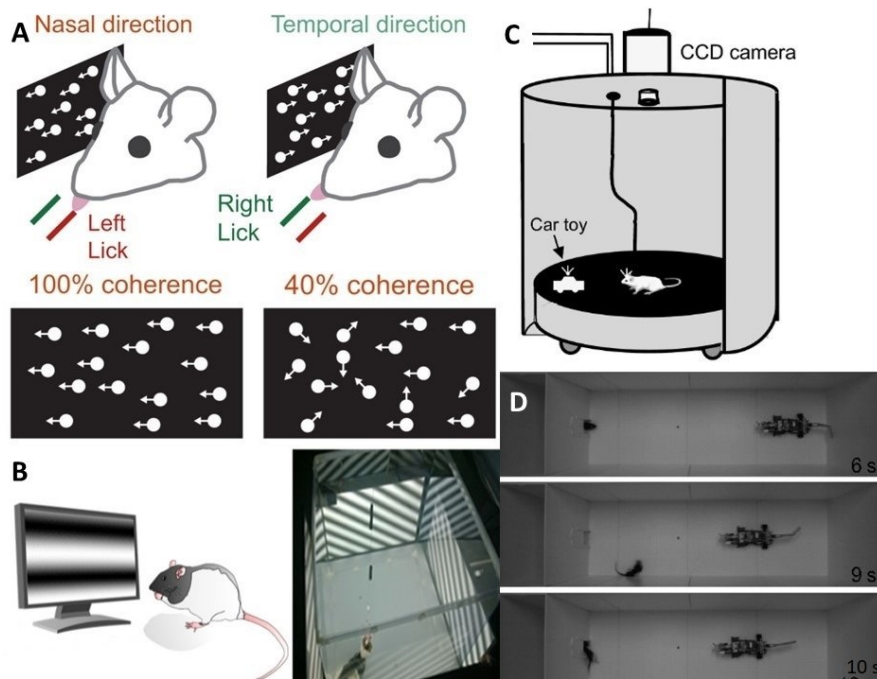


Figure 14: Examples of behavioral tasks utilizing dynamic environments. A: A task using computer screens for the presentation of random dot kinematograms (RDKs) to study motion perception in mice. Head-fixed mice were trained to discriminate the direction of global movement in RDKs with different coherences of movement. (Adapted from Marques et al., 2018). B: A behavioral task designed for studying the differences in the encoding of self-motion and self-independent motion of objects. Rats were first shown a moving pattern on a computer screen, and subsequently walked through a box with the same moving pattern on the walls. This experiment was combined with electrophysiological recordings from the striatum. (Adapted from Nagy et al., 2018). C: A spatial navigation task investigating the encoding of movement of other objects. In this task, the activity of hippocampal place cells of freely moving rats was recorded as they foraged through an environment with a moving toy car. The rats were trained to either chase or move independently of the car. (Adapted from Ho et al., 2008). D: A study investigating the role of amygdala in fear responses of rats foraging in an arena with a moving “predator-like” robot. (Adapted from Choi & Kim, 2010).

Dynamic scenes, by their nature, contain two aspects – space and time. While the encoding of space in the brain has been extensively studied for the last 50 years, up until the beginning of the 21st century, the encoding of time got very little academic attention.

4.2 Time cells

In the search for the neural substrate of time encoding, cells firing at successive moments during episodic experiences were found in the hippocampus (Pastalkova et al., 2008) and subsequently named the time cells (MacDonald et al., 2011). The hippocampus was targeted as a possible center of time encoding because of its importance in temporal organization of

memories in both animals (Fortin et al., 2002; Ergorul & Eichenbaum, 2006) and humans (Ekstrom & Bookheimer, 2007; Mayes et al., 2010).

The first study to discover hippocampal cells firing in sequence during specific times of the experiment was published by Pastalkova and colleagues in 2008. The discovery was made by recording from single CA1 hippocampal cells of rats that performed hippocampus-dependent spatial-alternation task. The left and right alternation trials were separated by a wheel running of a stable period. The authors found that, during the delay between trials, hippocampal cells fired at successive moments during the whole period of wheel running. Furthermore, the firing of these neurons differed depending on whether the animal turned left or right on the successive trial (Pastalkova et al., 2008).

The existence of cells firing successively during a delay between individual trials of a task was found in several other studies, both in studies utilizing mazes (Gill et al., 2011; Shimbo et al., 2021) and in studies using non-spatial tasks (MacDonald et al., 2011; Shahbaba et al., 2022). In one of these studies, the neurons were named time cells, inspired by place cells, and several of their properties were also named in accordance with the place cell terminology. The durations in which time cells fire were called their time fields and the change in the firing of these cells, found to happen after the extension of the inter-trial delay, was called the re-timing of these neurons (MacDonald et al., 2011).

Time cells were found to encode time over multiple timescales, ranging from the course of a few seconds to several days (Mau et al., 2018). The firing of time cells has also been observed in head-fixed animals, suggesting that translational movement is not important for the generation of time cell sequences (MacDonald et al., 2013). In another study, time-cells were recorded from rats running on a treadmill at varying speeds. The results indicated that time cells encode both the time and distance run, either conjointly or separately (Kraus et al., 2013). Time cells have also been observed in humans (Umbach et al., 2020; Reddy et al., 2021) and non-human primates (Naya & Suzuki, 2011).

Time cells and place cells can constitute the same population of hippocampal neurons. Although not all, many time cells have been found to also have place fields (MacDonald et al., 2011; Kraus et al., 2013; Salz et al., 2016). Moreover, many similarities in the properties of the firing of place cells and time cells were found, such as the fact that they remap and retime, respectively, after a context change (MacDonald et al., 2011), or the relation of their firing to the theta rhythm (Shimbo et al., 2021). The major difference in their activity is the size of their

fields. The sizes of place fields are equivalent across areas in the environment, whereas the sizes of time fields increase with the time of the delay (Kraus et al., 2013; Salz et al., 2016; Fig. 15). The notion that place cells and time cells are the same neurons points to a possibility that time and space are represented by similar neural mechanisms (Eichenbaum, 2014; Shimbo et al., 2021).

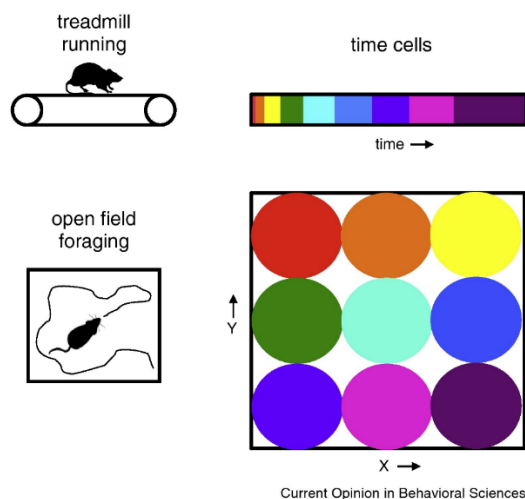


Figure 15: The similarities and differences between time cells and place cells. Time cells fire in distinct sequences during wheel running, with longer time fields later in time. Place cells, similarly, fire at distinct locations during random foraging in an environment, but their place fields are of a similar size in all positions. (Eichenbaum, 2017).

Time-encoding neurons have been found not only in the CA1 hippocampal area, but also in the CA3 (Salz et al., 2016), MEC (Heys & Dombeck, 2018), and LEC (Tsao et al., 2018). In the MEC specifically, grid cells were observed to signal time and distance traveled during wheel running (Kraus et al., 2015). One study found the inactivation of the MEC to disrupt time cell sequences in the hippocampus (Robinson et al., 2017). A subsequent study, however, contradicted these results and suggested that although MEC lesion disrupts hippocampus-dependent working memory, the firing of time cells in MEC-lesioned animals is left intact (Sabariego et al., 2019). A recent article indicated that time processing is dependent on the MEC only for delays extending 10 seconds (Vo et al., 2021).

The research on time cells has been growing dynamically in recent years but there are still many questions surrounding this topic and much knowledge to be uncovered. While many above-mentioned studies present convincing evidence, the existence of time cells is still merely a hypothesis.

4.3 Time compaction

Alike the hypothesis about the existence of time cells, the hypothesis of time compaction is based on the premise that both time and space are encoded in the hippocampus and, possibly, by similar mechanisms. The hypothesis was introduced to answer the question of how highly complex dynamic situations are represented in the brain in such a way that humans and animals can quickly and effectively respond to a sudden change in the environment. The hypothesis proposes that the brain does not encode time in itself, but rather embeds it into space, and therefore, represents static and dynamic situations by the same neural mechanisms (Villacorta-Atienza et al., 2010; Villacorta-Atienza et al., 2021). According to the hypothesis, a dynamic situation is neurally represented as a compact internal representation (CIR). This CIR works as a static map that encodes all possible interactions between elements in a given environment, and so eliminates time from the dynamic situation while containing all the necessary information for navigation (Fig. 16). It was proposed that the CIRs can also be stored as memories of dynamic situations and used for future navigation under similar circumstances (Villacorta-Atienza & Makarov, 2013).

Experimental results supporting the existence of time compaction have recently been found in humans performing a behavioral task based on stimulus generalization in a collision perception task (Villacorta-Atienza et al., 2021). The formation of CIRs has also been supported by neural network models and experiments with robots (Villacorta-Atienza et al., 2010; Villacorta-Atienza & Makarov, 2013). Experimental work searching for this process in animals has, however, not been done yet.

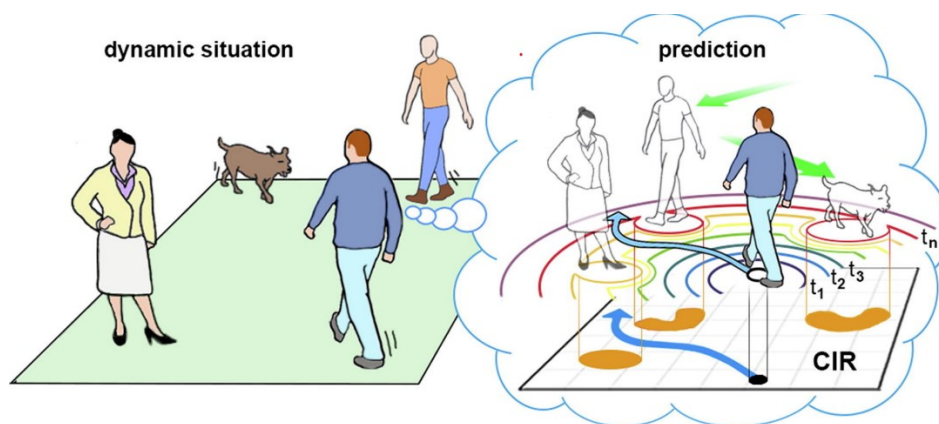


Figure 16: Illustration of the principle of time compaction. Left: A dynamic situation. Right: A CIR of the same dynamic situation. All subjects in the situation are represented as their predicted future positions (colored curves for respective times, orange shapes show the places of possible future collisions with the observer). The

CIR includes trajectories for the navigation between other moving (or static) subjects in the environment (blue arrow, only one possible trajectory is shown). (Villacorta-Atienza et al., 2021).

PRACTICAL PART

5 Aims of work

The hippocampus is known to have a crucial function in spatial cognition. Place cells – first discovered over 50 years ago (O’Keefe & Dostrovsky, 1971) and heavily researched since then – are an important population of cells in the hippocampus that encode the subject’s own position in space (O’Keefe, 1976). The role of place cells has since also been linked to the encoding of the positions of static objects (Manns & Eichenbaum, 2009; Deshmukh & Knierim, 2013; Vandrey et al., 2021), and even of moving objects (Ho et al., 2008; Omer et al., 2018), including other animals (Danjo et al., 2018; Omer et al., 2018). In addition, an inactivation study showed the importance of the hippocampus in the perception of a moving object’s position (Telensky et al., 2011).

All these studies, however, were done in an accessible space, with animals directly interacting with the experimental objects, or at least in a highly familiar and previously intensively explored space as was the case in the experiments leading to the discovery of social place cells (Danjo et al., 2018; Omer et al., 2018).

The neural representation of inaccessible situations, and especially inaccessible dynamic situations, has lacked research. Does the hippocampus also encode for these situations? And if it is so, then how? The goal of this thesis is to help answer these questions using behavioral experiments in combination with single-unit electrophysiological recording in freely moving rats.

The objectives of the research underlying this thesis were:

- 1) To compare the learning of inaccessible static and complementary dynamic visual spatial stimuli in rats.
- 2) To assess whether the acquired knowledge of visual spatial scenes is transferable into novel dynamic scenes.
- 3) To determine if the positions of inaccessible visual objects are encoded by the neurons of dorsal hippocampal CA1 area, and if the static and complementary dynamic visual scenes are internally represented using mutual mechanisms.

The research was done using an operant behavioral task in which rats had to discriminate static and complementary dynamic spatial visual stimuli by pressing a lever for getting a food reward. In the second part of the study, single-unit electrophysiological recording from the CA1 pyramidal layer of the rat hippocampus was performed while the rats solved the behavioral task.

6 Methods

6.1 Animals

The experimental subjects were eight outbred male Long-Evans rats three months old at the beginning of the experiment. The rats were obtained from the Velaz breeding colony in Germany and were housed in pairs, and individually after surgery. The animal room was kept at a constant temperature of 21 °C and a 12-hour light/dark cycle. All handling and experiments were performed during the light phase. Rats had free access to water but lived on food restriction in order to be maintained at 90% of their free-feeding weight. After transportation from the breeding colony, they were let to acclimatize for 10 days, and then properly handled every day for 1 week before the beginning of the experiment. Animal welfare complied with current legislation (the Animal Protection Code of the Czech Republic and EU Directive 2010/63/EC).

6.2 Behavioral apparatus

The apparatus consisted of an operant chamber with a lever and a feeder, a 24'' LCD monitor placed 37 cm in front of the chamber, and a computer (Fig. 17). The computer displayed the rewarded and non-rewarded stimuli on the screen, registered lever presses, activated the feeder, and synchronized events and stimulus presentations with the neural data recording. In the case of a rewarded lever press, a 45 mg chocolate-flavored pellet (Bio-Serv, USA) was delivered from the feeder to the operant chamber. The stimuli were displayed by a software written in C++.

During the training sessions, rats were trained in two identical apparatuses. Each rat was always trained in the same apparatus. For the recording sessions, the animals were placed in a third operant chamber, identical to the ones used for training. Above the operant chamber used for recording sessions was installed a camera to track the position of the animal and a tethered headstage connecting the implants on the animals' heads to the CED acquisition system.

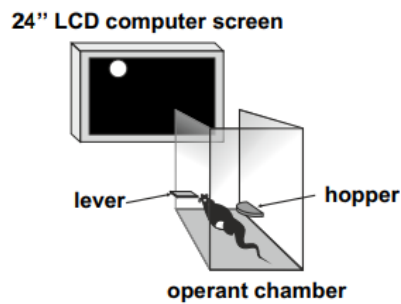


Figure 17: Behavioral apparatus. The animal was placed in an operant chamber with a lever and a feeder (hopper), and static and dynamic spatial scenes were displayed on the computer screen in front of the chamber. (Adapted from Klement et al., 2010).

6.3 Experimental design

The food-deprived rats were trained to press the lever during the presentation of the rewarded stimuli to obtain a food pellet. Before the first day of the training, they were left to familiarize with the operant chamber, pre-trained to press the lever for a reward, and then trained once a day, five days a week until they reached the performance criteria in the final configuration of the task.

There were two types of displayed stimuli in the task: static and dynamic. The static stimulus was a white circle in one of the four positions (corners) forming a rectangle on a black screen (Fig. 18-A). The complementary dynamic stimulus consisted of two such circles moving in the direction of the same corner implying future collision at that position, but with both circles disappearing in the middle of their trajectory. The starting positions of the circles were the corner above/below the circle in the complementary static stimulus and the center of the screen (Fig. 18-A). Out of the four total stimuli (two static and two dynamic), one of each type was rewarded, and one was non-rewarded. Although the rewarded and non-rewarded stimuli differed between rats, the combination of the two rewarded and non-rewarded stimuli, respectively, was always the same, with the imagined collision during the rewarded dynamic stimulus taking place in the same corner, in which the single white circle was located during the static rewarded stimulus for the respective rat (Fig. 18-A). The rewarded and non-rewarded stimuli were counterbalanced and for each rat were assigned randomly before the beginning of the experiment.

The behavioral training consisted of five consecutive configurations with increasing difficulty. One experimental session lasted approximately 30 minutes (see Table 1) and consisted of the presentation of the stimuli in fixed but pseudorandom order, each stimulus was

separated by a black screen displayed for 3 or 5 seconds. The individual stimuli were displayed for 90 seconds in the first (easiest) configuration, with the time of presentation decreasing in the consecutive configurations until it was only 15 seconds in the last, most advanced configuration. The number of correct presses needed for the reward to be delivered also differed between the configurations, with three presses needed on average, but never more than 5 presses during the final configuration (Table 1 shows the specifics for the individual configurations). The duration of a single dynamic scene was 1 second, so the scene was presented 15 times in a row in the last configuration. The laterally positioned circles were moving with the speed of 460 pixels/s and the circles starting in the center of the screen with the speed of 325.3 pixels/s. The sequence of the stimuli was pseudorandom in order to prevent the rats from using alternative strategies to solve the task. Simultaneously, the same stimulus was never displayed twice in a row, and neither were two rewarded stimuli. The non-rewarded stimuli were presented twice as often as the rewarded ones; therefore, the chance level performance was 33.3%.

The criterion for reaching the next configuration was a stable performance of 60% or more correct presses for at least four out of five previous sessions. During the last, most challenging configuration, this criterion had to be reached to allow for carrying out the generalization session and the subsequent implantation of rats with the microdrive for electrophysiological recordings.

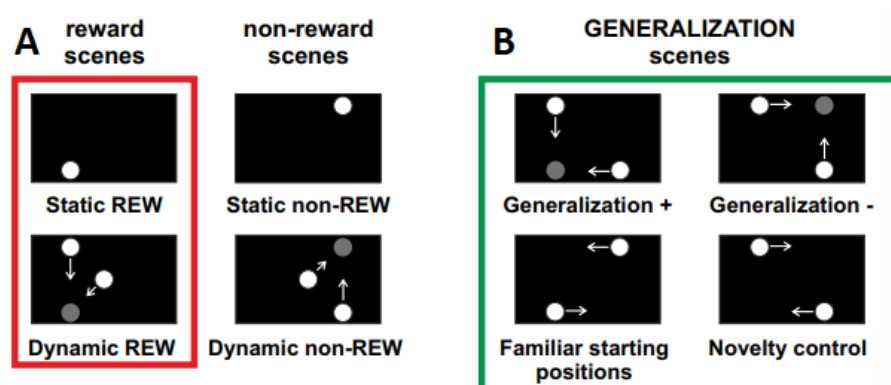


Figure 18: Experimental design of the behavioral task. A: Design of the behavioral task consisted of two rewarded stimuli – one static and one dynamic (Static REW + Dynamic REW), and two non-rewarded stimuli (also one static, one dynamic – Static non-REW + Dynamic non-REW). In the dynamic scenes, the two circles were moving towards the same location (equal to the position of the circle in the static scene) but disappeared before reaching it. B: Design of the generalization session. The four novel stimuli were displayed along with the four stimuli from the previous training sessions. Out of the four novel stimuli, one had the same location of the imagined collision as the Dynamic REW (Generalization +), one as the Dynamic non-REW (Generalization -),

and two were control stimuli. One of the controls shared the starting positions of the circles with the locations of the stimuli in Static REW and Static non-REW. The starting positions of the circles for the novelty control stimulus were on the opposite left/right side of the screen.

Protocol	Time/stimulus	Reward	Duration	Pauses	S REW	S non-REW	S GEN
1 st	90 s	CR	28 min 30 s	5 s	3	6	---
2 nd	45 s	FR2	30 min	5 s	6	12	---
3 rd	30 s	VR3	30 min	3 s	9	18	---
4 th	20 s	VR3	34 min 30 s	3 s	15	30	---
5 th	15 s	VR3	32 min 24 s	3 s	18	36	---
Gen	15 + 10 s	VR3*	45 min 24 s	3 + 5 s	18	36	12
Rec	15 s	VR3	45 min 36 s	3 + 5 s	24	48	---

Table 1: The properties of individual protocols for behavioral training. Protocol: 1st – 5th were the training configurations, Gen and Rec the configurations for generalization tests and electrophysiological recordings, respectively. Time/stimulus shows the duration of a single scene in the individual configuration. Reward: CR = continuous reinforcement, the reward was delivered after every correct lever press. FR2 = fixed ratio 2, the reward was delivered after every second correct lever press. VR3 = variable ratio 3, the reward was delivered after 3 presses on average but never after more than 5 correct presses. Duration means the total length of a single session in the respective configuration. Pauses (black screen presentations) lasted 5 seconds in the easier and 3 seconds in the more difficult configurations. For generalization and recording sessions, a combination of 3- and 5-s pauses between individual stimuli was used. S REW = number of displays of a single rewarded stimulus during the session, S non-REW = number of displays of a single non-rewarded stimulus during the session, S GEN = number of displays of each novel stimulus during the generalization session. * - novel stimuli in the generalization session were not rewarded.

6.3.1 Generalization

Once the rats had been trained in the behavioral task, they were tested in a generalization session to see if they were able to transfer the knowledge acquired in the behavioral training into novel dynamic scenes that shared the place of the imagined collision with the original dynamic scenes.

The design of the generalization session consisted of eight stimuli in total – four of them being the original stimuli, and four of them being novel. Out of the novel stimuli, two shared a part of the spatial information with the dynamic stimuli from the original task. The novel stimuli – same as the original dynamic ones – consisted of two white circles moving in the same direction. The starting positions were in the upper right and bottom left (or upper left and bottom

right, depending on the experimental setup for the respective rat) corners of the area on the screen this time. One of these stimuli shared the location of the imagined collision with the original rewarded stimulus, the second one with the original non-rewarded stimulus (Fig. 18-B). The premise was that if the animals solve the task depending on the position of the imagined collision (which was also the position of the familiar static stimulus), they should – by the process of generalization – prefer the novel stimulus resembling the previously rewarded situation over the other novel stimulus.

The two remaining novel stimuli served as control stimuli as well as to find out if familiar starting positions could be generalized. These stimuli were also dynamic with two moving circles, but they lacked the imagined collision. The starting positions of the circles were the upper right and bottom left corner for one stimulus, and upper left and bottom right corner for the other (Fig. 18-B). This way, one of the control stimuli shared the starting positions with the locations of the original static stimuli. Therefore, if the presence of initial static reward position is enough for generalization, this scene should be favored by the rats over the other control stimulus.

The individual novel stimuli were always displayed for 10 seconds. The black screen presentations were the same as in the final version of the original task, and one generalization session lasted 45 minutes 24 seconds in total (Table 1). Novel stimuli were not rewarded during the generalization task. The generalization session was performed two times, both of these sessions were separated by 5 standard training sessions.

6.4 Surgery

Before performing surgery, eight 12 μ m Ni-Chrome tetrodes with insulation were constructed using a lab-made tetrode twister. The tetrodes were then loaded into the VersaDrive-8 microdrive from Neuralynx, USA according to the publicly available manual. One day before the surgery, the recording tetrodes were electroplated with gold plating solution to final impedances of 200-300 kOhm using the NanoZ impedance tester by White Matter LLC, USA. Rats fully trained in the behavioral experiment were subsequently anesthetized with 2% isoflurane and chronically implanted with the array of eight 12 μ m tetrodes held by the microdrive.

The anesthetized rat was given injections of Carprofen (5 mg/kg/ml – 1 ml, i.p.), atropine (0.5 mg/ml – 0.5 ml, s.c.), and Mesocain (10 mg/ml – 0.1 ml, locally in the skin on the head).

Subsequently, the anesthetized animal was mounted into the stereotaxic frame, and the skin on the top of the head was removed to expose the skull. The whole area was well cleaned, six holes for screws were drilled into the skull, and a craniotomy was done above the dorsal hippocampus. The microdrive was implanted above the central part of the dorsal hippocampus at the coordinates: AP -4.0 mm, ML 2.2 mm from the bregma, in agreement with the stereotaxic atlas (Paxinos & Watson, 2007). The tips of the tetrodes were lowered 1.4 mm below the dura mater (the dura was carefully removed before lowering the tetrodes). Four anchoring screws were attached to the skull and served to secure the implant in its position. Two additional anchoring screws served as ground and reference electrodes. The microdrive was attached to and secured on the skull using dental acrylic (the tetrodes were previously covered in Vaseline which served as a protection from fixation in place by the dental acrylic). The area around the implant was treated with Betadine, Septonex (antiseptics), and Framykoin (local antibiotic), and the animal was injected with Carprofen (5 mg/kg/ml – 1 ml, i.p.) to prevent inflammation.

Rats were given antibiotics (Enrogal, 100 mg/ml – 0.5 ml/500 ml drinking water) three days prior and a week after the surgery. After the surgery, anti-inflammatory drugs (Nurofen, 20 mg/ml – 2.5 ml/500 ml drinking water) were also added to the water. The rats were allowed to recover from the surgery for seven days and monitored daily.

6.5 Electrophysiological recording

After recovering from the surgery, rats were retrained in the behavioral task, and the activity of CA1 hippocampal neurons during the solving of the task was recorded using the tetrodes. The tips of the tetrodes were lowered by 30 μ m at a time, and not by more than 120 μ m/day. The goal was to detect the activity of single neurons in the CA1 region of the hippocampus in a maximum number of the 8 tetrodes. The VersaDrive-8 allowed to move each tetrode separately, and therefore, to position each tetrode into the CA1 pyramidal layer.

Data recorded with the tetrodes were amplified (1000 x) with four Lynx-8 amplifiers from Neuralynx, USA. The raw signal was band-pass filtered at 300-9000 Hz and sampled at 31250 Hz with the CED Data Acquisition System (CED, UK). The position of the animal in the operant chamber was monitored using two LEDs on the headstage. The position of the LEDs was observed by the overhead camera operating at 25 frames/s. The rat's position was synchronized with neural data recording. Lever presses, pellet delivery and the onset of visual stimuli was recorded and time-stamped with the CED recording system.

6.6 Histology

24 hours before the perfusion, an electrolytic lesion was done by passing 30 μ A current to all electrodes for 30 seconds to visualize the final positions of the tetrodes.

After the completion of all behavioral experiments and the acquisition of electrophysiological data, the rats were anesthetized with a sublethal dose of a 50:50 mixture of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). They were transcardially perfused with 250-300 ml of phosphate buffer with pH 7.4, and subsequently with 250-300 ml of 4% paraformaldehyde (PFA) solution. The brains were removed and placed in 4% PFA solution for 24 hours, and then in 30% sucrose solution for 72 hours. After the 72 h, the brains were removed from sucrose and frozen. Later they were cut into 40 μ m thin slices and stained with cresyl violet to validate the position of the recording tips of the tetrodes.

6.7 Data analysis

The behavioral data from the learning phase of the experiment and the generalization session were analyzed in order to assess the probability of lever pressing during individual stimuli. The probability was calculated by normalizing the pressing rate for all rats. The real number of presses during each stimulus was divided by the mean pressing rate in the first four sessions. The calculated number was then divided by the duration of the stimulus. This was done to minimize the confounding effects of differences in pressing frequency between rats on the models. The calculated ratio represented the probability of pressing the lever within 100 milliseconds of the display of a given stimulus. Analysis was done by the means of a general linear model with mixed effects (GLMM) with Gaussian distribution. GLMM was used because the individual stimuli varied in the frequency of their presentation and – in the generalization session – also in their duration. GLMM takes all these possible random effects into account. As fixed variables in the models were included – depending on the particular model: the stimulus type, time (either the normalized duration of the whole 1st training protocol or the time from the onset of the stimulus), and the interaction of the stimuli and time. Time to the first lever press after the onset of the stimulus was calculated using a mixed effects cox regression model.

For the analysis of electrophysiological data, a high-pass filter was applied for extracting spikes from the raw recordings and was followed by wavelet artifact suppression for removing noise. The curated signal was transformed by taking its absolute value and averaging it over all channels in a given tetrode. The events that surpassed a given threshold for each tetrode (based

on the variability of signal across channels) were extracted and further curated around a 2-millisecond interval to avoid false positive counts in the refractory period. This process was repeated using first a high-pass filter at 800 Hz for the retention of real spikes and spike-like noise events, and then a high-pass filter at 2000 Hz for the retention of noise events only. Events detected at 2000 Hz were then subtracted from 800 Hz to minimize the effect of technical artifacts. The resulting spikes were up-sampled using interpolation and underwent a dimensionality reduction procedure based on Principal Component Analysis. This procedure was applied for every channel in each tetrode. The three most informative dimensions were then chosen based on their significance in the Hartigan's Dip test and the explained percentage of original variance. Based on the dimensions, spikes were sorted into clusters of cells applying either k-means or Gaussian Mixture Modeling.

For each principal cell, a GLMM was calculated to predict the firing rate of the respective cell (spikes/100-ms bins). Fixed factors in the model were the stimulus type, lever presses, and time from the onset of the scene. The GLMMs were calculated using the following equation:

$$\begin{aligned} \log(\text{fire}_{ij}) = & \beta_0 + \beta_1 \text{time}_{ij} + \beta_2 \text{type}_{ij} + \beta_3 \text{lever}_{ij} + \\ & + \beta_4 \text{time}_{ij} \times \text{type}_{ij} + \beta_5 \text{type}_{ij} \times \text{lever}_{ij} + \beta_6 \text{time}_{ij} \times \text{type}_{ij} + \beta_7 \text{time}_{ij} \times \text{lever}_{ij} + \\ & + \beta_8 \text{time}_{ij} \times \text{type}_{ij} \times \text{lever}_{ij} + u_{0i} + u_{1i} \text{time}_{ij} + e_{ij} \end{aligned}$$

, where fire_{ij} represents the predicted firing rate, each β_h term accounts for the fixed effect of the corresponding factor or interaction, u_{hi} refers to the random effects within each stimulus, and e_{ij} is a random error or residual of each observation.

To eliminate the effect of lever pressing activity and possible reward expectancy on the predicted firing rate, lever presses bins (± 200 ms intervals around the lever presses) and factor were removed from the final model.

Polynomial fixed factors models were also calculated to compare the predicted firing rate (calculated using the chosen fixed factors) with the real firing rate. Data models were plotted as means and 95% confidence intervals. Type II Wald Chi-Squared Tests were used to find out if fixed factors in a model are significant. Pairwise comparisons were calculated by means of Tukey-adjusted p-values, and the significance was considered at the 0.05 level. Spike sorting and data modeling analysis was done in collaboration with the group of J. A. Villacorta-Atienza from Complutense University of Madrid, Spain.

7 Results

7.1 Behavioral experiment – training phase

Out of the 8 rats trained in the behavioral experiment, 7 were able to reach a stable performance of > 60% in the final configuration of the task. It took 46 (± 2.4 SEM) sessions on average for the rats to be fully trained in the task (Table 2 shows the number of sessions it took individual rats to reach stable performance in the respective configuration).

Animal	1 st protocol	2 nd protocol	3 rd protocol	4 th protocol	5 th protocol	Total
Rat 39	17	5	5	10	10	47
Rat 41	22	5	7	5	5	44
Rat 42	11	5	5	14	5	40
Rat 43	31	5	5	6	11	58
Rat 44	30	5	5	5	5	50
Rat 45	22	5	5	5	5	42
Rat 46	21	5	5	5	5	41

Table 2: The progress of learning in the behavioral task. The numbers indicate the counts of sessions needed for each animal to achieve a stable performance in the respective configuration of the task. Total: total number of training sessions for each rat. Rat 40 was excluded from the training after 45 sessions as it was not able to reach a performance of > 60% in the first configuration of the task.

The behavioral data were analyzed, and performance was assessed as the probability of pressing the lever in 100-ms time bins, and as the probability of the first lever press during the presentation of a particular scene. Learning was the most significant during the first configuration of the task when clear learning could be seen for all four presented stimuli. During the next four configurations, learning was slower and mostly selective for a particular stimulus in each rat. These configurations served to solidify the learning process and to increase the cognitive demands of the task by decreasing the time of single scene presentations. Since the learning process was the most significant during the first protocol, only data from the first configuration and from the very last training session (i.e., when the rats had already been fully trained in the task) are presented here.

Type II Wald Chi-Square test was calculated for the learning data from the first configuration, and both the stimuli type ($p < 2e^{-16}$) and the interaction of stimuli and time ($p < 2e^{-16}$) were found to be significant factors in relation to the change of the lever pressing rate.

When comparing the learning of static and dynamic stimuli, we found that rats preferred the dynamic over the static stimuli (Fig. 19). This was concluded as the probability of lever pressing was higher for the dynamic rewarded stimulus than for the static rewarded stimulus among rats ($p < 0.0001$). The probability of lever pressing was also higher for the dynamic non-rewarded stimulus than for the static non-rewarded stimulus ($p < 0.0001$) and although the preference for both the non-rewarded stimuli declined over the course of learning, preference of the dynamic stimulus over the static one was present during the first learning configuration (Fig. 19-A). Individual stimuli were compared with each other by means of Tukey post-hoc tests and the differences between all stimuli were significant, with all p values < 0.0001 .

In the last session of the final configuration (i.e., at the end of the training phase), Type II Wald Chi-Square test again showed that the type of stimulus was a significant factor changing the lever pressing rate ($p < 0.0001$). Tukey post-hoc tests revealed that there were significant differences in the lever pressing rate between the dynamic rewarded stimulus and all other stimuli ($p < 0.0001$), and between the static rewarded stimulus and all other stimuli ($p < 0.0001$). There was no difference between the two non-rewarded stimuli ($p = 0.7733$) (Fig. 19-B).

Visual scene types also had a clear effect on the time of the first lever press during a single scene presentation ($p < 2.2e^{-16}$ in Type II Wald Chi-Square test). Tukey post-hoc tests again showed differences in the first lever press between the dynamic rewarded stimulus and remaining three stimuli ($p < 0.0001$), and between the static rewarded stimulus and remaining three stimuli ($p < 0.0001$). There were no differences between the two non-rewarded stimuli ($p = 0.9992$). The time of the first lever press was shorter for the dynamic rewarded stimulus than for the static rewarded stimulus, and the average first lever press happened much later for both non-rewarded stimuli compared to the rewarded ones (Fig 19-C).

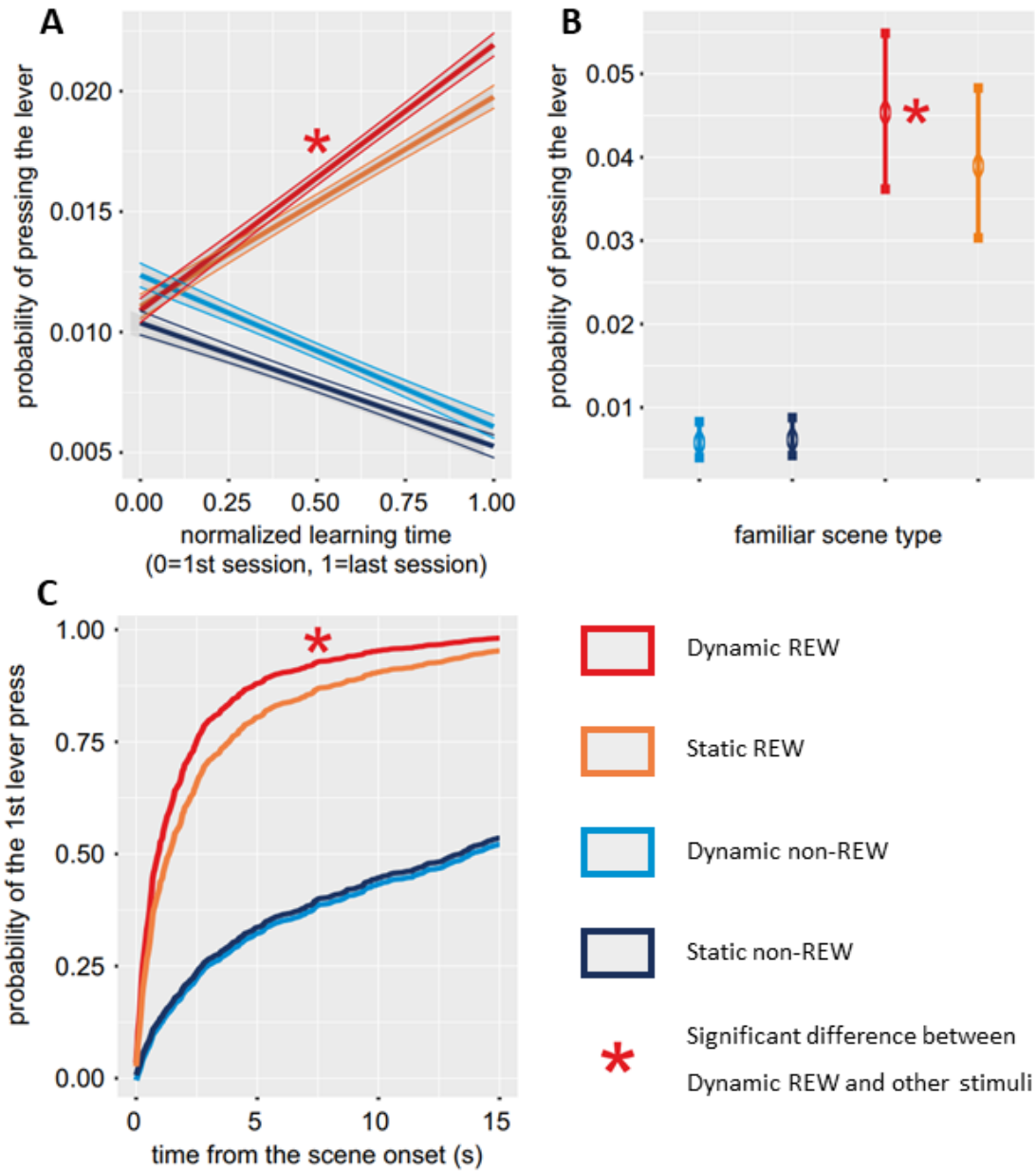


Figure 19: Results from the behavioral training. A: The average learning curves for the individual stimuli in the behavioral training. The X axis shows normalized time from the first session until the last session of the first configuration, the Y axis shows the probability of pressing the lever within 100 milliseconds of the display of a given stimulus. These data show a steadily growing probability of pressing the lever for the rewarded stimuli and a steadily declining probability for the non-rewarded stimuli. There are significant differences between the preferences for all stimuli. Data from the first configuration only were used because the learning was the most significant during this protocol. B: Probability of pressing the lever during the final session in the last configuration (i.e., when the rats had already been fully trained in the task). The probability was significantly higher for the rewarded than the non-rewarded stimuli. There was also a significant preference of the dynamic rewarded over the static rewarded stimulus. C: Probability of the first lever press during a single scene of the last configuration (15 s presentations of individual stimuli). This graph shows that the animals pressed the lever significantly faster in case of appearance of a rewarded stimulus. Red asterix in all graphs marks significant differences between the dynamic

rewarded stimulus and all other scenes. Data are pictured as means and 95% confidence intervals. The results show that the learning was faster and more precise for dynamic than static stimuli.

7.2 Generalization

The novel stimuli from the generalization sessions were analyzed separately from the familiar stimuli. This was done to prevent the confounding effects of novelty on the results and because we primarily wanted to assess the differences between individual novel stimuli.

When performing Type II Wald Chi-Square test for the lever pressing for the novel stimuli only, we found that there was a clear effect of the stimuli on the probability of lever pressing ($p < 0.0001$). The rats preferred the novel stimulus that shared the place of the imagined collision with the previously rewarded stimuli from the training protocol (Generalization +) over the other three stimuli ($p < 0.0001$ for pairwise comparisons of the Generalization + stimuli with all other novel stimuli, Tukey post-hoc test). The probability of lever pressing was significantly lower for the Generalization - stimulus (this stimulus shared the place of the imagined collision with the previously non-rewarded stimuli from the training protocol) than for the control with familiar starting positions ($p = 0.0001$, Tukey post-hoc test). There was no difference between Generalization - and the novelty control ($p = 0.1244$, Tukey post-hoc test) or between the two control stimuli ($p = 0.0734$, Tukey post-hoc test) (Fig. 20-A).

In contrast to the stimuli from the training sessions, there was no difference between the probability of the first lever press after stimulus onset among the novel stimuli ($p = 0.2347$, Type II Wald Chi-Square test) (Fig. 20-B).

The probability of lever pressing during the presentation of individual scenes was affected both by the type of the stimulus ($p < 2.2e^{-16}$, Type II Wald Chi-Square test) and by the interaction of stimuli and time ($p < 5.107e^{-08}$, Type II Wald Chi-Square test). The probability of lever pressing increased in time in case of the Generalization + stimulus and decreased in time in case of the Generalization - stimulus. The probability of lever pressing for both control stimuli stayed constant during the scene presentations. Polynomial models were calculated to compare real pressing rate with the predicted pressing rate for each stimulus (Fig. 20-C).

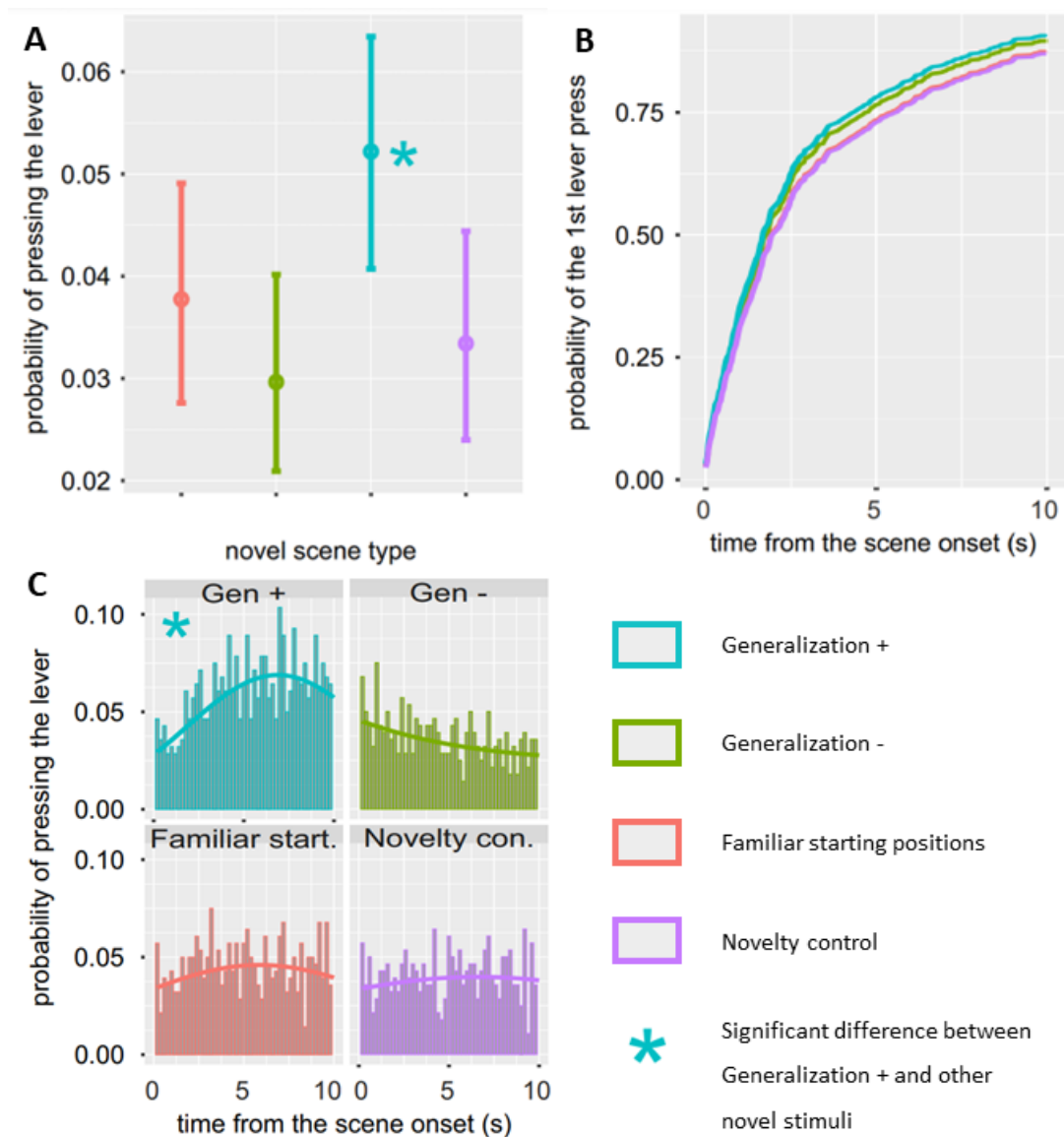


Figure 20: Results from the generalization session. A: Probability of pressing the lever within 100 milliseconds of duration for the novel stimuli in the generalization session. The probability was significantly higher for the Generalization + stimulus than for the other three stimuli. The probability of lever pressing was significantly lower for the Generalization - stimulus than for the Generalization + and Familiar starting positions stimuli. There was no difference between Generalization - and Novelty control stimuli and between Novelty control and Familiar starting positions stimuli. B: Probability of the first lever press during the presentation of a single scene (10 s presentations of individual stimuli). The probability was comparable for all novel stimuli. C: Polynomial models calculated for the individual stimuli to compare the calculated probability of pressing the lever with the real pressing rate. The models show pressing during the display of a single scene (10 s). The calculated probability is in accordance with the real lever pressing values. The pressing rate increased in time for the Generalization + stimulus, decreased for the Generalization - stimulus and stayed constant for the control stimuli. Turquoise asterisk in graphs A and C marks significant differences between Generalization + stimulus and all other novel scenes. Data are pictured as means and 95% confidence intervals.

7.3 Electrophysiology

For single-unit electrophysiological recordings, 5 out of the 7 trained rats were implanted with the tetrodes, the 2 other rats died during surgery. This thesis contains electrophysiological data from 4 rats, as there were no recordings from the last rat obtained due to a defect in the implanted microdrive.

The recorded cells were sorted into tentative principal cells (Fig. 21-A) and interneurons (Fig. 21-B) based on their inter-spike interval histograms, wave-form shapes, and average firing rates. Interneurons typically have narrower wave-form shapes and higher firing rates than principal cells. Since they fire in individual spikes and not in bursts like pyramidal cells, they also have broader spectrums of inter-spike intervals (see also Fig.5 and Fig.6). The median firing rate of principal cells was 1,08 (MAD 0,75) Hz, and the median firing rate of interneurons 13,97 (MAD 6,99) Hz. 132 neurons were recorded in total, 119 (90.15%) of them being principal cells and 13 (9.85%) interneurons (Fig. 21-C).

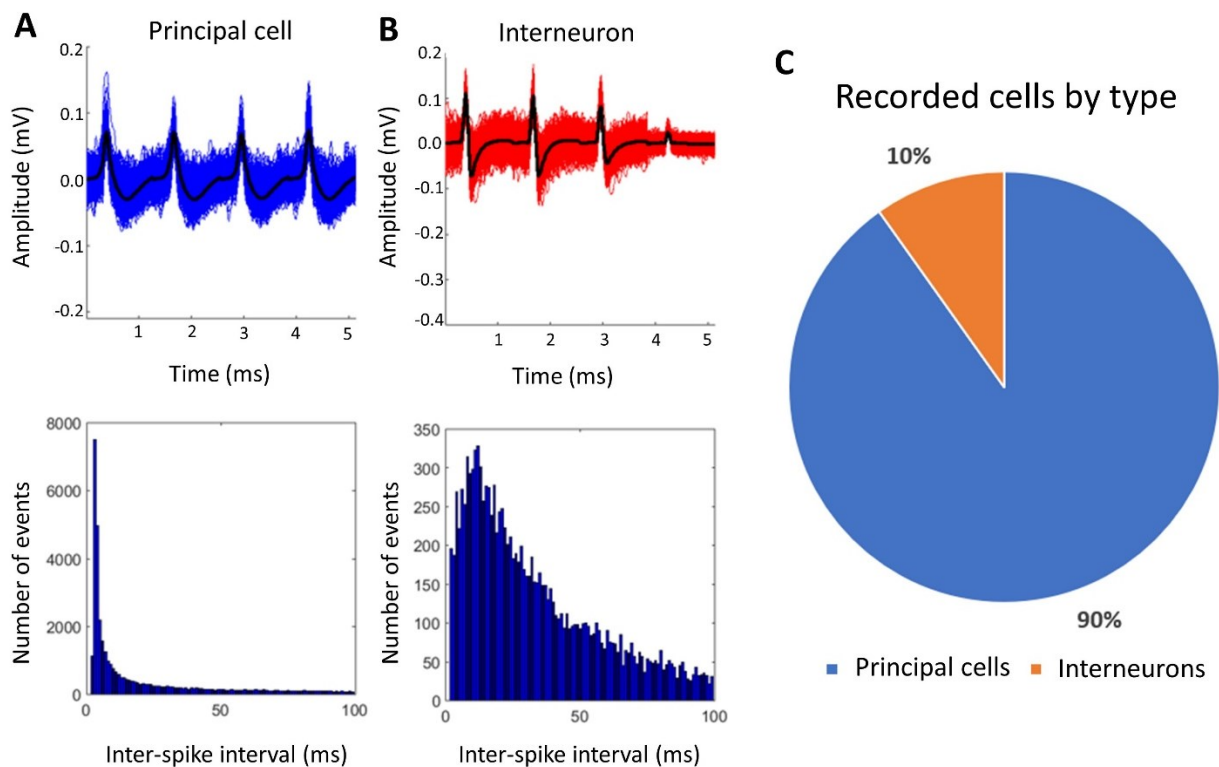


Figure 21: Types of recorded cells. A: Example of a principal cell recorded from rat 43. The top picture shows the waveform of a typical shape as recorded from each electrode of a tetrode. Time window shown for each channel is 1.25 milliseconds. The bottom picture shows the distribution of inter-spike intervals of this cell in milliseconds. This is a characteristic distribution for pyramidal complex-spiking cells which fire in bursts of spikes. B: Example of an interneuron recorded from rat 46. The top picture shows a waveform that has a typical shape and is narrower

than the one of the principal cell. Time window for each channel of a tetrode is 1.25 milliseconds. The bottom picture shows the distribution of inter-spike intervals in milliseconds. The distribution is broader than in the case of the principal cell because interneurons fire in individual spikes rather than in complex bursts. C: The distribution of cell types in the recorded sample. Out of the 132 cells recorded in total, 119 (90.15%) were principal cells and 13 (9.85%) were interneurons.

Out of the 119 pyramidal cells recorded in total, 108 changed their firing rate during the task. However, the firing rate of 36 of these neurons was influenced by either lever pressing or the duration of the stimuli presentation but not by the stimuli type. The firing rate of the remaining 72 neurons was modulated by the visual stimuli, constituting 60.5% of all the recorded pyramidal cells. We found 7 neurons that differentiated between the rewarded and non-rewarded stimuli (type RxNR), 3 neurons that differentiated between the rewarded and non-rewarded stimuli and also between the two rewarded stimuli (type R), and 8 neurons discriminating single scenes. Out of the 8 single scene-recognizing neurons, 1 discriminated the static rewarded stimulus (type S REW), 4 the dynamic rewarded stimulus (type D REW), and 3 the dynamic non-rewarded stimulus (type D non-REW). The firing rates of the remaining 54 neurons were also influenced by the visual scenes but this modulation was non-specific or rather incoherent, with these neurons falling out of all the above-described categories. For example, one non-specifically modulated neuron recorded from rat 43 discriminated between the static non-rewarded scene and both dynamic scenes but not between the static non-rewarded scene and static rewarded scene or between all other scenes (see Fig. 22-C). The proportions of stimuli-modulated neurons for individual animals are listed in Table 3.

Rat	Total	Modulated	Non-specific	RxNR	R	S REW	S non-REW	D REW	D non-REW
Rat 43	33	14	12	---	---	---	---	2	---
Rat 44	33	17	12	2	1	---	---	2	---
Rat 45	28	22	16	3	1	---	---	---	2
Rat 46	25	19	14	2	1	1	---	---	1

Table 3: Neurons by activity modulation by stimuli for each rat. Total: number of all recorded pyramidal cells from the respective animal. Modulated: number of cells responding to stimuli type. Non-specific: cells responding non-specifically. RxNR: cells discriminating between rewarded and non-rewarded stimuli. R: cells discriminating between rewarded and non-rewarded stimuli and between both rewarded stimuli. S REW – static reward, S non-REW – static non-reward, D REW – dynamic reward, D non-REW – dynamic non-reward: cells discriminating single scenes.

A GLMM was calculated for every recorded neuron. Polynomial models were also calculated to compare real firing rates with predicted firing rates calculated using the same fixed factors used in linear models. Below are pictured the results and examples of models for individual rats. Linear models for all specifically modulated cells are shown along with one model for a non-specifically modulated cell and one model for a cell showing no modulation by the visual stimuli for each rat.

Rat 43: 33 principal cells were recorded in total, the firing rate of 14 of them was modulated by the visual scenes. 12 of these cells were modulated non-specifically and 2 responded to an individual scene type, specifically to the dynamic rewarded scene (D REW) (Fig. 22). An example of polynomial models for one D REW cell (Fig. 22-A) is shown (Fig 23).

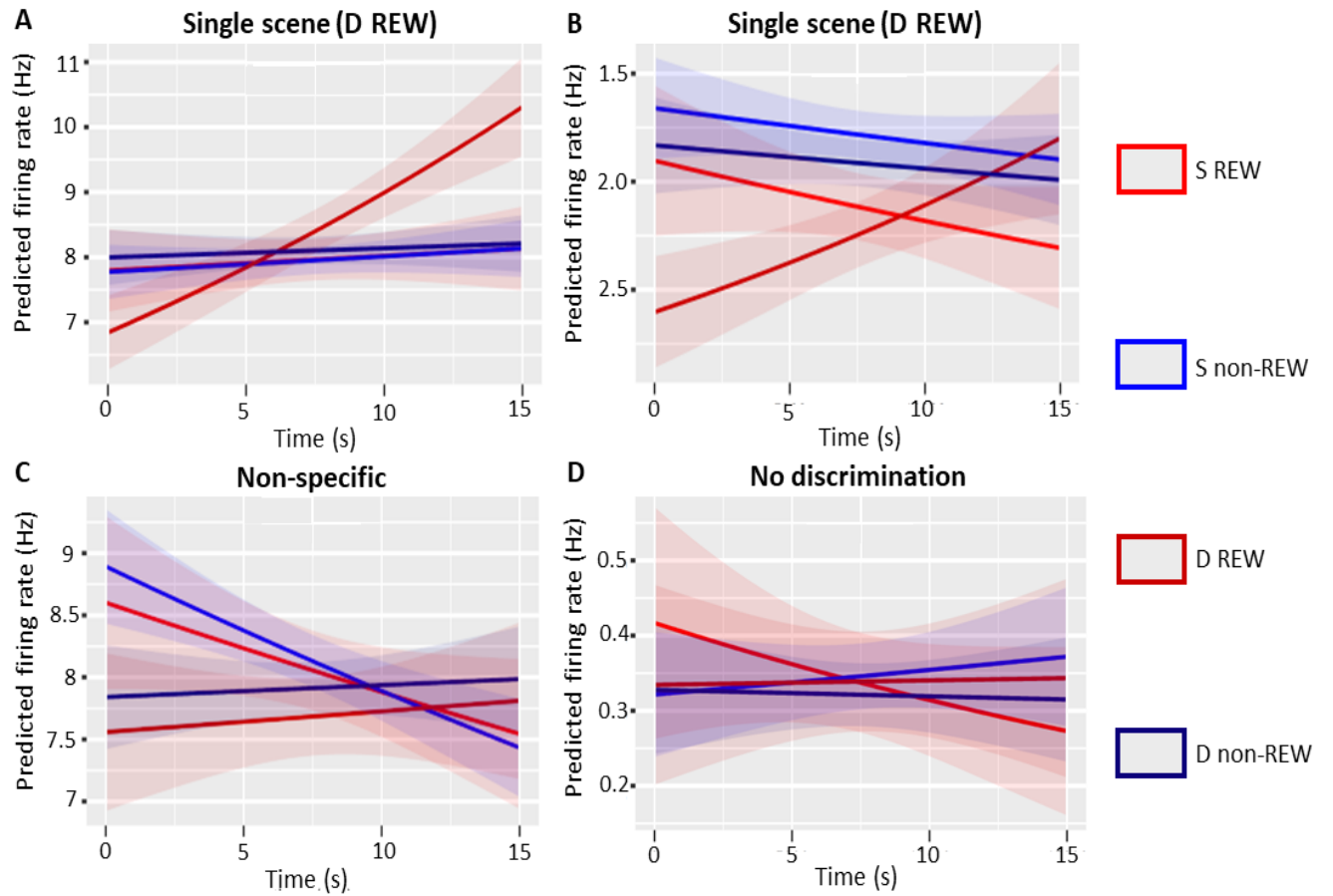


Figure 22: GLMMs for 4 principal cells recorded from rat 43. A: Neuron that increased its firing rate during the presentation of the dynamic rewarded scene and did not change its firing rate during the presentation of any other stimulus. B: Neuron that increased its firing rate during the presentation of dynamic rewarded stimulus and slightly decreased its firing during the presentation of the other three stimuli. C: Example of a non-specifically modulated neuron. D: Example of a cell that did not respond to any visual stimuli. S REW = static rewarded, S non-REW = static non-rewarded, D REW = dynamic rewarded, D non-REW = dynamic non-rewarded. Time in

all graphs shows the duration of stimulus presentation in seconds. The predicted firing rate is pictured in Hz. Lever presses bins were removed from the analysis in all models.

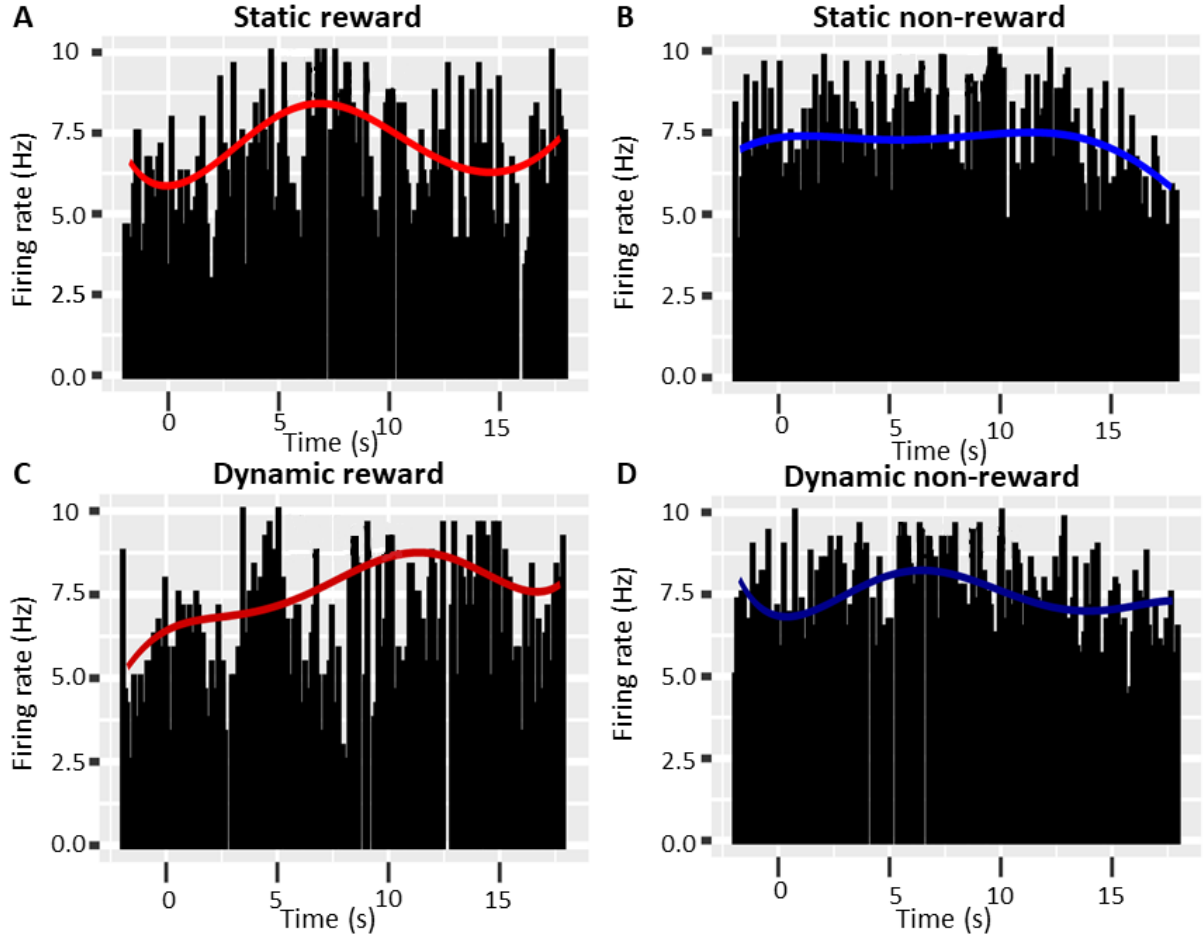


Figure 23: Polynomial models for D REW cell recorded from rat 43 (Fig. 22-A). A: Real firing rate (x axis, in Hz) and predicted firing rate from the polynomial model (light red line) during the presentation of the static rewarded stimulus. B: Real firing rate (x axis, in Hz) and predicted firing rate from the polynomial model (light blue line) during the presentation of the static non-rewarded stimulus. C: Real firing rate (x axis, in Hz) and predicted firing rate from the polynomial model (dark red line) during the presentation of the dynamic rewarded stimulus. D: Real firing rate (x axis, in Hz) and predicted firing rate from the polynomial model (dark blue line) during the presentation of the dynamic non-rewarded stimulus. Time in all graphs shows the duration of stimulus presentation in seconds with 0 marking the onset of the stimulus. Firing rate is pictured in Hz.

Rat 44: 33 principal cells were recorded in total, out of which 17 changed their firing rate in relation to the visual scenes. The modulation of 12 of these cells was non-specific, 2 cells discriminated a single scene (D REW), 2 cells were of type RxNR, and 1 cell of type R (Fig. 24).

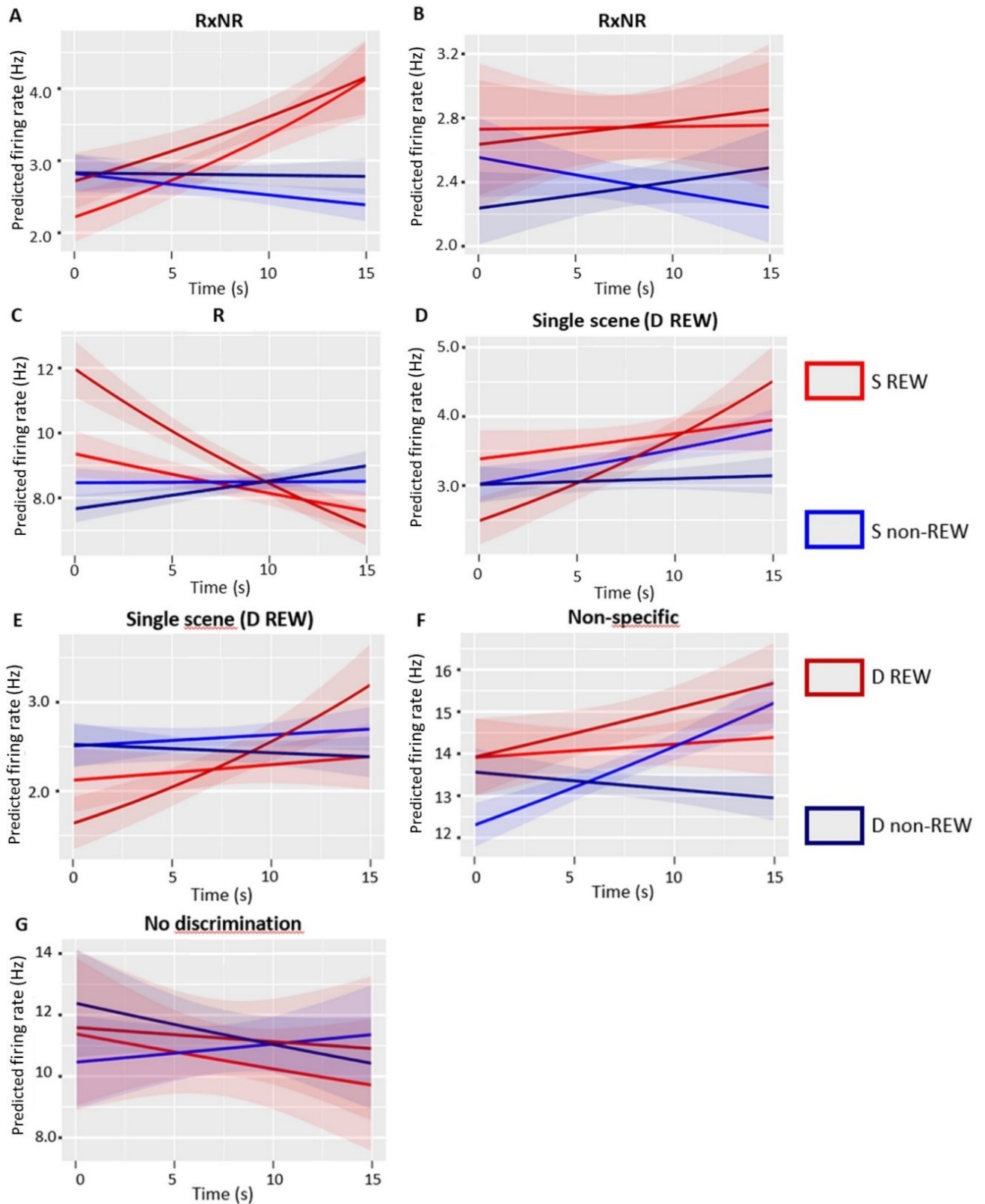


Figure 24: GLMMs for 7 principal cells recorded from rat 44. A: Neuron that increased its firing rate during the presentation of rewarded stimuli and slightly decreased its firing rate during the presentation of non-rewarded stimuli. B: Neuron with significantly higher firing rate in the presence of rewarded than non-rewarded stimuli. C: neuron discriminating rewarded and non-rewarded stimuli and both rewarded stimuli. This neuron decreased its

firing rate during the presentation of both rewarded stimuli, and the decrease was more significant in the case of the dynamic rewarded stimulus. D+E: Two neurons that significantly increased their firing rates in relation to the dynamic rewarded stimulus, the firing rates of these neurons remained unchanged in the presence of the other stimuli. F: Example of a non-specifically modulated neuron. G: Example of a neuron showing no modulation in firing rate in relation to the displayed stimuli. S REW = static rewarded, S non-REW = static non-rewarded, D REW = dynamic rewarded, D non-REW = dynamic non-rewarded. Time in all graphs shows the duration of stimulus presentation in seconds. The predicted firing rate is pictured in Hz. Lever presses bins were removed from the analysis in all models.

Rat 45: Total of 28 principal cells were recorded and 22 of them changed their firing rate in relation to the visual stimuli. The firing rate of 16 of these cells was modulated non-specifically, 3 cells were of type RxNR, 2 discriminated the D non-REW scene, and 1 was type R (Fig. 25).

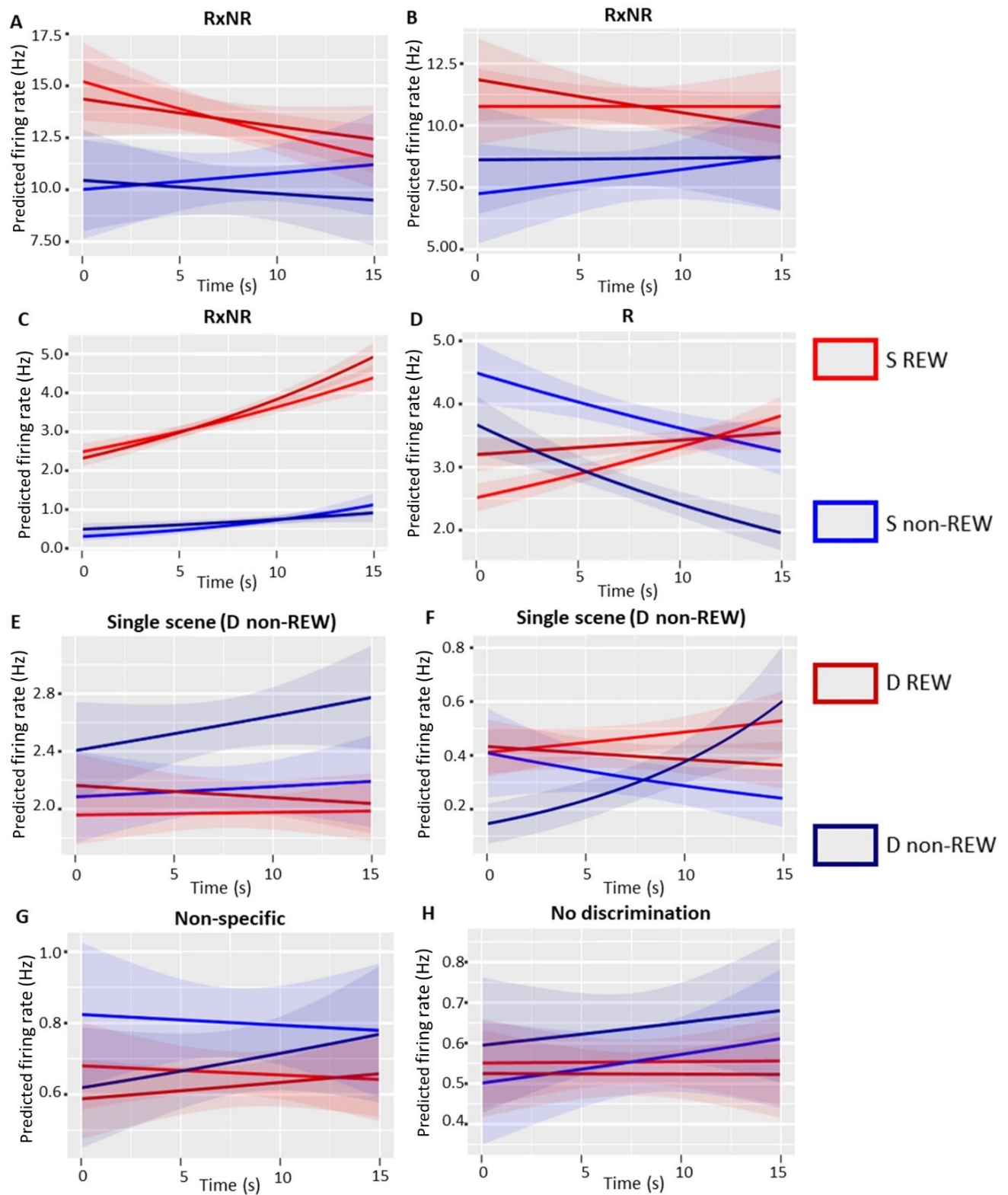


Figure 25: GLMMs for 8 principal cells recorded from rat 45. A+B+C: Three neurons discriminating between rewarded and non-rewarded spatial visual scenes. The firing rates of the first two neurons decreased in relation to the rewarded stimuli and did not change in relation to the non-rewarded ones. The firing rate of the third neuron was higher and significantly increased in the presence of the rewarded stimuli compared to the non-rewarded ones. D: Neuron discriminating rewarded and non-rewarded and both rewarded stimuli. The firing rate of this neuron

decreased in the presence of both non-rewarded stimuli and increased in the presence of rewarded stimuli, more significantly in the case of the static rewarded stimulus. E+F: Two neurons representing the dynamic non-rewarded scene. These neurons rapidly increased their firing rates during the presentation of the dynamic non-rewarded stimulus. G: Example of a non-specifically modulated neuron. H: Example of a neuron that showed no modulation by the spatial visual stimuli. S REW = static rewarded, S non-REW = static non-rewarded, D REW = dynamic rewarded, D non-REW = dynamic non-rewarded. Time in all graphs shows the duration of stimulus presentation in seconds. The predicted firing rate is pictured in Hz. Lever presses bins were removed from the analysis in all models.

Rat 46: 25 principal neurons were recorded, 19 of them responded to the visual stimuli. 14 cells changed their firing rate non-specifically, 2 were RxNR, 1 R, and 2 responded to single scenes (1 S REW and 1 D non-REW) (Fig. 26).

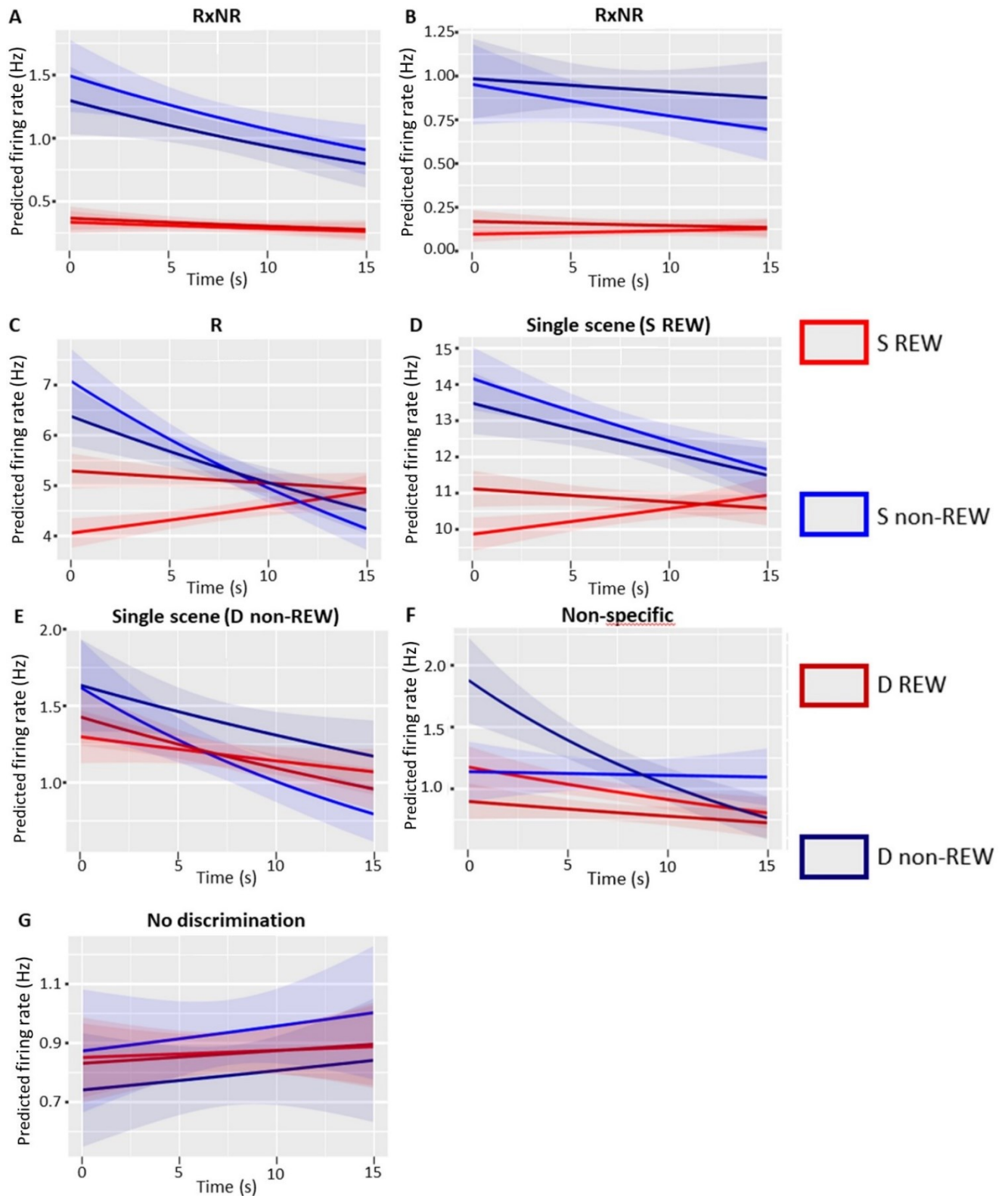


Figure 26: GLMMs for 7 principal cells recorded from rat 46. A+B: Two cells whose firing rates were significantly higher and decreased during the presentation of non-rewarded stimuli but remained unchanged during the presentation of rewarded stimuli. C: Principal cell discriminating between rewarded and non-rewarded and both rewarded stimuli. The firing rate of this neuron decreased in relation to non-rewarded stimuli, stayed unchanged in the presence of the dynamic rewarded stimulus and increased in relation to the static rewarded

stimulus. D: Neuron representing the static rewarded scene. The firing rate of this neuron increased during the presentation of the static rewarded stimulus and decreased during the presentation of the three remaining stimuli. E: Neuron representing the dynamic non-rewarded scene. This neuron had a significantly higher firing rate for this scene than for the other three scene. F: Example of a neuron with non-specific modulation of firing. G: Example of a neuron that did not change its firing in relation to the spatial visual scenes. S REW = static rewarded, S non-REW = static non-rewarded, D REW = dynamic rewarded, D non-REW = dynamic non-rewarded. Time in all graphs shows the duration of stimulus presentation in seconds. The predicted firing rate is pictured in Hz. Lever presses bins were removed from the analysis in all models.

7.4 Validation of tetrode positions

To validate the final positions of the tetrodes, an electrolytic lesion was done 24 hours before perfusion of the animal. Brains were then frozen, cut, and stained with cresyl violet for visualization. Obtained samples validated the localization of tetrodes in the CA1 area of the hippocampus (Fig. 27), but some brain slices unfortunately got damaged during the processing. However, validation of the tetrode position was also possible during the recording, when we observed theta rhythm and bursts of complex spikes, which are both electrophysiological phenomena typical for the pyramidal layer of the hippocampus.

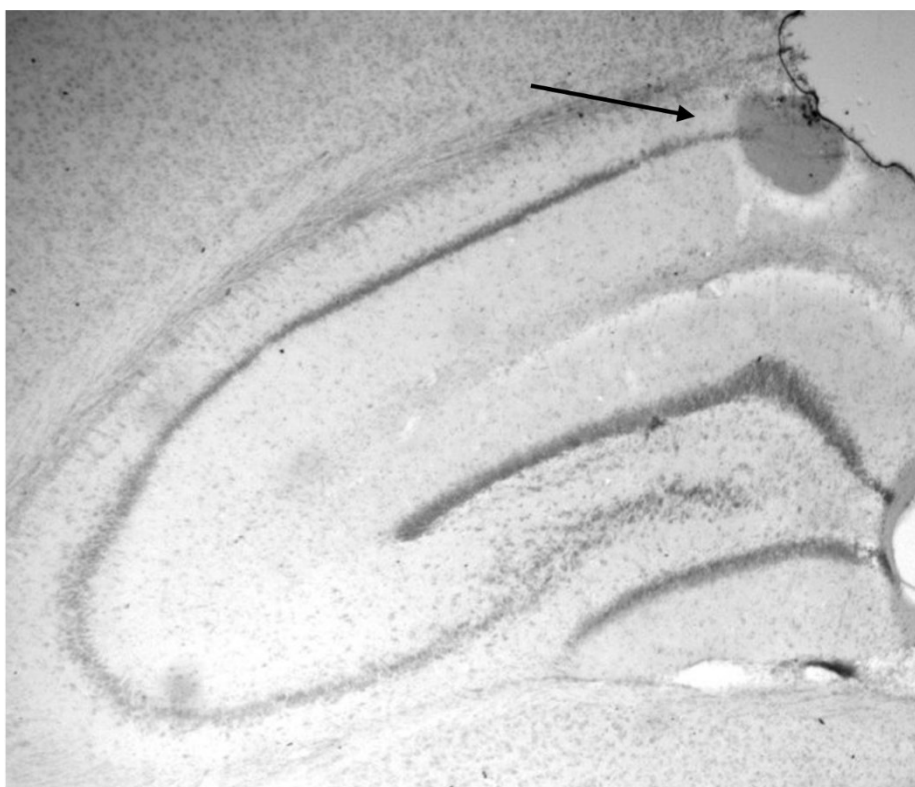


Figure 27: An example of a cresyl violet stained brain slice with visible lesion in the CA1 hippocampal area. This brain slice was obtained from rat 43. Arrow points to the location of the lesion.

8 Discussion

The aim of our experiments was to compare the learning of inaccessible static and dynamic visual stimuli and to find out if the positions of these visual objects are encoded by the neurons of the hippocampus. To study this, we designed a behavioral task, in which rats were placed in an operant chamber and were trained to discriminate between pairs of static and dynamic visual stimuli. Subsequently, we performed single-unit electrophysiological recordings from the CA1 area of the hippocampus of the rats while they solved the behavioral task.

8.1 Experimental set-up

The design of the behavioral task that we used combined two aspects of spatial situations that have previously been only scarcely researched. The first of them was the dynamic character of the presented scenes, and the second one the inaccessibility of the space where the scenes were presented. Furthermore, the design of our task allowed us to combine static and dynamic stimuli that were complementary in their spatial aspects due to the place of imagined collision of the dynamic stimuli.

For a long time, hippocampal place cells were thought to only represent a subject's own location in space. In an attempt to answer the question of how the positions of other objects are encoded by the brain, a few studies linked the role of hippocampal cells to the representation of other objects in space. Some of these studies recorded the activity of hippocampal pyramidal cells of rats freely foraging in environments (arenas, tracks) where they encountered various static objects. In one experiment, rats ran in laps on a circular track with objects placed in different locations on every trial (Manns & Eichenbaum, 2009). In another, rats were placed in one of two interconnected arenas and presented with static objects of different contexts (Komorowski et al., 2009), or in a simple familiar arena filled with multiple various objects in different configurations (Deshmukh & Knierim, 2013; Vandrey et al., 2021). All these studies found some modulation of the activity of hippocampal pyramidal neurons related to the positions of static objects.

Ho and colleagues performed a behavioral task, in which rats were placed in an arena containing a moving car and were trained to either chase the car for a reward or move independently of the car. This study found the modulation of place cells' activity by the moving object but not direct encoding of the moving object by place cells (Ho et al., 2008). Danjo and colleagues and Omer and colleagues created observational behavioral tasks for rats and bats,

respectively. In these experiments, one animal (observer) watched another animal of the same species (demonstrator) performing the learned behavioral task while electrophysiological recording from the CA1 pyramidal neurons of the observer animal was obtained. Both of these studies found the existence of cells directly encoding the position of the demonstrator animal. These cells were named social place cells (Danjo et al., 2018; Omer et al., 2018). On top of that, Omer and colleagues also recorded the activity of hippocampal cells during the observation of an inanimate moving object and discovered cells that encoded the position of this object (Omer et al., 2018). Recently, a study that found spatial selectivity of hippocampal cells to a moving bar of light in immobile rats was published. In this experiment, rats were head-fixed on a treadmill and surrounded by a cylindrical screen, on which a bar of light moving at a constant speed in the horizontal plane was presented. Single-unit recording revealed the modulation of firing rate of hippocampal neurons by the angle, distance, and direction of the moving stimulus (Purandare et al., 2022).

Most of the studies mentioned above were done in accessible space. The few exceptions were the two studies that discovered the existence of social place cells, and the most recent study discovering hippocampal cells' selectivity to a moving bar of light. In the social place cells studies, however, while the observed object (demonstrator bat/rat) was located in an immediately inaccessible space, this space was still highly familiar to the observer as animals visited it many times over the course of the experiment. In the study of Purandare and colleagues, the experimental space was inaccessible and unfamiliar, but – in contrast to our task – the rats were immobile. In addition, the visual stimulus was only moving in one direction, whereas in our task the dynamic stimuli were moving in various directions across the screen. Another difference was that in this task (in contrast to our experiment), rats were not trained to learn the positions or movement of the visual stimulus. Yet, the authors found vectorial representation of this stimulus in the hippocampus. It could be an interesting future prospect to study, whether the neural representation of stimuli in our task would already be found in the hippocampus in the beginning of the training. To assess this, we would need to record from rats naïve to the task. However, in the present version of our task, the behavioral training served to create a stable representation for the individual stimuli, and to ensure that the rats discriminated the stimuli.

In contrast to previous studies concerned with the neural representation of objects in space, we designed a behavioral task that combined static and dynamic spatial visual stimuli in inaccessible, never visited space with single-unit electrophysiological recording from the

pyramidal layer of the CA1 area of the hippocampus in freely moving rats. To our knowledge, this is the first such task within the field of spatial navigation research.

8.2 Behavioral training phase

In the behavioral training phase of the experiment, we found that rats were able to successfully learn to discriminate both the static and dynamic spatial visual scenes. We also observed that they discriminated the dynamic stimuli faster and more precisely than the static ones. Moreover, they preferred not only the dynamic rewarded stimulus over the static rewarded one, but also the dynamic non-rewarded stimulus over the static non-rewarded one. One possible explanation for these results is that it could be more important for survival to react quickly and accurately to dynamic situations than to static situations. Real environments are always changing and both predators and prey must react quickly in order to survive. While it is important to pay attention and react to stable features of the environment, it is arguably even more important to appropriately react to a sudden change in the environment (e.g., an attacking predator, a moving prey, a falling tree, etc.). Therefore, it would be ecologically advantageous to have shorter reaction times to dynamic than static situations. Studies with human subjects focusing on the difference in perception of static and dynamic objects also found that humans detected moving objects sooner and with higher accuracy than static objects. One experiment presented static and moving objects to people in a virtual environment simulating natural scenes and found a clear advantage of dynamic objects on object detection (Vuong et al., 2006). Another experiment simulated a vehicle driving situation with moving and static objects (pedestrians, cyclists) and tested the times to the detection of different categories of objects. This study found that test subjects detected moving objects sooner than static objects (Bhagavathula et al., 2012). Our results, together with the results from these studies, suggest that the more accurate discrimination of dynamic over static stimuli could be a widespread phenomenon important for survival in the real world.

The preference of dynamic situations could also be caused by animals' higher attention to moving objects than to static objects. This could again be explained by the great ecological importance of noticing abrupt changes in the environment. The difference of attention to static and moving objects was researched in early studies with human infants (McKenzie & Day, 1976) and later with adult human subjects (Abrams & Christ, 2003; Franconeri & Simons, 2003), and mice (Braida et al., 2013). All these experiments found increased attention of the experimental subjects to moving stimuli compared to stationary ones. One study proposed that

it is the onset of movement and not movement itself that captures attention (Abrams & Christ, 2003). This hypothesis could be in line with our findings as in the dynamic stimulus in our task, the scene was repeated every second – the two circles were moving towards the collision point and then disappeared. Thus, there was an initiation of movement every second of the dynamic stimulus duration. It might be particularly important to pay attention to the onset of movement as that is the crucial period indicating potential danger by moving objects (Abrams & Christ, 2003).

Another possible explanation for these results could stem from the fact that rats are known to be highly curious animals. This can be experimentally tested by introducing novel objects together with familiar objects into an arena. Rats tend to explore and pay more attention to the novel objects than to the familiar ones (Bevins & Besheer, 2006). This curiosity could also be the reason for their preference of the moving stimuli as it is possible that the rats found them more interesting than the static ones. This explanation, however, does not seem very likely as the rats saw the static and dynamic stimuli many times over the course of the training. Hence, all stimuli were highly familiar to the rats at the end of the first configuration of the training phase, but the preference of dynamic over static stimuli persisted.

8.3 Generalization

In the generalization sessions, we found that rats pressed the lever more during the appearance of Generalization + stimulus (stimulus that shared the place of the imagined collision with the place of imagined collision in the dynamic rewarded stimulus and with the location of the static rewarded stimulus in the training phase). The probability of lever pressing of the rats was lower in the case of Generalization - stimulus (which shared the place of imagined collision with the non-rewarded stimuli from the training phase) compared to Generalization + and the control stimulus with familiar starting positions. These results suggest that rats were able to generalize the Generalization + and Generalization - stimuli and associate them with the rewarded and non-rewarded stimuli from the training session, respectively.

There was no difference in the probability of lever pressing between the novelty control and the control with familiar starting positions which suggest that showing the rats a scene that (among its other features) contains a component of the familiar rewarded stimuli (rewarded static stimulus as the starting position of one circle) is not sufficient for stimulus generalization in our task. This might have been caused by the fact that although the starting positions of the circles presented in this scene were familiar, the information represented in them was

contradictory – in the beginning of the scene, there was one circle in the position identical to the static rewarded and the other to the static non-rewarded stimulus from the training phase. Moreover, they were moving but in the “opposite” direction than the dynamic stimuli from the training phase, with the circles starting in the positions of the imagined collisions and moving from them, not towards them. This might have confused the rats and caused them to generalize this stimulus at a lower level than the Generalization + stimulus. These results might also suggest that familiar starting positions of the objects are not enough for generalization, and that the crucial factor of the presented scenes – on the basis of which rats generalize the situations – is the imagined collision. I give reasons for this interpretation below.

When comparing the lever pressing rate during single scene presentations, we found that the pressing rate was equivalent for all four novel stimuli in the beginning but changed in time. For the Generalization + stimulus, the lever pressing rate increased during the presentation, and for the Generalization - stimulus it decreased. The rate of pressing stayed constant for both control stimuli. These results lead us to a possible interpretation that rats pressed the lever at a constant rate whenever they saw a novel stimulus – a phenomenon that can be explained by their curiosity and natural preference for novel objects – but modified their pressing once they generalized the stimulus. These results support the previously mentioned interpretation that the element of prediction of the collision place might be crucial for the discrimination and generalization of the visual stimuli. I discuss available literature that might support this assumption in the next two paragraphs.

It has been proposed that time and space might be encoded by similar mechanisms in the brain (Buzsáki & Tingley, 2018). Several findings point to this possibility. First, time cells (pyramidal neurons of the hippocampus that fire in sequences during the delay of a behavioral task that were discovered in the first decade of the 21st century and have been heavily researched since then (Pastalkova et al., 2008; MacDonald et al., 2011)) have been suggested to constitute the same population of cells as place cells that encode an animal’s own position in space (Eichenbaum, 2014). It might therefore be possible that place cells and time cells are in fact the same neurons that encode both space and time. Second, a theoretical hypothesis (supported by results from a behavioral experiment with human subjects (Villacorta-Atienza et al., 2021)) called the time compaction has been put forward in an attempt to explain how dynamic situations are encoded in the brain. Time compaction proposes that dynamic situations are internally represented as static maps containing all possible interactions between the objects in a given environment. According to this concept, dynamic and static situations are encoded by

the same mechanisms (Villacorta-Atienza et al., 2010). Hence, time and space are also encoded by the same mechanisms as dynamic situations combine both of these aspects in themselves. Since this internal map encodes all possible collisions between the elements in the environment and possible ways to avoid them, it is also concerned with the subject's ability to predict future positions of moving objects in the environment (Villacorta-Atienza et al., 2021).

In accordance with the time compaction hypothesis, we propose that rats were able to predict future positions of dynamic stimuli in our task and imagine their potential collisions. Time compaction is not, after all, the only hypothesis that connects the prediction of future positions/trajectories to the neural representation of space. Theta phase precession, a phenomenon that ties together the firing of place cells with the hippocampal theta rhythm, has also been suggested to play a role in the prediction of future movement (Huxter et al., 2008). Moreover, place cells have been found to fire in sequences indicating the prediction of one's future trajectory during periods of sharp wave-ripples, another electrophysiological pattern found in the hippocampus (Pfeiffer & Foster, 2013; Widloski & Foster, 2022). In a recent study, the authors recorded from the pyramidal cells of the hippocampal CA1 area of freely flying bats and found neurons that fired in relation to nonlocal places in the environment. This way, the cells represented future locations of the bats, both during random foraging and during goal-directed navigation. Moreover, the majority of these cells had their firing fields located at the intersections of the flying paths (Dotson & Yartsev, 2021). The last finding is particularly interesting as the encoding of intersections of paths and collision sites of moving objects in our study could potentially share similar features. Finally, a new concept that stems from the above-described findings hypothesizes the role of the hippocampus as a predictive map. This hypothesis revisits the concept of a cognitive map but takes into account the role the hippocampus plays in the prediction of future movement (Huxter et al., 2008), time (Pastalkova et al., 2008), and reward (Gauthier & Tank, 2018) coding. Then it proposes that the hippocampus does not create a purely spatial cognitive map, but rather a predictive map that represents the environment in successive time periods and combines this representation with prediction of possible reward and future movement of self and others (Stachenfeld et al., 2017). The predictive map has been studied in behavioral experiments with humans and rats (de Cothi et al., 2022).

Lastly, although the generalization stimuli in our task were different from the dynamic stimuli presented in the training phase, they shared the trajectory of one of the circles participating in the scene with them. For this reason, we cannot rule out the possibility that rats

recognized this trajectory rather than the place of the imagined collision. However, after considering the results of studies listed above, we lean towards the interpretation that the main factor for stimuli recognition was indeed the imagined collision.

8.4 Electrophysiology

In the electrophysiological recordings from four rats, we found 119 pyramidal cells. 72 (60.5%) of them changed their firing rate in relation to the visual stimuli. This sample included cells discriminating single scenes, cells differentiating between rewarded and non-rewarded scenes, and cells differentiating between rewarded and non-rewarded scenes and between the two rewarded scenes. The majority of the spatial stimuli-modulated neurons (54/72) were modulated non-specifically. One reason for these results could be that the sample of recorded cells was not big enough and therefore, we were not able to observe full modulation of the cells' activity. One possible way to fix this issue could be maximizing the number of repetitions of single scene presentations. The main obstacle of doing this is the length of the individual sessions. The length of the recording session was already 45 minutes 36 seconds and if we extended it, we could face the problem of decreased attention of the rats and their saturation with food pellets. A possible solution for this obstacle might be decreasing the length of the individual scene presentations (15 seconds for each stimulus in the training phase and 10 seconds for the novel stimuli in the generalization sessions in the current protocol). This way, the length of the session could stay practically unchanged while the number of repetitions would increase. This alteration might be a useful prospect for future experiments investigating the neural representation of inaccessible objects.

Another reason for the predominance of non-specifically modulated cells (and for the absence of cells that would fire solely in the presence of the stimuli) in the recorded sample could be that these inaccessible spatial scenes are not encoded by single neurons but rather jointly by a population of neurons. This kind of a population coding is known from many cognitive and behavioral variables, such as encoding of shape of visual objects (Pasupathy & Connor, 2002), expectation of reward probability (van Duuren et al., 2009), arm movement (Shenoy et al., 2013), categorization of sounds (Bathellier et al., 2012), or speech and audio recognition (Pan et al., 2019). In a population code, a single stimulus is represented by a distinct pattern of firing of multiple neurons in a population. This way, every neuron carries complementary information to that of other neurons (Panzeri et al., 2015). We speculate that stimuli in our inaccessible spatial task could also be represented by a population code, although

we currently do not possess any results that could support this hypothesis. Potential future utilization of large-scale recordings of local circuits (Berényi et al., 2014; Buzsáki et al., 2015) in a similar experiment could, however, help obtain more data relevant to this proposal.

We found a portion of cells that discriminated pairs of complementary static and dynamic stimuli. Out of the 18 specifically modulated cells, 7 (39%) differentiated between the rewarded and non-rewarded stimuli. These results could point to a mutual mechanism of the encoding of static and complementary dynamic situations, which would be in agreement with the time compaction hypothesis mentioned in the previous subchapter. In future experiments, a bigger sample of recorded cells would be needed to support this hypothesis. A control experiment could also be designed in which two rewarded and two non-rewarded stimuli would be used again, but this time with no shared spatial component.

9 Conclusion

In our experiments, we found that rats can discriminate inaccessible static and complementary dynamic spatial visual scenes, and that they prefer dynamic over static stimuli. We also observed that with the knowledge of familiar visual stimuli, they can generalize novel dynamic visual stimuli. I argue that the crucial aspect of the dynamic scenes that is necessary for generalization could be the location of the imagined collision.

In the recording phase of the experiment, we found cells with firing rates specifically and non-specifically modulated by the spatial visual stimuli. Based on our results and related results in the available literature, we propose that the static and complementary dynamic spatial visual stimuli could be encoded by mutual neural mechanisms.

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Figures

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