HIPPOCAMPAL NEURAL REPRESENTATIONAL DYNAMICS: DIFFERENTIAL EFFECTS OF VISUAL INFORMATION ON PLACE FIELD DIRECTIONALITY, POSITION, AND SIZE

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A thesis submitted
in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

NEUROSCIENCE

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Date of Defence: July 15, 2020

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ABSTRACT

Stored hippocampal spatial maps differ substantially from ones the hippocampus creates on initial acquaintance with a new environment. During the first few laps on a new maze, spatially tuned hippocampal ‘place cells’ develop an experience-dependent direction specificity and a backward shift of the place field centre of mass, normally associated with place field expansion. In this work, we used reliance on visual cues as a tool to test the putative functions and mechanisms of these three adjustments. We found that place field directionality was not affected by the availability of visual cues and therefore is not a primary mechanism to associate spatial and nonspatial information, as was previously hypothesized. Contrary to expectations, however, the place field expansion and backward shift appeared to be two distinct, independent phenomena with different reliance on visual cues. We propose new mechanisms for the observed phenomena taking into account their reliance on visual cues.

ACKNOWLEDGMENTS

First I'd like to thank my supervisor, Dr. Bruce McNaughton, for inviting me over and making this possible. I'm grateful for his supervision and for letting me explore paths outside of my main project. I met a lot of nice people in CCBN, who directly or indirectly contributed to my work. When I came to the lab, I was privileged to learn electrophysiology from experienced postdocs: Aaron Wilber, Jake Ormond, and Hendrick Steenland. Hiroe Yamazaki and Adam Neumann helped me with my first surgeries. Zaneta Navratilova shared the source code for place field analysis. Rui Pais provided an enormous help with behavioural experiments.

I'm grateful to the veterinarians for their help with animal care.

Thank you Naomi and Amanda for walking me through the regulations, paper work, supply ordering, and many more.

Thank you to the scientists who helped me to shape my project into something feasible and served as members of my Committee: Drs. Robert Sutherland, Artur Luczak, and Masami Tatsuno. Your expertise was invaluable.

I'm also grateful to Dr. Majid Mohajerani, Dr. Edgar Bermudez, Dr. JianJun Sun and Jennifer Tarnowsky for a side quest that resulted in a paper about simultaneous VSDI and electrophysiological recordings.

I'm thankful to Aubrey Demchuk for proofreading the text.

For last, I want to thank my 'PhD significant other', Dr. Olga Mineyeva, for her love and support.

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CAD	Computer-aided design
COM	Centre of mass
DG	Dentate gyrus
DI	Directionality index
EIB	Electronic Interface Board
FR	Firing rate
LEC	Lateral entorhinal cortex
LFP	Local field potential
LTP	Long-term potentiation
MEC	Medial entorhinal cortex
PBS	Phosphate-buffered saline
PF	Place field
SWR	Sharp-wave ripple

1 General Introduction

Memories are essential for animal survival. To a large extent, recollections of individual experience also create a unique human personality. In neuroscience, the mechanisms of memory formation, retrieval and transformation are of particular interest. In the ever-changing brain, where many processes occur in the second-millisecond timescale, memories are the result of long-term changes that are more accessible for experimental research.

Most parts of the brain demonstrate surprisingly little or indirect importance for memory formation and retrieval (Lashley 1950). A key brain region for memory was found following surgical resection of the hippocampus and adjacent brain parts in an attempt to cure epilepsy (Scoville & Milner 1957). The resultant memory deficit was so apparent that it was first noticed even without a dedicated search. Further research depicted the hippocampus as a crucial brain structure for long-term memory in both animals (Jeffery 2018, Lisman et al 2017, Zemla & Basu 2017) and humans (Burgess et al 2002, Maguire et al 2016, Sheldon & Levine 2016).

Brain functions are executed mostly through the firing of neurons. The distinct role of the hippocampus in memory formation is mediated by neurons with unique firing properties. When recorded from awake, behaving animals, hippocampal principal cells display characteristic spatially tuned activity, which led them to be called “place cells” (O'Keefe 1976, O'Keefe & Dostrovsky 1971). A subset of place cells active in a given environment and their corresponding firing fields, called “place fields”, form a “cognitive map” of that environment, the stability of which correlates with a behavioural estimate of hippocampal function (Barnes et al 1997).

1.1 Hippocampal cognitive maps

Early understanding of hippocampal neural properties was tightly bound to progress in recording techniques. Recordings from awake, behaving animals became possible upon adoption of implantable microelectrode advancers (Ainsworth et al 1969, Wall et al 1967). They were used to hold metal, glass-insulated microelectrodes and record single cells within the spinal cord and medulla. The subsequent discovery of 'hippocampal maps' stemmed from an unusual strategy for scientific thinking. This chapter recapitulates the history of the entitlement of the hippocampus as a 'cognitive map' as well as the paradigmatic shift and technical advances that allowed for it.

The discovery of place cells by pioneers in hippocampal maps (O'Keefe & Dostrovsky 1971) was preceded by hippocampal studies with a completely different conceptual focus. The first recordings of single units from the hippocampus of awake animals were made in attempt to define tuning curves of hippocampal neurons by presenting the animals with a set of light and sound stimuli (Semenova & Vinogradova 1970, Vinogradova 1965, Vinogradova 1966). In these studies, O.S. Vinogradova and her colleagues found a rather high (>60%) response rate in dorsal and ventral hippocampi and the majority of the units had low selectivity in terms of nature of the stimuli (light or sound) and the quality of the stimuli (tone of the sound). The responses manifested as tonic excitation or tonic inhibition and were prone to habituation, which allowed for the conclusion that hippocampal cells served as novelty detectors.

Vinogradova's approach was in line with the dominant Pavlovian tradition, which aimed at testing neuronal reaction to an introduced stimulus. This essentially required tightly controlled experimental conditions and a well-articulated hypothesis to be tested.

While these requirements are also essential in modern era studies, they inadvertently produce a discovery-limiting experimental strategy. In this approach, an animal was exposed to a single and usually simple stimulus and the body response was measured. Although this strategy is advantageous when defining a fine aspect of a body part function, it is impractical when the broad function is unknown. John O'Keefe and colleagues benefited from another approach: to place an animal in an environment as ecologically relevant as possible and allow it to behave naturally. Instead of testing neuronal response to a predefined set of stimuli, the units were described individually with their reaction to a wide range of natural stimuli.

This new experimental approach resulted in an article (O'Keefe & Dostrovsky 1971) describing the hippocampus as a 'cognitive' or 'spatial map'. Surprisingly, most of the recorded units in this paper were not spatially tuned and were described as either 'arousal' units (14/76), 'movement' units (21/76), or 'uninterpretable' (31/76). Thus, a new function for the hippocampus was assigned based on only 8 single units recorded from total of 23 rats. This extraordinary claim was justified by extraordinary evidence, namely by a completely novel type of unit response – they fired only when the animal was in a particular place and faced a particular direction. We now know that the recording environment (a platform of 24 by 36 cm) was far from ideal for detecting place cells and the single metal wires were barely usable for isolating hippocampal single units. However, to a large degree, this pioneering work shaped the landscape of neuroscience for the next several decades.

The second paper on place cells (O'Keefe 1976) described hippocampal unit firing in a more relevant behavior task with a larger environment to explore: a two-arm maze.

This task was more suitable for place cell detection since the rat was able to make longer runs, meaning the proportion of detected 'place units' was substantially larger (26/50). The properties of the 'place units' were therefore able to be described in more detail. Their activity was not found to be triggered by local olfactory, tactile clues or auditory cues. Importantly, this early report suggested that the firing rate of a place cell can depend on the direction of running through the place field (PF), and that the place cell properties remain relatively unchanged with the lights on and off. Interestingly, the animal tracking in the dark for this study was usually made by gently holding the rat's tail. Notably, some results of this study were actually confirmed by an earlier study (Ranck 1973) that was conducted before the spatially-selective firing of CA1 cells was confirmed. Ranck described the hippocampal units as 'approach-consummate-mismatch cells' because, although he did not observe the spatial component of cell tuning, he demonstrated the lack of cellular response to simple sensory stimuli, similar to the subsequent O'Keefe paper (O'Keefe 1976).

These early researchers acknowledged the limitations they faced in their work. First, there was the unit isolation problem. The hippocampal pyramidal layer is comprised of several layers of densely packed neurons, which complicates the distinction of nearby neurons by amplitude and waveform. This problem was almost solved in the mid-90s with the adoption of the tetrode technique. Another issue was unit stability: the electrodes were lowered to the pyramidal layer minutes before the recording session, which resulted in inferior unit stability (tens of minutes) and limited time for an experiment to be completed. This problem was later solved by gradual advancement of the electrodes.

The first major attempt to resolve the unit isolation problem was made with the invention of stereotrodes (two wires twisted together) (McNaughton et al 1983b), which allowed for disambiguation of adjacent neurons based on the differences in amplitude recorded from two closely spaced electrodes. Further improvement in unit isolation was achieved with the adoption of tetrodes (four wires twisted together, as was described in the concept first given in (McNaughton et al 1983b)) (Gray et al 1995, O'Keefe & Recce 1993, Wilson & McNaughton 1993). Nevertheless, the uncertainty in spike sorting remains a problem in electrophysiology studies of the hippocampus, mainly due to the lack of a ground truth for spike assignment. The isolation problem is less critical when cell activity is assessed by the changing fluorescence of a calcium sensor (Ziv et al 2013), but the convenience of unit isolation in this case comes at the price of temporal resolution.

Early studies of place cells used a subjective method of assigning neuron firing to behavioural acts: a researcher heard the spikes, observed the behavior, and registered both using a paper and a pen. Objective registration of neural activity and behaviour became a state-of-the-art approach in 1983 (McNaughton et al 1983a), which allowed for the correlation of neuron firing rate with the animal velocity.

A couple of early studies used multi-day single-unit electrode or microdrive recordings to demonstrate the long-term stability of place cells. One such study demonstrated place field stability across days (Muller et al 1987), and another demonstrated a stable place field for 153 days (Thompson & Best 1990). Calcium imaging with microendoscopes (Ziv et al 2013) later proved to be a more useful technique to evaluate the long-term stability of place cells. Although the first report (Ziv et al 2013) was somewhat misleading because the flexibility of mouse hippocampal maps was not taken

into account, a more cautious study (Kinsky et al 2018) subsequently confirmed the long-term stability of hippocampal maps.

The implantable microdrives were convenient for ensuring the precise location of the electrode tips in the pyramidal layer but provided a limited yield of single units. Microdrive arrays, like the Hyperdrive (Gothard et al 1996, Wilson & McNaughton 1993), allowed for insertion of multiple probes into a limited area, substantially increasing the yield of hippocampal single units. This, in turn, made population analysis of hippocampal maps possible, leading to the implementation of such methods as principal component analysis and Bayesian decoding.

Electrophysiological studies frequently suffer from restrictive experimental settings. Particularly, the size of the explorable enclosure is limited by the cables connecting the microdrive array on the animals head to the connector on the ceiling of the recording room. As a result, the animals explore a much smaller environment than a natural habitat. Two approaches were employed to overcome this limitation. The Moser group used movable acquisition systems to transport both the rat and the equipment from one room to another (Alme et al 2014). This study demonstrated that the hippocampus creates a new map for every enclosure without recycling them. In another study, a rat explored a 48-meter long maze and was followed by a researcher pulling a stand with cables and cable tensioning mechanisms (Rich et al 2014). This study described the stochastic nature of place field distribution in large environments.

A particularly challenging and fruitful line of research stemmed from intracellular recordings of hippocampal neurons in freely moving animals (Lee & Brecht 2018, Lee et al 2006a, Lee & Lee 2017, Lee et al 2014, Long & Lee 2012). This method allowed

researchers to register subthreshold depolarizations of hippocampal cells, which demonstrated the intrinsic differences between the place cells and silent cells: place cells appeared to be predetermined to represent a new environment by their spike threshold and propensity to burst before the start of exploration (Epsztein et al 2011). It has also been found that a spatially uniform depolarization of a silent cell during spatial exploration leads to the emergence of place fields (Lee et al 2012), suggesting a presence of spatially tuned input even to silent cells.

Thus, advances in experimental strategies, data acquisition technologies, and computational techniques have brought us to the current understanding of the properties of hippocampal neurons. A hippocampal cognitive map can now be described using three main parameters: the identity of active place cells, the place field locations, and the firing rate of the engaged place cells. All three features change simultaneously every time the animal enters a new environment (Alme et al 2014, Leutgeb et al 2004). The firing rate can also be affected independently when subtle changes are introduced into the context, including changes to the colour of the walls (Jeffery & Anderson 2003, Leutgeb et al 2005), the intentions of the animal (Bower et al 2005, Frank et al 2000, Ji & Wilson 2008, Wood et al 2000), or the time that has passed since the last exposure to a familiar context (Mankin et al 2012). Thus, the subset of active cells and the place field (PF) locations encode the spatial component of a scene, whereas the rate map encodes the non-spatial components. Whereas the classic map only accounted for PF locations (referred to as the ‘field location map’ in this manuscript), a hippocampal spatial map is now described by the identity of PCs, the PF locations, and the firing rate.

Place cells systematically shift their firing in relation to the phase of theta cycle as the animal passes through the place field (Maurer & McNaughton 2007, O'Keefe & Recce 1993, Skaggs et al 1996). This phenomenon is known as ‘phase precession’ can be used for proper delineation of place field boundaries. The first spikes within the field occur later in the theta cycle, but the following spikes occur on earlier phases of the theta cycle, making almost 360-degree shift along the theta cycle, but never more.

1.2 The rate map in the hippocampus

The field location map and the rate map differ a lot in terms of their stability in new environments. The field location map becomes established during an animal’s first traverse through a new enclosure and stays relatively stable for the duration of the experiment (Hill 1978, Navratilova et al 2012b, Wilson & McNaughton 1993). In contrast, the rate map exhibits strong variations within the first few episodes of exploration but then stabilizes (Navratilova et al 2012b). Thus, different neural mechanisms are expected to be involved in establishing the field location map and the rate map in the hippocampus.

According to the path integration theory, the field location map is based on a path integration mechanism (McNaughton et al 1996, McNaughton et al 2006) which employs internal, egocentric signals as the main source of information to establish the map. External stimuli are of minor importance and are used to correct the accumulating error of the path integrator. This theory accurately describes the place field properties where spatial cues are dominant. However, there is evidence for firing of hippocampal cells that is specific to certain non-spatial stimuli, like objects and cues (Wood et al 1999, Young et al 1994), in behavioural tasks where non-spatial stimuli prevail.

Unlike the field location map, the rate map is affected by numerous, often uncontrollable internal and external cues. A detailed description of rate code perturbations is lacking since many of the contributing factors were discovered just recently (and it is likely that many more remain undiscovered). However, the importance of the rate component in the hippocampal cognitive map can be inferred from the theoretical point of view. The stability of a field location map is considered the basis for the maintenance of spatial memory (Barnes et al 1997). It is expected that each time the animal enters a familiar enclosure, the same set of place cells will be activated producing the same set of place fields (Barnes et al 1997, Kinsky et al 2018, Muller et al 1987, Thompson & Best 1990) (but see (Jeffery et al 2003, Ziv et al 2013)). Since the place cells have an arguably causal role in navigation (de Lavilléon et al 2015), a failure to reactivate the field location map should result in aberrant spatial memory. If the preservation of spatial memory requires the subset of activated cells and the locations of corresponding place fields remaining unchanged during every visit to the enclosure then the mechanism to remember different episodes within the same enclosure should employ some additional feature of the cognitive map. The most probable candidate mechanism to be superimposed over the field location map without disrupting it is the place cell firing rate. Speaking in terms of episodic-like memory in rodents, which assumes remembering what-where-when details of an event, the 'where' component is reflected in the field location map, whereas the 'what' and 'when' should be encoded by means of the rate map.

Rate remapping, or the establishment of a new rate map, can be induced by diverse factors, each of specific interest. To study the basic features of rate remapping mechanisms, we used one of the most simplistic and ecologically relevant models: that where a rat runs

on a restricted linear path and rate remapping appears as an increasing difference between the within-field firing rates for the two opposite directions of running (Battaglia et al 2004, Navratilova et al 2012b).

1.3 Establishment of the Hippocampal Map on a Constrained Path

For the hippocampus, establishing a new map can be a proxy for learning. Generally, learning something new, such as a new skill, takes a considerable number of repetitions to enable the gradual adoption of the new information. Even simple associative learning, like blinking in response to a tone, requires a few trials to learn. Considering the number of unique episodes of experience in an animal's life, the hippocampus (the structure evolved to store episodic memory) cannot always rely on repetitions for learning. To memorize events that happen only once, the hippocampus must be implementing a unique mechanism that enables fast learning, including the use of preconfigured blocks to create new memories (Dragoi & Tonegawa 2011, Dragoi & Tonegawa 2013) and fast synaptic modifications.

The changes exhibited by the hippocampal map during the first few minutes after its creation are the consequences of this fast learning. Therefore, the map features evolving during the first few passes through the place field are of particular interest, since they reflect the processes enabling fast learning and fast stabilization of the hippocampal map. Namely, these features are the place field directionality, place field expansion and the place field backward shift.

While passing the same spot on a constrained path in opposite directions, like on a linear or circular track, a rat gets repeatedly exposed to two arguably distinct sets of stimuli. In such a case, the distant cues and the head direction (HD) signals are unique for each

direction. Alternatively, the path integration mechanism provides sufficient information to confirm that the animal is traveling through the same spots, maintaining the stability of the place field locations. Electrophysiologically, this appears as a place cell having overlapping place fields in both directions but with the number of spiking events differing for each direction (Battaglia et al 2004). The difference in firing rate between the two directions can be expressed in terms of the directionality index (DI), which will be described in detail in the Methods section. The higher the DI, the more different the firing rate is between the two directions. During the first run in an unfamiliar maze, the DI values are low and gradually increase until they plateau after several laps. However, when the rat explores a familiar maze, the DI is high (Navratilova et al 2012b). The gradual increment of DI probably reflects a learning curve where the path integration information is becoming associated with additional non-spatial stimuli. It is likely that the additional stimuli are the distant cues used to correct for the cumulative error of the path integrator (McNaughton et al 1996).

Notably, if salient local cues are present within the maze, the place fields near them show reduced directionality (Battaglia et al 2004). This observation suggests that whatever information is encoded in terms of place field directionality, it can be overridden by the invariant representation of a salient local stimulus. The presence of direction independent components increases the correlation between the two input vectors for two directions.

In addition, the place field directionality cannot be explained merely by the effect of the maze walls along a constrained path. In an open field, if a rat runs back and forth following a linear path, the directionality of the place fields is similar to that on a constrained path (Navratilova et al 2012b).

The field location map can be observed just after one pass through the environment remains relatively stable starting from the very first pass through the environment (Hill 1978). However, some changes to the field size and position can be observed. Place fields expand and their centre of mass (COM) shifts slightly backward as the animal spends more time on a maze (Mehta et al 1997, Navratilova et al 2012b). Both adjustments develop over the course of several laps and, while more pronounced in a new maze, are also significant in a familiar maze (Mehta et al 1997). The place field shift was also observed after just one lap in an environment with altered cues (Lee & Knierim 2007, Lee et al 2004c), emphasizing the speed of learning in the hippocampus. A biological phenomenon proposed to explain experience-dependent place field shift and expansion is the formation of asymmetric functional connections between place cells with adjacent place fields that represent the order of PF activation along a route in one direction.

2 General Objectives

This study attempts to address several questions that arose from previous studies and to investigate the adjustments of the hippocampal map during the first few laps on a new maze.

It is still unclear what drives rate remapping on a constrained path resulting in place field directionality. In the simple task of running in a cue-poor circular maze, the main candidate drivers for rate remapping are the distant cues around the maze or the HD signals from the HD cells (Taube et al 1990) or conjunctive cells in the medial entorhinal cortex (MEC) (Sargolini et al 2006a). In this study, the distant visual cues were made temporarily unavailable by switching the lights off to test if the distant cues were necessary for the

development of the place field directionality. Thus, the first few laps in a new maze were run in darkness. Distant cues were also made temporarily unavailable on subsequent days of training to ascertain if the continuous presence of distant cues is important for the manifestation of place field directionality.

Another important question is whether or not there is long-term retention of the rate map in the hippocampus. The rate map is known to be successfully retrieved on the following day after training (Navratilova et al 2012b), but consistent retrieval after longer periods of time remains unexplored. To test this, the rat was re-exposed to the same environment 5 days after the end of training to check if the rate map was successfully retrieved or developed *de novo*.

A third objective was to determine if visual cues (and manipulation of the visual cues) could affect place field expansion and backward shift. The current view suggests an asymmetric strengthening of the synapses between the place cells with adjacent place fields simply requires coactivation of these place cells with no regard for the stimuli driving the activation (Mehta et al 1997, Mehta et al 2000). Considering the sparseness of the CA1 recurrent network (Thomson & Radpour 1991), asymmetric synapse modification should occur somewhere upstream in the hippocampal network, either in CA3 where rate remapping associated with visual cue manipulation is more pronounced (Leutgeb et al 2004), in the dentate gyrus (DG) where the allocentric and egocentric pathways from LEC and MEC converge, or in the Schaffer collateral synapses. These considerations warranted the investigation of the effects of visual cues on CA1 place field size and position.

Lastly, hippocampal place cells were recorded bilaterally to investigate possible interhemispherical differences in these place field properties.

3 General Methods

Animals

The data were collected from 4 male Fisher/Brown Norway hybrid rats. Rats were housed in pairs before surgery and were housed individually after the hyperdrive implantation. The animals were kept on a 12 h light / 12 h dark cycle. Training and recording sessions took place during the light phase. All protocols were approved by the Animal Welfare Committee of the University of Lethbridge.

Recording Environment

The rats ran laps on a plain circular track, 1 meter in diameter, with no salient local cues and a single barrier requiring the rat to turn around and run in the opposite direction, as described by (Navratilova et al 2012b). The track was placed in the centre of the recording room with numerous salient distant cues, including tables, a computer monitor, etc. The turn-around point was illuminated by a dim red bulb during the dark laps. During the light laps, the maze was illuminated by a moderately bright LED array placed on the ceiling of the room.

To change the context, two curtains were used to cover two adjacent corners of the room and change the apparent geometry of the room. A rat was covered with a cardboard box when the changes were applied; therefore, the animal did not witness the changes to the visual cues. The cardboard box was removed only after the lights were turned off and immediately before the start of the dark laps.

Noise level in the room was measured from the centre of the maze against 4 room walls using a M500 Pettersson ultrasound microphone (Pettersson Elektronik AB, Uppsala, Sweden). The frequency-amplitude profiles were similar for all 4 directions with the exception of a sharp peak at 25 kHz. For the 4 directions, the peak was 6 dB, 6 dB, 2 dB and 0 dB above the background level of 35 dB. The sound appeared to come from the fluorescent lamps located in the hallway outside the room and can be defined as a distant audible cue.

Foraging Behavior

The rats were trained to run on a linear track for food reward. To promote running, food restriction was applied for 7-10 days before the start of recording sessions, and the rats were kept at 85-95% of free-feeding body weight throughout the experiment. The rats were pretrained to run for a food reward in mazes of different shapes. All mazes used for pretraining differed from the experimental maze in size and/or shape: 2 linear mazes, 1.2 meter and 2 meter long, one circular maze, 40 cm in diameter. The pretraining sessions never took place in the experimental room, but were performed in the control room or different recording room. The pretraining involved running for food reward in darkness and under illumination. The rats were habituated to the experimental room during tetrode turning while sitting in a flowerpot in the centre of the room.

Microelectrode assembly

The rats were implanted with a dual-bundle Hyperdrive, similar to that previously described (Lansink et al 2007). The hyperdrive was comprised of 21 individually movable microdrives each loaded with a tetrode made of four, 12.5 µm, polyimide insulated,

nichrome wires which were twisted together. The tips of the tetrodes were gold-plated to reduce the impedance of each electrode down to 200-500 kΩ. A general description of the tetrode fabrication, hyperdrive design, and assembly can be found elsewhere (Gothard et al 1996, Kloosterman et al 2009, Lansink et al 2007, Nguyen et al 2009). The distance between centres of the bundles was 5 mm for 3 animals and 6 mm for 1 animal.

Several changes were introduced to make the Hyperdrive more robust and facilitate its recovery and repeated use. These improvements included a headpiece, internal ground and the use of metal hexnuts for the microdrives (Fig. 1).

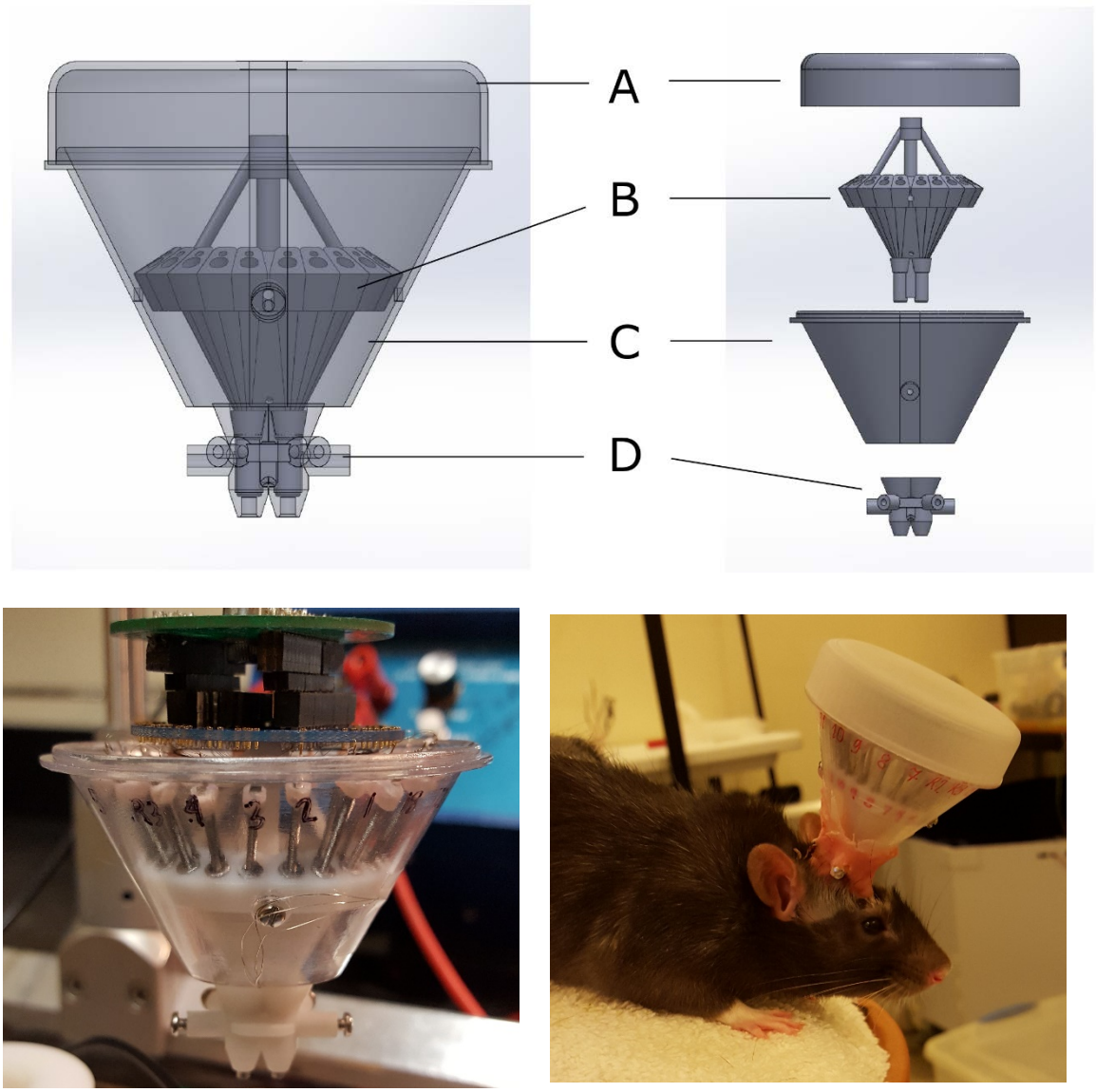

Figure 1. The Hyperdrive.

Top: Computer-aided design (CAD) rendering of the 3D printable parts for the dual-bundle Hyperdrive. Bottom left: the Hyperdrive loaded with the tetrodes. Bottom right: rat and the Hyperdrive after implantation. A – cap, B – Hyperdrive body, C – protective cone, D – headpiece.

The headpiece allowed for easy recovery of the hyperdrive. Following euthanasia, the hyperdrive could be released by loosening 4 screws on the headpiece and removing the

portion of dental acrylic holding the cone. Following that, the hyperdrive bundles came off cleanly with the tetrodes sticking out. One of the Hyperdrives was fully recovered by submerging the bundles into a lens cleaning solution and distilled water. Afterwards, the tetrodes were gold-plated to ensure proper impedance of the electrode and the hyperdrive was implanted into another animal. The data from rat P3103 was obtained using this recovered hyperdrive.

The hexnuts provided smoother and more precise travel of the microdrive, in comparison to a thread made in the plastic body of the hyperdrive. Unlike the thread in plastic, hexnut performance did not deteriorate with repeated use, which is particularly useful for a hyperdrive intended for multiple repeated uses. The same approach proved its robustness in a microelectrode array that was repeatedly used in acute experiments in mice (Bermudez-Contreras et al 2018).

Another improvement we introduced to the hyperdrive design was the internal grounding. As a standard procedure, the ground wire is soldered to the ground screw implanted over the cerebellum. Another end of the wire is soldered to an electronic interface board (EIB). Implantation of a standard hyperdrive with external ground requires the ground screw and a part of the ground wire to be covered with dental acrylic, so the recovery of the standard hyperdrive requires re-soldering of the ground wire with potential to damage the EIB and warranting replacement of all the wiring (including the EIB). To facilitate improved hyperdrive implantation and recovery procedures, we routed the ground wire inside a metal cannula forming one of the bundles. Since all guide cannulas for the tetrodes were also metal and contacted the ground cannula, they were also grounded. This solution appeared to be both convenient and reliable; no grounds were lost in any animals

over the course of the experiments and referencing to this ground variation was just as good as that to an electrode in the corpus callosum. Another advantage of using the internal ground as a reference is that it does not need to be lowered into the corpus callosum before use, it picks up no neurons, and its signal does not get contaminated by the volume conducted sharp wave ripples (SWR).

Surgery

Stereotaxic implantations were performed under isoflurane anesthesia. Two circular craniotomies were performed with the centre coordinates of -3.5 mm posterior to Bregma and 2.5 or 3.0 mm lateral from the midline to accommodate the bundles of the hyperdrive. Dura mater was removed, and the bundles were placed so that they just touched the surface of the brain to ensure proper contact with the internal ground wire. The craniotomies then were sealed with the low viscosity “Kwik-Sil” silicone.

To increase the stability of the implant, 7 titanium anchor screws (1.14 mm in diameter) were driven through the holes in the skull drilled with a 0.9 mm drill bit. Our prior experience indicates that it is crucial for implant stability to ensure complete penetration of the screws through the skull. After the animal was fixed in the stereotaxic apparatus, the eyes were covered with artificial tear ointment and soft gauze. An improvised aluminum foil mask then was secured on the top of the gauze to prevent eye damage from the lamps used to illuminate the surgical field. At the end of the surgery, all tetrodes were turned down by 1 mm to ensure they entered the brain. The rats were injected subcutaneously with Baytril and Metacam daily for 3-5 days to control inflammation, pain, and infections. The rats were allowed to recover for 7 days before any further manipulations and procedures.

Electrode placement and data acquisition

Tetrodes were lowered to CA1 or CA3 areas of the hippocampus over the course of 2-4 weeks. The signal was referenced against an electrode in the corpus callosum or the internal ground wire. One of the tetrodes was lowered to the hippocampal fissure, 300 μm below CA1 pyramidal layer, for local field potential (LFP) recordings and off-line extraction of the theta signal. The signal was preamplified using three 32-channel (HS-27) headstages (Neuralynx, Bozeman, Montana) and then filtered, digitized, timestamped, and recorded using a Neuralynx digital Cheetah system. For spike detection, the signals were digitally filtered between 600 and 6 kHz and sampled at ~32 kHz. A threshold algorithm was used to detect the candidate spike events with peaks of >50 μV in at least one of the channels. For LFP channels, the signals were digitally filtered between 1 and 1000 Hz and sampled at ~2 kHz. The animal's position was tracked using Cheetah software and a video camera positioned above the maze, with a resolution of about 3 pixels per centimeter. When the lights were on, the position was tracked using red and green reflective tape (3M). In darkness, the position was tracked using infrared LEDs (Fig. 2).

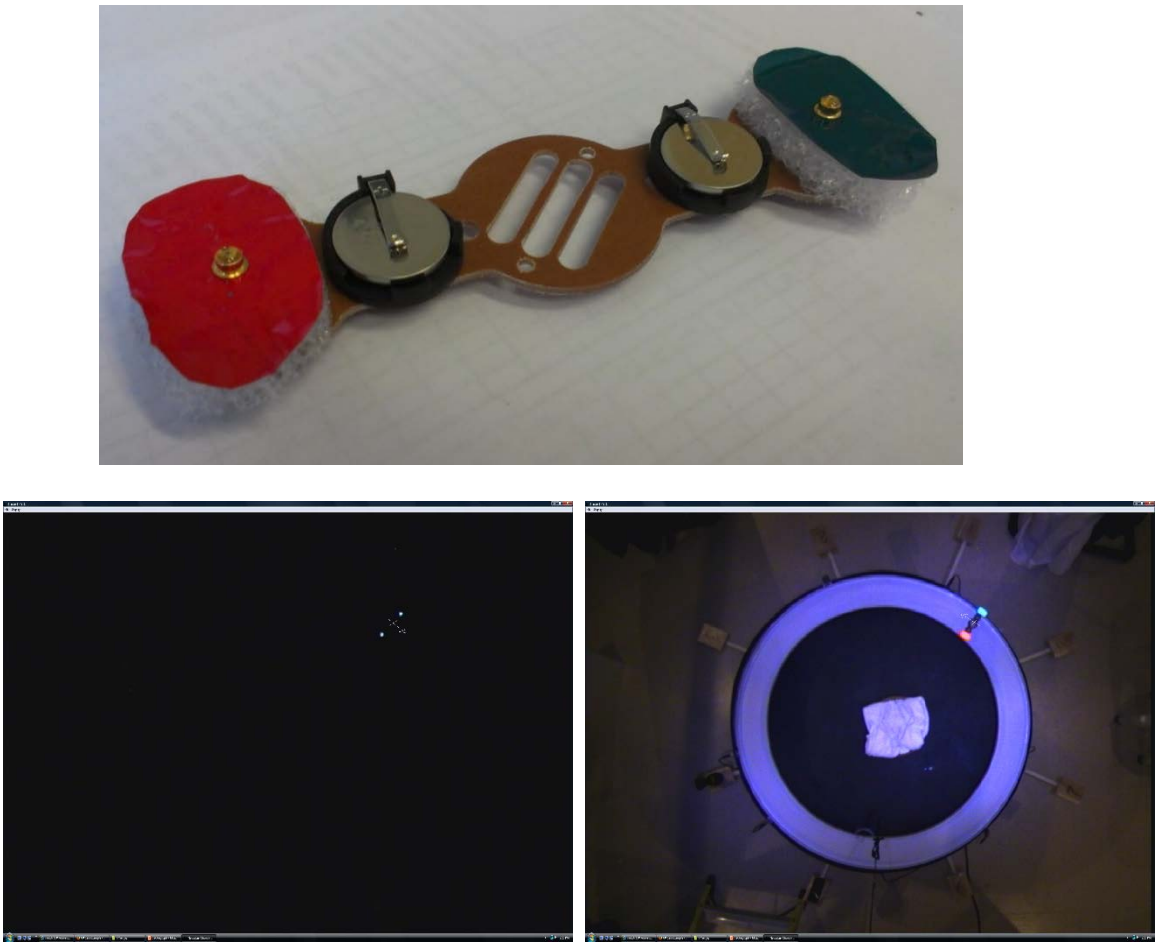

Figure 2. Animal video tracking in darkness and under illumination using a custom head mount.

The distance between the infrared LEDs was 14 cm. Tracking based on reflective tape and infrared LEDs resulted in detection of the same centre of mass by the tracking software.

Recording sessions

The recording sessions consisted of 4 dark laps followed by 4 light laps with a possibility of running 2 dark and 2 light laps after a 30-minute break (Fig. 3). On the 3rd day of recording, the 30-minute break was followed by a context change and the rat ran 4 dark and 4 light laps in context B with altered distant visual cues. The context change

involved curtains that were placed to cover two adjacent corners and visually change the geometry of the room. In addition, a few objects, including a big bucket and a metal stand, were moved to different positions. The new context was used for two more days of recordings (days 4 and 5). The dark laps involved a dim red light bulb illuminating the turn-around point and the food wells on the circular track. On Day 10, the rats were again exposed to the first context, 5 days after the last training session and 7 days after the last exposure to the first context. The design of the test session was the same as that of the first training session, beginning with 4 dark laps and followed by 4 light laps.

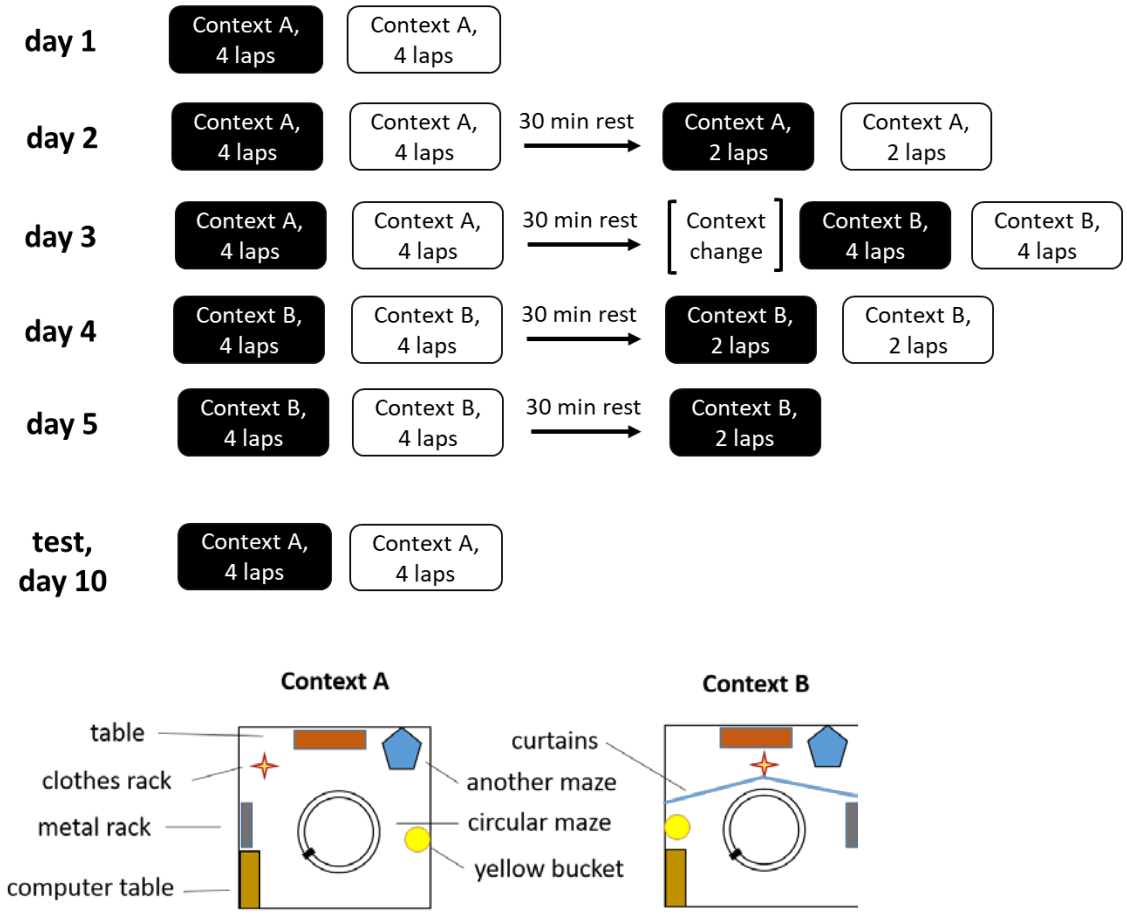

Figure 3. Recording session design.

Black boxes represent laps in darkness, and blank boxes represent laps with lights on. On day 3, the visual context was changed after a 30-minute break: curtains were placed to

cover two adjacent corners and visually change the geometry of the room. Running sessions on days 4 and 5 were conducted in this altered context. The test session was held on day 10, 5 days after the last training day, in the original context.

Perfusion and histology

At the end of the physiological recordings, positions of the tetrode tips were marked with a small direct electric current (5 μA, 10 s, positive to electrode, negative to the tail) ~7 days before the animals were perfused. The rats were deeply anesthetized with isoflurane, intraperitoneally injected with a lethal dose of pentobarbital, and then perfused first with chilled 1X phosphate-buffered saline (PBS) and then with chilled 4% (wt/vol) paraformaldehyde in PBS. Extracted brains were post-fixed overnight in 4% paraformaldehyde and then cryoprotected in 30% sucrose in PBS. Coronal 40um sections were subsequently prepared using a sliding microtome and slide-mounted brain sections spanning the region of interest were then stained with 0.5% cresyl violet to histologically confirm the positions of the electrode tips (Fig. 4).

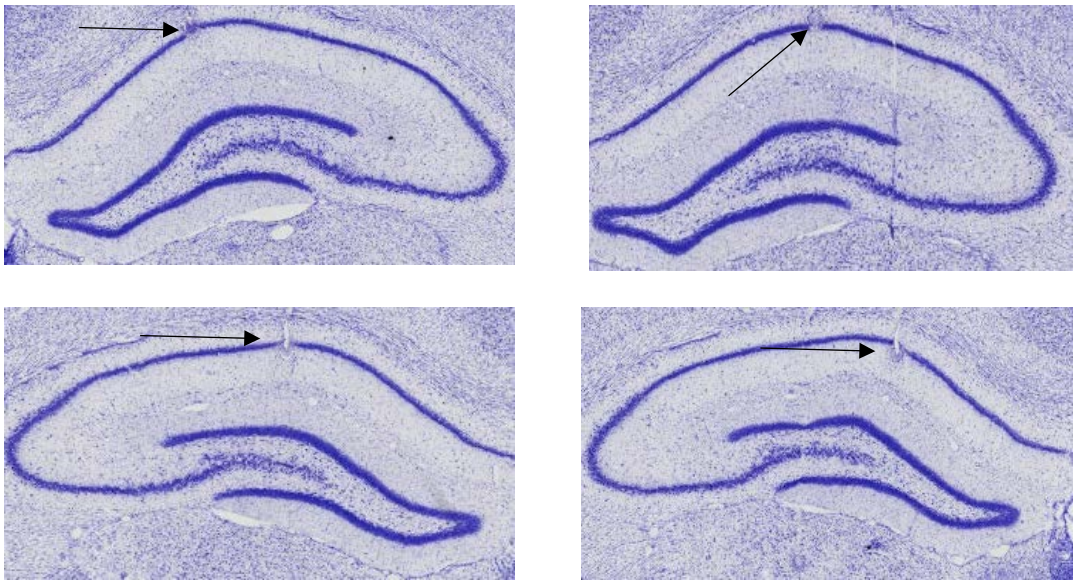

Figure 4. Examples of cresyl-stained coronal sections used to verify electrode positions in the hippocampus. Arrows point to electrode tips.

Data preprocessing

Spike sorting was done in two steps. First, the spike data were automatically processed using KlustaKwik (K. D. Harris, http://klustakwik.sourceforge.net/) to find clusters that were then manually curated in a custom version of MClust 3.1 (A. D. Redish, http://redishlab.neuroscience.umn.edu/MClust/MClust.html). Only well isolated clusters with <0.4% of the noise spikes within the absolute refractory period (2 ms) were incorporated into the analysis.

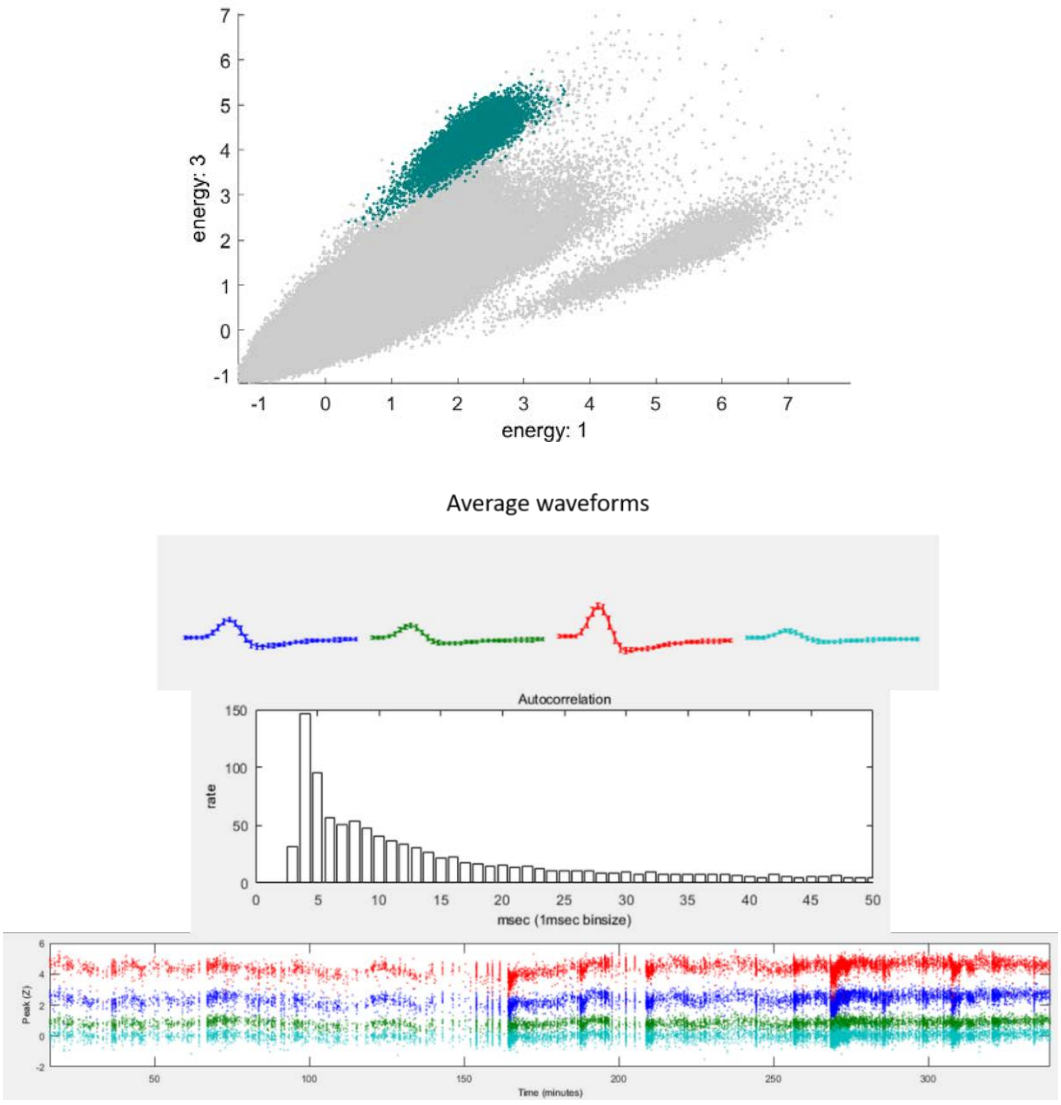

Figure 5. An example of a well-isolated cluster on an energy plot.

The green cluster had zero spikes in the 2 ms absolute refractory period window on the autocorrelation plot and had stable amplitudes throughout the recording session.

Place field analysis

Animal position was determined based on bright pixels originating either from reflective tape or from the infrared LEDs. The 2D position of the rat running on the circular track was then linearized to a 1D position. Periods of immobility were eliminated by finding the

epochs in which the animal's speed was less than 3 cm/s for more than 1 second. The velocity was calculated using smoothed (1s hamming window) positional data. The analysis only included laps in which the rat covered at least 80% of the maze moving in the correct direction. Otherwise, the laps in the clockwise and in the counterclockwise direction were removed from the analysis. The positional data were binned into 2-deg bins and then the smoothed (5 bin hamming window) firing rate data were assigned to the positional bins. The place fields were required to have at least a 0.7 Hz peak firing rate to be included in the analysis, and the boundaries of the fields were assigned to the bins where the firing rate (FR) first dropped to 0.05 Hz. The boundaries were assigned individually in each running direction (Fig. 6). If the field had few or no spikes in one of the directions, the boundaries for the opposite direction were used. The fields that occupied less than 20 degrees of the track (17.5 cm) were excluded from the analysis in agreement with the threshold used in previous study (Navratilova et al 2012b). All place field boundaries were checked manually using the phase precession plot, and adjusted if needed (Maurer & McNaughton 2007). Most adjustments were made to include a few spikes on the phase precession plot excluded by the algorithm, or to separate two overlapping fields, or to exclude spurious spikes. Overlapping fields that could not be separated and the fields overlapping the food dish location were excluded. The direction in which the field exhibited more spikes over the recording session was assigned as the higher FR direction, as opposed to the lower FR direction.

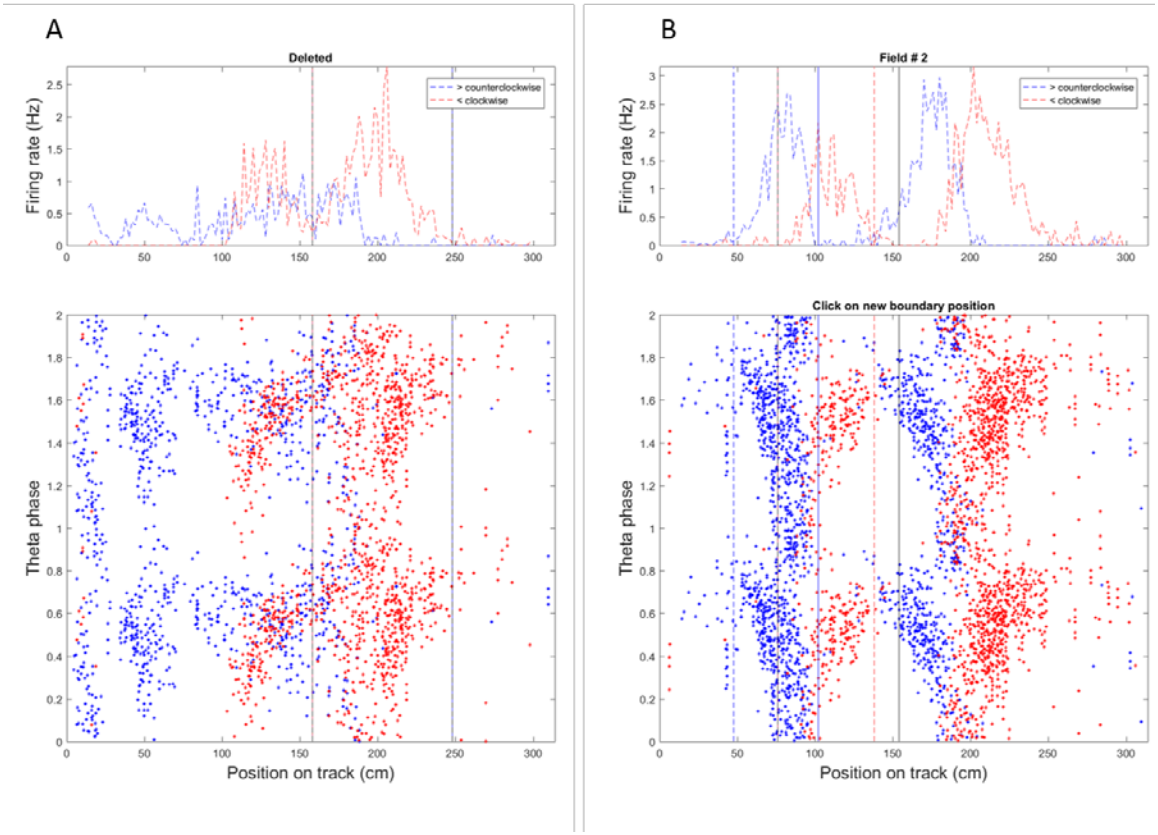

Figure 6. Assignment of the place field boundaries.

A – an example of inseparable place fields. These fields were excluded from the analysis.
B – an example of separable place fields.

The direction-specific firing of the place cells was assessed by means of a Directionality Index (DI) (Navratilova 2012). The DI is calculated as the difference in the number of spikes between the two directions of running divided by the total number of spikes. The lap-specific field size was measured as the distance between the first and the last spikes emitted during a given pass through the place field. The place field centre of mass (COM) was calculated as the mean position of all spikes emitted during a given pass through a place field.

Statistics

The normality of distributions was tested using Lilliefors test.

A *t*-test was employed for paired and independent samples as a significance measure for the parameters that followed normal distribution, and Wilcoxon's test was used for parameters that didn't follow normal distribution. The Cohen's *d* was used as an effect size measure. The Cohen's *d* for paired and independent samples was calculated using the Matlab function 'computeCohen_d(x1, x2, varargin)' (Ruggero G. Bettinardi, 2019) (https://www.mathworks.com/matlabcentral/fileexchange/62957-computecohen_d-x1-x2-varargin).

4 Role of Distant Cues in the Establishment and Maintenance of Place Field Directionality

In mazes with constrained paths, the directional specificity of place cells gradually develops by means of rate remapping during the first few traverses through their place fields. It was hypothesized that the gradual changes in firing properties could reflect a learning process during the course of which some supplementary information becomes associated with path integration signals. It is reasonable to assume that this additional information is used to differentiate between two directions and originates either from the perception of distant cues or HD signals. We tested the importance of distant visual cues to place field directionality by making rats run the first laps in a new maze in darkness. We also tested the immediate importance of distant cues to the manifestation of rate remapping by temporarily switching lights off during the subsequent days of training.

We demonstrated that the absence of distant visual cues had no significant effect on the development and manifestation of place field directionality. Therefore, gradual rate remapping and place cell directionality must be driven by HD signals or non-visual distant cues.

4.1 Background

Place cells show direction specificity when a rat traverses place fields in only two opposite directions, as opposed to omnidirectional traverses during random foraging in an open field. This phenomenon has been observed in radial arm mazes (Markus et al 1994, Markus et al 1995, McNaughton et al 1983a, Muller et al 1994), one-dimensional tracks

(Battaglia et al 2004, Navratilova et al 2012b), and even during a directed search in an open field when, despite the lack of physical constraints, the rat moves along a linear trajectory (Markus et al 1995, Navratilova et al 2012b).

Place field (PF) directionality develops gradually and is affected by both local and distant cues. Place cells exhibit little to no directional specificity during the first run in a new maze but promptly begin to distinguish between two directions after as few as 4-5 traverses through their place fields (Navratilova et al 2012b). The presence of salient local cues within the maze reduces the directional specificity of place cells (Battaglia et al 2004), whereas the complexity of the cues surrounding the maze has no effect on directional firing (Markus et al 1995). A complete absence of distant visual cues, as during running in darkness, also has no significant effect on place cell directionality (Markus et al 1994). The latter is surprising considering that manipulation of distant visual cues resulted in pronounced rate remapping in more recent studies (Leutgeb et al 2005), and rate remapping is the mechanism driving place field directionality. It is likely that the discrepancies in the effects of distant cues lie in the complexity of the behavioural task. In Markus et al., the rats performed a forced-choice task in an 8-arm maze, whereas in Leutget et al., animals randomly foraged in a square box. If this is true, then the role of distant cues can be minimized by increasing the complexity of the task. Besides, it has been shown that visual cues are capable of producing directional firing of place cells in the absence of a differentiating HD signal, like when head-fixed or in a virtual reality setup (Acharya et al 2016).

Accumulated data suggest the following mechanism of place field appearance and transformation during a rat's first few runs in a new maze. From the very first run, the path

integration mechanism defines the place field locations (McNaughton et al 2006). The location of these place fields will remain largely unchanged, with only a slight backward COM shift taking place during the next few laps (Mehta et al 1997, Navratilova et al 2012b). Within the first few laps, additional environmental and path integration information then becomes associated with the place cells and represented by changes in firing rate. If this information is directionally invariant, like salient local stimuli on the maze, the firing rate for two running directions will remain the same. If the information for each of the two directions is different, like the relative position of distant cues or HD signals, the place fields will develop directionality by means of rate remapping.

The hippocampus is a crucial brain structure for forming new explicit long-term memories (Scoville & Milner 1957, Squire 1992, Squire & Dede 2015) and reactivation of episodic memories long after the initial acquisition (Corkin 2002, Pilkiw & Takehara-Nishiuchi 2018). The cognitive map, comprised of place cells, serves as a cellular foundation for these long-term memories (Barnes et al 1997, de Lavilléon et al 2015, Muller et al 1987, Thompson & Best 1990).

Rodents demonstrate a type of memory that is very similar to episodic memory in humans. Episodic memory in humans involves re-experiencing the events and scenes from the past, a phenomenon that we are unable to prove occurs in other animals. Therefore, this type of memory in animals is called episodic-like memory. It includes “what”, “where”, and “when” components and depends on an intact hippocampus. Each of these components is represented in a cognitive map. “Where” is encoded in the subset of recruited place cells and the locations of their respective place fields. The rate map is supposedly able to reflect all other important memory components, including the “what” and “when” components of

episodic-like memories. Demonstrating that the rate component of the cognitive map can be retrieved from long-term memory would be an important piece of evidence for the proposed model of the cognitive map as the basis for episodic memory.

4.2 Study Objectives

The importance of distant visual cues for place cell directionality was tested in two ways:

1. The necessity of distant visual cues for the acquisition of PF directionality was tested by recording the first laps in a novel maze in darkness.
2. The necessity of distant visual cues for maintenance of directionality was tested by temporarily switching the lights off during the 2nd and 3rd days of recording.

If distant visual cues are necessary for acquiring direction specificity then the directionality index will remain at low levels during dark laps and then will increase as soon as the room becomes illuminated. If direction specificity is constantly dependent on distant visual cues, then their temporary unavailability will result in a decrease in the directionality index values. All directionality measures were calculated separately for the left and right hippocampus to test for possible interhemispheric differences.

It was shown in a previous study (Navratilova et al 2012b) that the DI shows only a slight decline over a 24-hour period, but retention over a longer period remained unknown. Thus, another objective of the study was to test whether place cell directionality is preserved in long-term memory on the scale of several days.

4.3 Study design

The first laps in the new maze were critical to determine the initial direction specificity of the place cells. Thus, to promote running in a new maze without unnecessary turn-arounds in the middle of the track, the rats were pretrained to run for a food reward on several linear tracks in different rooms. The design of the recording sessions is described in detail in the Methods section. Briefly, the recording sessions consisted of 4 dark laps followed by 4 light laps with a possibility of running 2 dark and 2 light laps after a 30-minute break (refer to Methods). On the 3rd day of recording, the 30-minute break was followed by a context change and the rat subsequently ran 4 dark and 4 light laps in the new context with altered distant visual cues. The new context was used for the next two days of recordings. The rats were then exposed to the same maze 5 days after the last training session. The design of the test session repeated the design of the first training session.

4.4 Results

Over the recording sessions, the following numbers of bidirectional place fields were isolated in CA1: day 1 - 171 fields (104 in left CA1, 67 in right CA1); day 2 - 146 (93 left, 53 right); day 3 - 112 (81 left, 31 right); day 4 - 94 (60 left, 34 right); day 5 -108 (73 left, 35 right); test session - 167 (90 left, 77 right). The number of CA3 fields was low and inconsistent across days so these fields were excluded from the analysis. Of note, some fields in the analysis had spikes only in one direction of running. The fields with overlapping locations in two directions of running were count as one bidirectional field.

Table 1. Number of bidirectional place fields included in analysis (for left/right hemisphere)

day / rat	**3102**	**3103**	**3762**	**0162**	**total**
day 1	19 (11/8)	44 (17/27)	71 (49/12)	47 (27/20)	171 (104/67)
day 2	37 (15/22)		63 (48/5)	56 (30/26)	146 (93/53)
day 3			48 (43/5)	64 (38/26)	112 (81/31)
day 4			55 (51/4)	94 (60/34)	149 (111/38)
day 5			48 (42/6)	60 (31/29)	108 (73/35)
test (day 10)	14 (12/2)	43 (7/36)	43 (40/3)	66 (31/35)	166 (90/76)

Visual cues were previously claimed to be necessary and sufficient for acquisition of directional specificity (Acharya et al 2016). However, that study employed a virtual reality task in which the HD signal is invariant. In addition, the findings contradicted an early study (Markus et al 1994) that demonstrated a lack of visual cue effects on place cell directionality. This same study (Markus et al 1994) showed that place field properties differ depending on the initial conditions for maze exploration (darkness or light). We therefore ensured that the initial exploration began in the dark to test if place cells acquire and maintain directional specificity when visual cues are unavailable. Similar to a previous study (Navratilova et al 2012b) in which the visual cues were available at all times, a substantial rise in DI values was observed during the first few laps in a new maze despite the lack of visual cues available. After the first 4 laps in darkness, the rats continued exploration under illuminated conditions for another 4 laps. Switching to illuminated conditions presumably resulted in a drastic increase in optical flow and distal landmark information, but produced no changes in DI values. Overall, the results from all training days confirmed that visual cues had no effect on place cell directionality (Fig. 7).

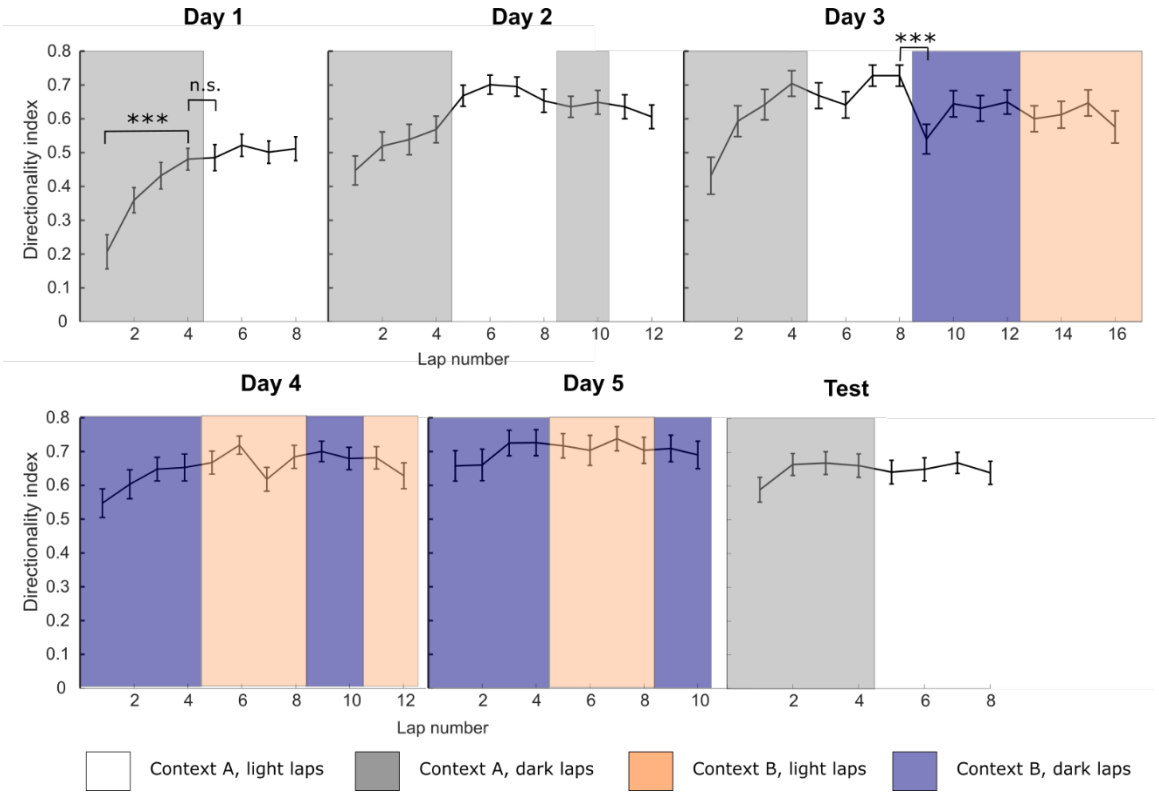

Figure 7. Place field directionality does not depend on visual cues.

Directionality index, M ± SEM. On day 1, DI values increased and reached a plateau by the last dark lap. The difference between 4th and 1st laps: +0.27, p= 0.00019, d= 0.35. No changes in DI values were observed between the 4th and 5th lap on any of five training days. On day 3, the DI dropped following the context change between 8th and 9th laps, but before the lights went on in the new context: -0.19, p<0.000001, d=-0.50 (medium effect size), explanation for this unexpected result is in the text.

Switching lights on and off had minimal effect on DI fluctuations over the five days of training (Fig. 7). However, it doesn't imply a complete lack of a rate remapping associated with change in illumination (see Fig. S3 for details). When comparing two adjacent laps a new track, there are always some PFs that change the sign of directionality index. Among these PFs, most change the directionality sign from negative to positive. The first illuminated lap was distinguished by having a substantial fraction of PFs which

changes the directionality sign from positive to negative (Fig. 3c). Though it hasn't been reflected in mean DI values, a fraction of PFs responded to introduction of visual cues by reducing the DI values.

After initial acquisition of direction specificity, DI values remained high even for the first laps on the subsequent days of training. The change of context following the 30-minute rest period on the third day of training was accompanied by a significant drop in the DI. The 30-minute breaks on the second, fourth, and fifth days were associated with no changes in the DI values.

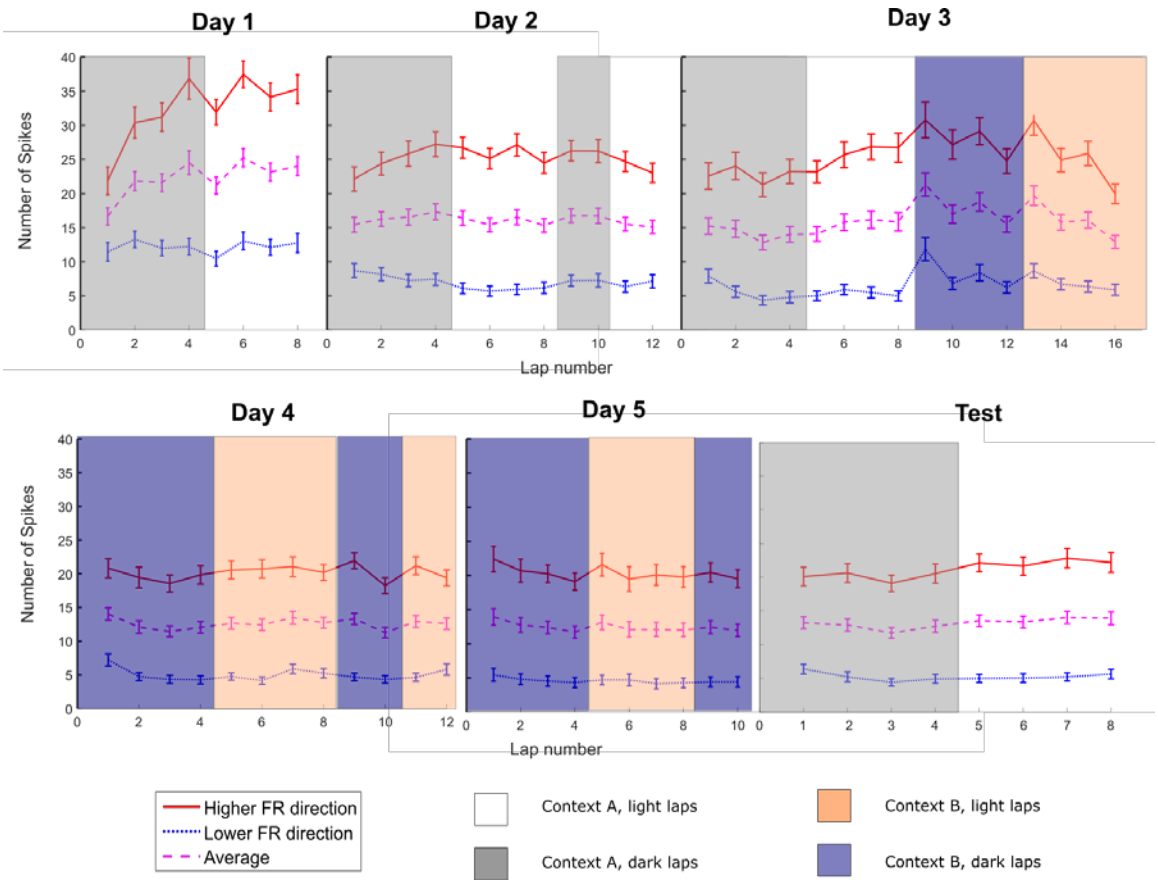

Figure 8. Spikes per lap during training and test sessions.

N, Mean ± SEM. There was no effect of visual cue availability on the FR. The firing rate on the 1st day of training is considerably higher than on other days. On the third day of training, the change of context results in a small increase in FR on the 3rd day of training.

The DI could remain constant if rate remapping is associated with a simultaneous and proportional change of FR in both directions. This possibility was tested and the number of spikes per lap also showed no response to changing illumination in the recording room (Fig. 8). Notably, the FR on the first day was higher than on all other days and another increase in FR was observed on the 9th lap of the 3rd day, immediately after the change in context. These elevated FR values are likely associated with agitation or stress during exposure to the new maze on the first day and due to the noise and room manipulations that accompanied the context change on the 3rd day, which occurred while the animal was still in the recording room.

There are notable differences between the firing rates underlying the DI changes during day 1, day 2, and day 3. On day 1, the DI increased due to a FR upsurge in the direction with a higher FR. On day 2, the DI increase was due to changes in FR in both running directions. On day 3, the initial slight increase and subsequent abrupt decrease in FR were mainly a result of FR changes in the direction with a lower FR. These results may suggest that the increase in the DI in a novel maze occurs due to elevated activity in the preferred (higher FR) direction, whereas in a familiar maze the increase in the DI is due to disinhibition of neuronal activity in the non-preferred (lower FR) direction.

It is highly unlikely that the decline in the DI on the 3rd day was associated with the altered visual environment. First, the initial 4 laps in the new context (context B) were run with the lights off. Second, the DI values did not change in response to visual cues at any other point over the 5 days of training (that is, when the lights were turned on after the first 4 dark laps each day). The decreased DI also cannot be explained by the 30-minute break, as the rest periods between laps 8 and 9 on the second, fourth, and fifth days were

associated with no changes in the DI values. A plausible explanation for the decreased DI is that stress levels were elevated due to disturbance of the animal during the context change. Importantly, the change in the DI was not a consequence of global remapping since no place fields emerged or disappeared after the 30-minute break. Together, these results imply that gradual development of directional specificity does not require visual cues and that variations in context and illumination have little effect on the stability of this directionality.

Interestingly, the change in context on day 3 resulted in certain minor changes in hippocampal rate map. Figure S4 shows that the mean number of spikes for light laps in context A and context B substantially differs for a small proportion of PFs.

The DI, as a measure of directional specificity, is susceptible to noise originating from place fields with a low number of spikes. If, during one lap, a place field has 1 spike in the higher FR direction and 0 spikes in the lower FR direction, the DI will be 1. If, on another lap, the field has 0 spikes in the higher FR direction and 1 spike in the lower FR direction, the DI will be -1. The impact of the place fields with zero spikes in any direction during the 1st lap on the DI dynamics was tested by performing a separate analysis excluding these fields (Fig. 9). This analysis showed a minimal effect of the 'zero spikes' field exclusion on the dynamics of DI values. Moreover, the changes in DI values in the 'zero spikes' fields generally followed the same patterns as the fields with 1 or more spikes in each direction during the 1st lap.

The number 'zero spike' fields on day 1 (65/171) was much higher than on the subsequent day (4/146). Some of the observed changes, e.g. increase in the number of

spikes in preferred direction on day 1, were predominantly driven by these ‘zero spike’ fields (Fig. 9).

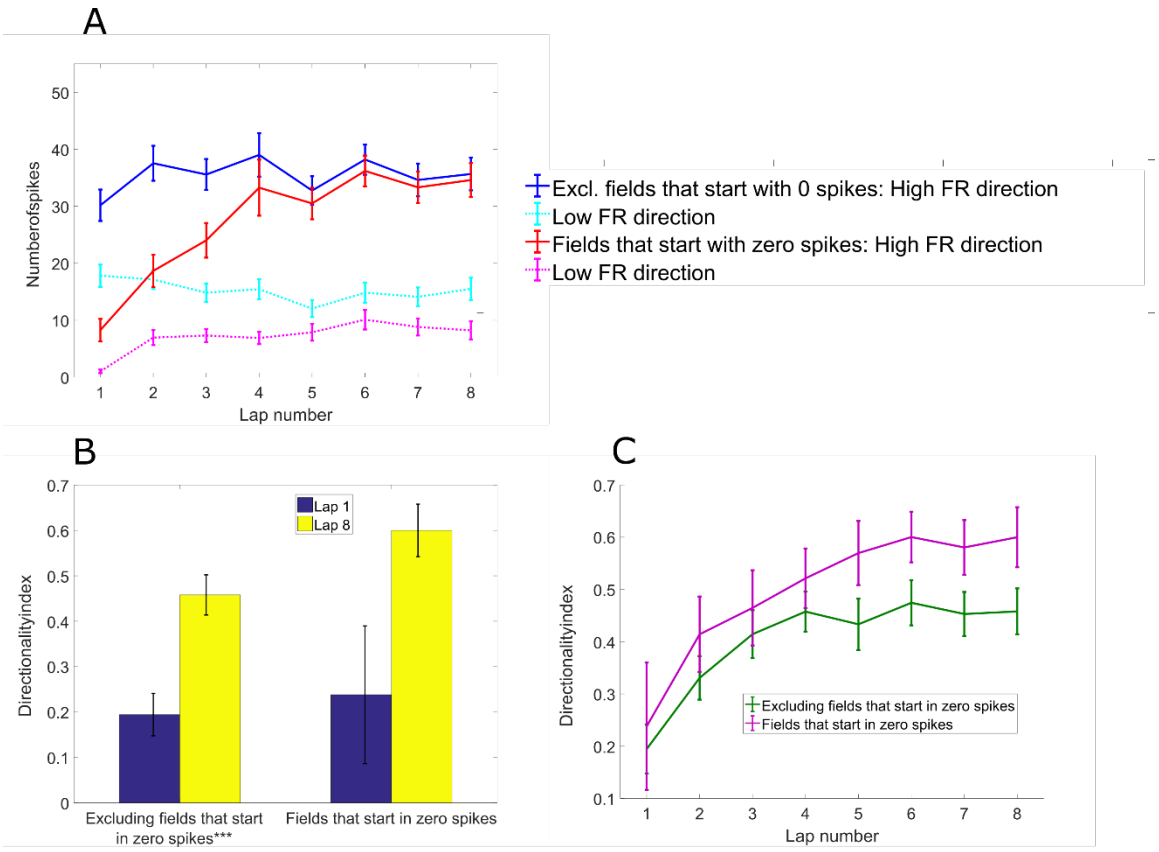

Figure 9. Directional specificity of the place fields that start with zero spikes in any direction on the first lap.

A: Number of spikes by laps for the fields that start with zero spikes in any direction versus the number of spikes for the fields that start with more than zero spikes. B: Statistics for directionality index change by the 8th lap, ***p<0.0001. C: Directionality index by laps for the fields that start with zero spikes in any direction versus the fields that start with more than zero spikes.

Interhemispheric differences in place cell directionality

In certain cases, the detection of parameter changes can be precluded upon averaging the values across functionally heterogenous bilateral structures. Acknowledging possible functional lateralization in hippocampus, the changes in DI values were analyzed

separately for left and right CA1. Prolonged training, availability of visual cues, and change of context exerted generally similar effects on place cell directionality in both the left and right hippocampi (Fig. 10).

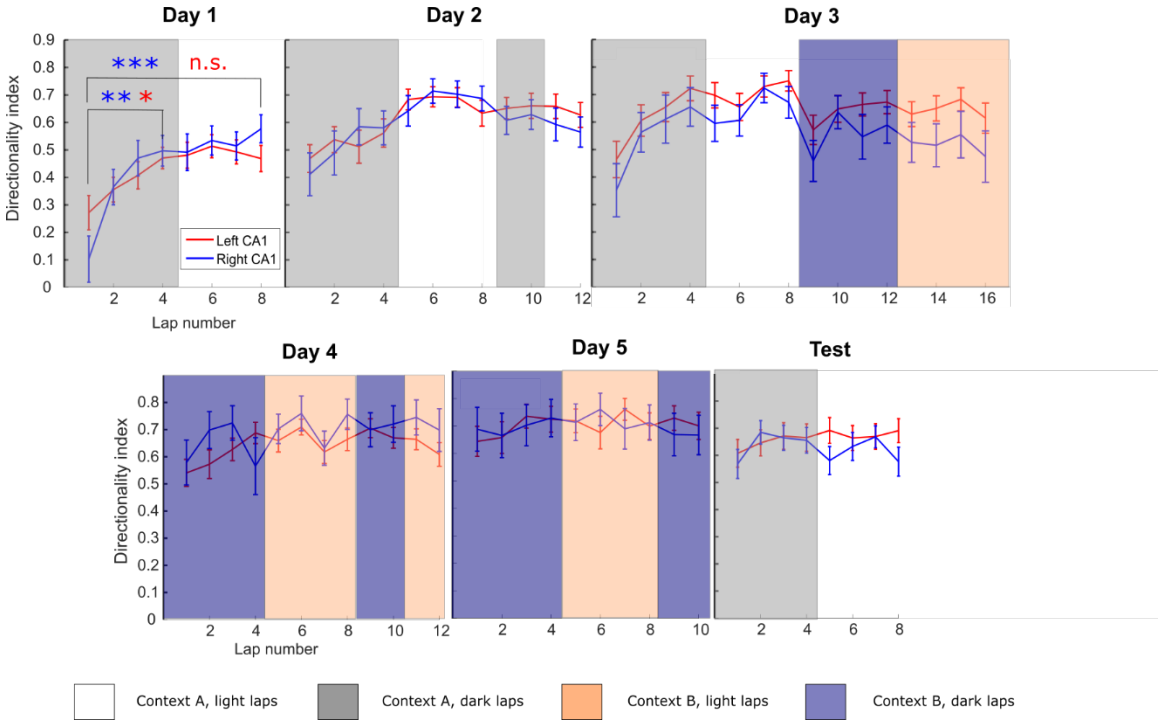

Figure 10. Changes in directionality in a new maze are marginally more pronounced in right CA1.

Directionality index, M±SEM. The bar graph shows the differences in DI between the first and the last laps of the 1st day in the left and right hippocampi: left CA1 p=0.06; right CA1 ***p<0.001. The difference in the DI on the 1st lap of the 1st day between left and right hippocampi were insignificant: Δ=0.17, p=0.13, d=0.26. The DI increase during dark laps on the 1st day was significant for both hemispheres: left hippocampus Δ=0.20, p=0.02, d=0.26; right hippocampus Δ=0.39, p=0.003, d=0.48.

Notably, even the aforementioned minor drop in DI values on the 3rd day after the context change was observed in both hemispheres. The only difference between left and right hippocampi was noticed on the first day of training: the DI values in the right hippocampus appeared lower on the first lap (0.1 vs. 0.27) and higher on the last lap (0.58

vs. 0.47) producing a significantly larger DI change (0.39 vs. 0.2, $p<0.05$) than for the left hippocampus. As a result, the increase in DI over 8 laps on day 1 was statistically significant in the right hippocampus ($p<0.001$) and nonsignificant in the left hippocampus ($p=0.06$) (Fig. 10). Overall, the cells in both hippocampi demonstrated direction specificity after a few laps on a novel track and maintained this directionality on subsequent days of training. The left CA1 cells showed substantial direction specificity even without training, therefore the experience-dependent changes in left CA1 are less pronounced than in right CA1.

The effect of place fields with zero spikes in any direction on the 1st lap of training was tested separately for left and right hippocampi and showed no differences between hemispheres (Fig. 11). The exclusion of the 'zero spikes' fields did not affect the statistical significance of DI values in either of the hippocampi.

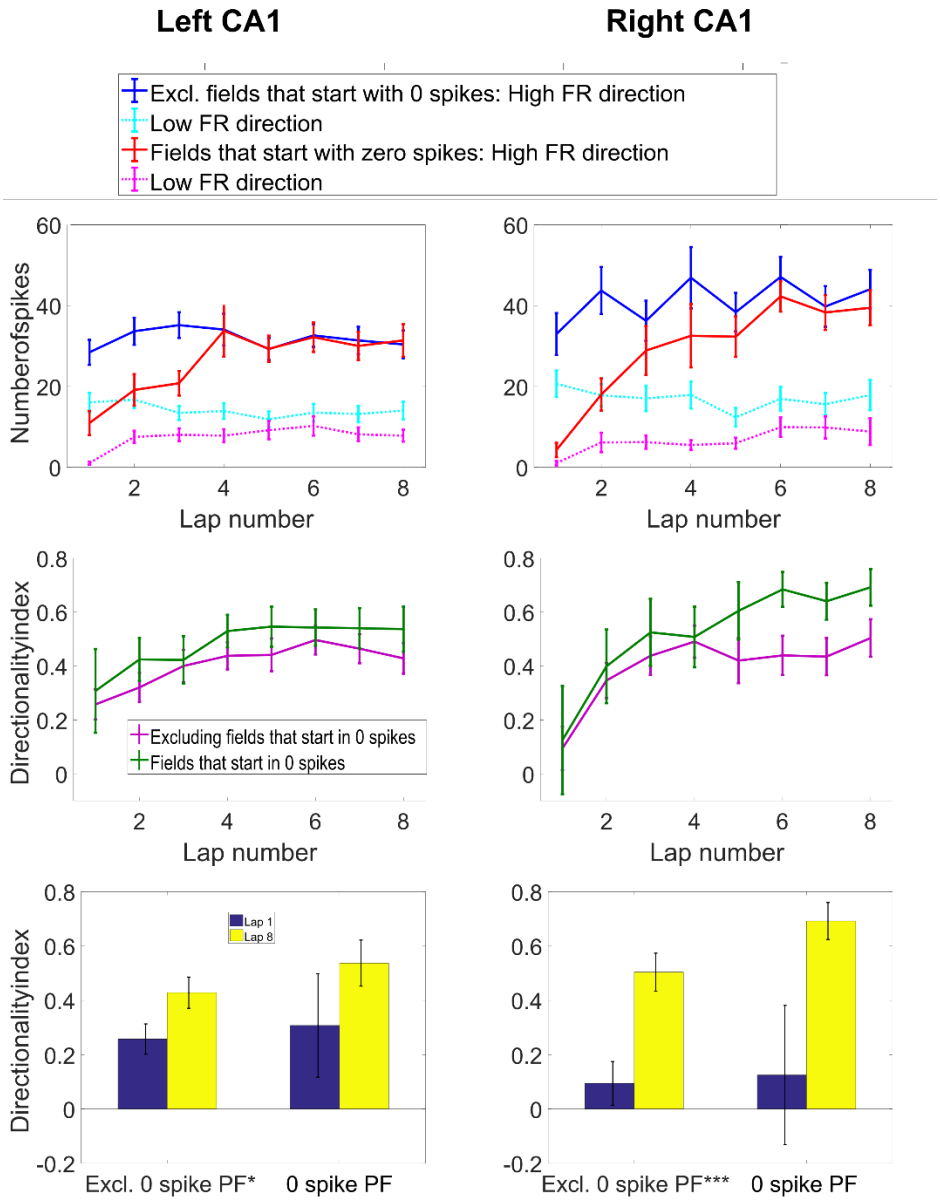

Figure 11. Place field directionality in right and left CA1 for the place fields that start with zero spikes in any direction versus the number of spikes for the fields that start with more than zero spikes.

Top panel: Number of spikes by lap, M ± SEM. Middle panel: Directionality index by lap, M ± SEM. Bottom panel: Directionality index increase, 8th lap versus 1st lap, t-test, *p<0.05, ***p<0.0001.

The changes in the hippocampal maps in response to introduction of salient visual cues could also manifest as the appearance of new place fields. Figure 12 shows the changes in the number of spikes for place fields with less than a threshold of 12 spikes in the first 3 laps. This threshold of 12 spikes corresponds with fields having, on average, less than 2 spikes per pass through the field over the 3 laps. In fact, the number of spikes for these fields fluctuated around 0 for the first 3 laps and slightly increased on the fourth lap. The introduction of salient visual cues was associated with a pronounced increase in the number of spikes in both the high FR direction and the low FR direction in these fields.

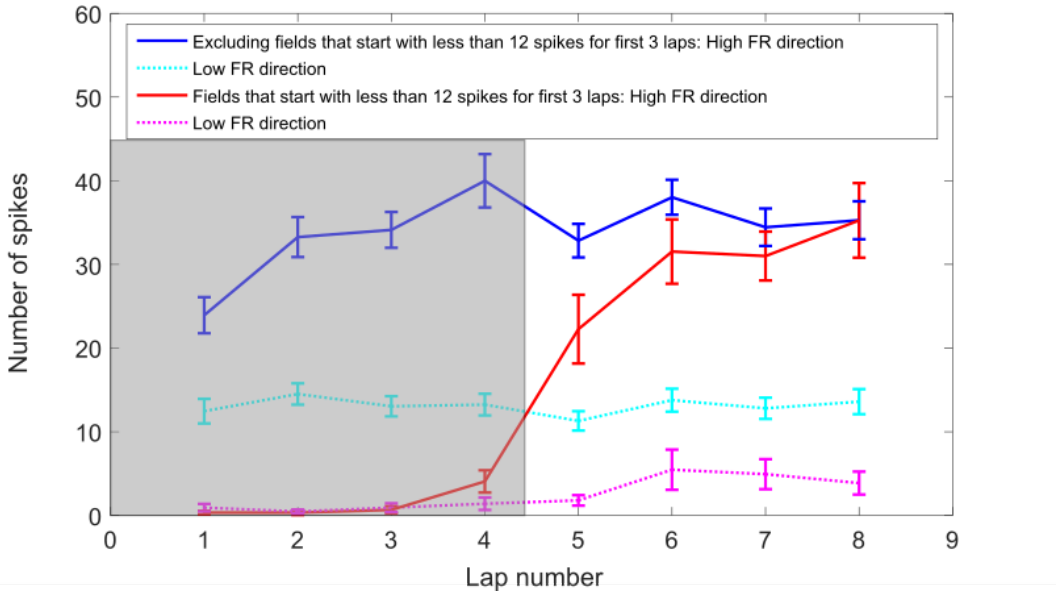

Figure 12. The number of spikes for the fields with less than 12 spikes in the first 3 laps on Day 1 and excluding those fields.
M±SEM. Grey area corresponds to dark laps.

There were 15 out of 171 fields that drastically increased FR in response to light. In comparison, there was only one field with less than 12 spikes for the last 3 laps. Overall, the fields with pronounced rate remapping in response to the introduction of the salient visual cues were observed on all the training days, but their numbers gradually reduced with experience (Table 2).

Table 2. Emerging place fields by the number of spikes they had in the darkness

	<24 in 3 laps	<16 in 3 laps	<12 in 3 laps	<6 in 3 laps	0 in 1st lap	0 in 2 laps	0 in 3 laps	0 in 4 laps
Day 1	27 (16%)	16 (9%)	15 (9%)	11 (6%)	23 (13%)	8 (5%)	5 (3%)	4 (2%)
Day 2	25 (17%)	20 (14%)	13 (9%)	9 (6%)	4 (3%)	3 (2%)	2 (1%)	1 (1%)
Day 3	18 (16%)	10 (9%)	7 (6%)	5 (4%)	4 (4%)	3 (3%)	3 (3%)	2 (2%)
Day 4	18 (19%)	10 (11%)	6 (6%)	8 (9%)	0	0	0	0
Day 5	21 (19%)	10 (9%)	5 (5%)	2 (2%)	5 (5%)	2 (2%)	0	0

The number and percentage of place fields with little or no spikes during the first few laps.

4.5 Discussion

Visual cues have been reported to affect the hippocampal rate map (Acharya et al 2016, Leutgeb et al 2005). Place cell directionality, as one of the consequences of rate remapping, was hypothesized to reflect changes in visual cues to a certain extent. The current study showed a complete lack of DI response to the availability of visual cues. Place cell direction specificity was successfully acquired in darkness and, moreover, the DI showed no changes later when the visual cues became available. Even temporary unavailability of visual cues in an already familiar maze produced no effect on the DI values.

In a single report, the directionality of place fields was reported to be independent of illumination in the recording room (Markus et al 1994), but this dataset was collected from overtrained animals performing a complicated task on a radial arm maze. It is conceivable that the degree of place cell reliance on visual cues is modulated by the requirements of behavioural task, the history of training, and the methods used to analyze place cell properties (especially comparing before and after recent advances in unit isolation and field detection). The current study employed a very simple behavioural task where a rat was only required to learn to run for a reward and to turn around at the ends of the maze. Most of the learning leading to direction specificity was incidental and could not be correlated with the effectiveness of the task execution (e.g. speed of running, see Fig. 21). Therefore, in the absence of proper controls, we can only speculate on the factors driving the place cell directionality.

In our experiment, where animals started novel maze exploration in darkness, the most probable factor driving place cell directionality was the HD signal. The path integration theory suggests that the appearance of place cell firing is based on an overlapping multiscale grid signal and the HD signal provides for transfer from one place field to another (McNaughton et al 2006). Distant cues of various natures may serve as another driving factor for place cell directionality. These distant cues may include odor or sound cues coming from within the experimental room (for example, the smell of the trash bin or the sound of a heavy-breathing experimentalist) or outside the room (environmental noise). The latter was detectable and direction specific: a narrow-band 25 kHz noise produced by the fluorescent lamps in the hallway was strong enough to penetrate into the recording room through the closed door. It is conceivable, then, that the gradual rise in DI

values reflects an association of the path integrator defined place field locations with the direction-specific acoustic cue.

The DI values took several days to reach a stable plateau. During the first 4 days of training, the DI slightly increased each day starting with values lower than those on the last lap of the previous day. This may suggest that most of the information driving place cell directionality is stored in long-term memory, but some depends on short-term memory. The underlying mechanism for this maybe similar to LTP which becomes more persistent with repetitions (Barnes 1979). The half hour break after 8 laps on days 2, 4, and 5 produced no decline in the DI values, suggesting that this short-term memory dependent component must be stored for longer than 30 minutes.

The only instance when the DI demonstrated a decreased response to changes in the environment was the 9^{th} lap on day 3, after the change of context. Notably, the change was observed during the first dark lap when the rat could not see the contextual changes. Therefore, this slight DI decrease is probably due to the stressful events associated with the context change and the cardboard box used to cover the animal.

The data analyzed separately for the two hemispheres on day 1 showed a statistically significant DI increase in the right hippocampus, but not in the left hippocampus. In addition, the differences in DI values between the last and first laps in right hippocampus were significantly ($p<0.05$) higher than in left hippocampus. Notably, previous research on place cell directionality (Battaglia et al 2004, Navratilova et al 2012b) was done with unilateral recordings from the right hippocampus. However, a lack of significant differences in DI values between left and right hippocampi at any time point on day 1 and an only marginal significance in DI increase ($p=0.0485$) gave us no confidence

in claiming this difference as real. There is a possible lateralization in hippocampal functions (see (Jordan 2020) for a comprehensive review), but the inactivation data in rodents (De Hoz et al 2005, El-Gaby et al 2016, Klur et al 2009, Shinohara et al 2012) is contradictory. We believe that if lateralization of hippocampal functions is present in rodents, it is unlikely that it is reflected in hippocampal map adjustments, described in this work. Separate analysis of COM shifts and PF expansions for left and right hippocampi did not reveal any interhemispherical differences (Fig. S6).

Despite most of the rate remapping occurring during dark laps, a subset (15; 9%) of place fields showed a pronounced change in FR upon maze illumination on the first day of training (Fig. 12). The proportion of these fields slowly declined over the following days, reaching 5% on the last day of training. The appearance of new fields in response to introduction of salient visual cues was not associated with the disappearance of other fields. There was only 1 field with less than 12 spikes on the last three laps on the first day of training. The appearance of new place fields in response to additional stimulation has been reported before in association with natural scanning behaviours (Monaco et al 2014) and after artificial electric stimulation of a silent hippocampal cell (Epsztein et al 2011). Intracellular recordings showed that silent hippocampal cells display spatially tuned activity but depolarisations are subthreshold (Epsztein et al 2011). If acute artificial depolarization drives the innate depolarization above threshold, the cell forms a place field that remains present even without further artificial stimulation (Epsztein et al 2011). A similar process might be responsible for new place field formation in response to increased salience of external landmarks.

Successful retrieval of information encoded in place cell direction specificity implies that the rate code can be reliably stored in long-term memory. Place cell directionality is an example of a rate map, and other rate maps dependent on other factors might be less stable after prolonged periods without training. The presented data is the first evidence for the long-term stability of a rate map. Previously, the reactivation of a rate map was only shown in sleep (Schwindel et al 2016).

The stability of hippocampal maps is a challenging topic for experimental research. The problem lies in the reliability of electrophysiological tracking of large numbers of neurons over long periods of time. The natural drift of a probe, the limited distance from which a neuron can be detected (Buzsáki 2004, Buzsaki et al 2012), and associated difficulties with spike sorting (Carlson & Carin 2019, Gray et al 1995, Navratilova et al 2016) means that most of the electrophysiological research to date is based on neurons recorded for only tens of minutes to several hours. Thus, the concept of a stable hippocampal map as the basis for long-term memory relies on only a handful of studies.

The first study to demonstrate the stability of place field location was presented as an abstract in 1984 and published in 1990 (Thompson & Best 1990), where single nichrome wires were used to record single hippocampal units with stable place fields for up to 153 days. Another study used similar single wires and showed numerous hippocampal cells with stable place fields during recording sessions separated by 1 or 2 days (Muller et al 1987). However, these early studies employed a methodology in which it was impossible to confirm the recording of single units. The tetrode technique demonstrated (Gray et al 1995) that separate hippocampal cells may appear very similar and indistinguishable when recorded with single wires or with stereotrodes. The invention of microendoscopes coupled

with calcium imaging (Ghosh et al 2011) markedly increased the number of sampled neurons and the stability of the recording/imaging. The first longitudinal calcium imaging study of CA1 place field stability over 30 days included >600 cells from 4 mice (Ziv et al 2013), whereas the previous electrophysiological study (Thompson & Best 1990) included only 5 cells (from 21 rats) reliably recorded for 30 days or longer. The longitudinal microendoscope study showed limited stability of the hippocampal place fields in mice (Ziv et al 2013) with a ~15-25% recurrence probability for the place fields on the sessions separated by 5 days or 30 days. A more recent study using similar imaging techniques but a different recording environment (an open field instead of a linear track) showed that the apparent instability of the hippocampal map can be explained by the coherent rotation of CA1 place fields rather than by global remapping (Kinsky et al 2018). In fact, even the original study (Ziv et al 2013) mentioned that fields that reappeared after several days frequently had the same location as before.

Thus, current knowledge concerning the stability of the place field location map in the hippocampus is based on evidence from calcium imaging in mice and anecdotal or indirect evidence from electrophysiological recordings in rats. Calcium imaging provides reliable data over multiple days but hippocampal maps in mice are less reliable than in rats (Agnihotri et al 2004, Kentros et al 1998). The decreased stability of hippocampal maps in mice can be associated with the lack of incidental spatial learning in mice: with increased attention, they are able to maintain more stable maps (Kentros et al 2004, Muzzio et al 2009). The lack of calcium imaging experiments in rats complicates the direct translation of imaging data to electrophysiological phenomena.

Acknowledging the difficulties associated with determining field location stability, the use of a DI as a proxy for a rate map is currently justified. It is expected that, in the near future, improved methods with either stable multichannel silicon probes (Chung et al 2019, Jun et al 2017) or calcium imaging (Keinath et al 2020) will better enable reliable quantification of place cell FR and direct multiday observation of the hippocampal rate map.

4.6 Conclusion

The availability of visual cues does not affect the acquisition and maintenance of direction specificity in majority hippocampal place cells, at least in the behavioural paradigm when animal starts novel maze exploration in darkness. In such a case, the acquired directionality likely relies on non-visual distant cues or HD signals.

The introduction of salient visual cues on the first day of training affects the firing rate of a small proportion of place cells by drastically ramping up the firing rate in place fields that were previously virtually silent.

Acquired place field directionality results in plasticity changes that persist for days and allow for rate map retention at least 5 days after the last training session.

5 Effects of distant visual cues on the expansion and backward shift of place fields

5.1 Background

The experience-dependent expansion and backward shift of place fields were described simultaneously (Mehta et al 1997) and were deemed to be two sides to a single process of sequence learning in the hippocampus. After a few laps on a maze, place fields get larger and expansion is more pronounced in the direction opposite to rat movement. As a result, the centre of mass (COM) of the expanded place field appears to be shifted backward. The formation of forward associations between neurons with overlapping place fields was proposed as a plausible mechanism for the phenomena (Blum & Abbott 1996, Mehta et al 2000). These associations create spatiotemporal sequences of neuron firing, which may be the basis of the hippocampal engram.

The experience-dependent COM shift is blocked in the presence of NMDA receptor antagonists (Ekstrom et al 2001) and the experience-dependent expansion of place fields is lost in aged rats (Shen et al 1997). These data provide additional evidence for the COM shift and PF expansion being memory-related phenomena.

After a prolonged break (e.g. 24 hours) between recording sessions, the shift starts anew but is less robust on the subsequent days of recordings (Knierim 2002, Mehta et al 1997), suggesting that the forward associations are formed gradually over the course of several days. Interestingly, manipulation of visual cues by means of rotating distant cues in one direction and the maze with salient local cues in the opposite direction results in a new and pronounced shift of the place fields even if the rat had already run the maze during the recording session (Knierim 2002). There was a surprising dissociation between the

COM shifts in CA1 and CA3 in this study. The CA3 cells experienced backward shift on the first day in the cue-altered maze, but not on subsequent days. The CA1 cells showed backward shift on the second and subsequent days in cue-altered maze, but not on the first day (Lee et al 2004b).

5.2 Study objectives

This study aimed at exploring whether or not the expansion and backward shift of place fields are controlled by distant visual cues. The complex phenomenon of place field size and position adjustment with extended experience on new and familiar tracks can shed light on the processes of sequence learning and prediction in hippocampal circuits. Multiple days of exposure to the same maze in light and dark conditions, a change of context, and a memory test several days after training provided a useful design to test the effects of visual cues on the expansion and backward shift of place fields. The use of a bilateral hyperdrive also enabled observation of any possible interhemispheric differences. Since PF perturbations of interest are different for new and familiar mazes, test sessions several days after training could also address the quality of memory retention for the information driving PF expansion and backward shift.

5.3 Study design

This chapter describes an additional analysis of the data collected for Chapter 4. The behavioural procedure has been already described in the corresponding sections.

The centre of mass (COM) was calculated as an average position of all place field spikes emitted during a given lap. The size of a place field was calculated as the distance between the first and the last spikes for a given lap within the place field.

5.4 Results

Similar to PF directionality, the most striking changes to PF size were observed on the 1st day of training. The first exposure to the novel maze (day 1, context A) resulted in a significant PF expansion in the higher FR direction (in which the PF had more spikes) but not in the lower FR direction (in which the PF had less spikes) (Fig. 13). Most of the expansion on day 1 took place primarily during the dark laps. The introduction of salient visual cues on the 5th lap of day 1 had no effect on the PF size in either direction. During the first 4 dark laps of day 1, the average position of the place field shifted in the opposite direction of running by 9.5 cm when running was in the higher FR direction. The backward shift when running in the lower FR direction was more modest at 4.1 cm. Unlike the PF size, the backward shift was attenuated when the lights were turned on, with shifts of only 5.8 and 2.5 cm in the higher FR and lower FR directions, respectively. Both COM shifts followed normal distributions in terms of the number of cells per magnitude of shift (Fig. 14), which probably reflects a unitary driving factor for each shift.

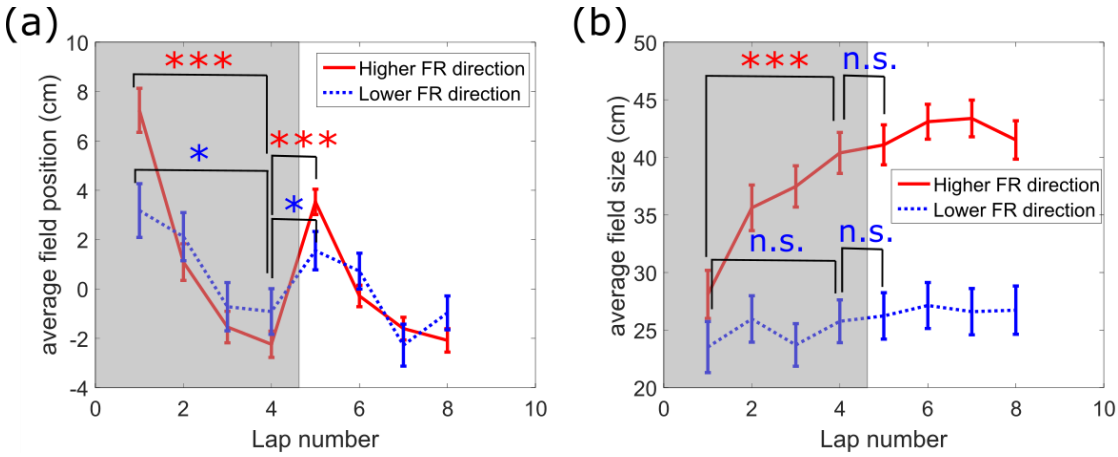

Figure 13. Introduction of visual cues on the 1st day of training diminished place field backward shift but not expansion.

Average PF position (a) and size (b), M±SEM. The first 4 dark laps on the 1st day of training were distinguished by a marked backward shift of place field COM in both the higher FR direction (-9.5 cm, $p<0.00001$, $d=-0.7$, medium effect size) and the lower FR direction (-4.1 cm, $p=0.0017$, $d=-0.25$, small effect size). The backward shift was accompanied by place field expansion in the higher FR direction (12.3 cm, $p<0.0001$, $d=0.47$, small effect size) but not in the lower FR direction (2.2 cm, $p=0.26$, $d=0.09$, very small effect size). The switch to illuminated conditions approximately halved the observed COM backward shift (higher FR: 5.8 cm, $p<0.00001$, $d=0.57$, medium effect size; lower FR: 2.5 cm, $p=0.03$, $d=0.17$, very small effect size) but had no effect on PF expansion (for both directions: <1 cm, $p>0.5$, $d<0.05$).

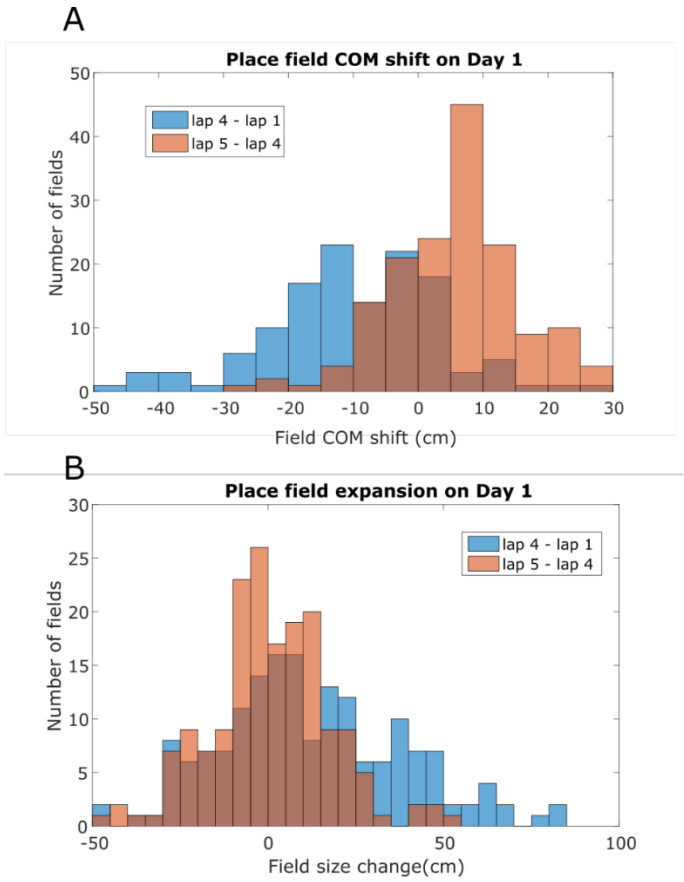

Figure 14. Histograms of place field COM shifts and place field expansion on the 1st day of training.

A: number of fields as a function of COM shift in cm by the 4th dark lap relative to the 1st lap (blue) and by the 5th lap relative to the 4th lap (orange). B: number of place fields as a function of the change in PF size in cm by the 4th dark lap relative to the 1st lap (blue) and by the 5th lap relatively to the 4th lap (orange). All distributions were unimodal.

The analysis of place field size by laps on different days of training revealed heterodromous and time-dependent changes of PF sizes in different FR directions (Fig. 15). On the first day, PF expansion in the higher FR direction was associated with stable PF size in the lower FR direction. Following extended training, PF size in the higher FR direction became stabilized with values similar to those before PF expansion on the 1st lap

of day 1. In contrast, the average PF size in the lower FR direction gradually declined throughout the 5 days of training and finally stabilized at values smaller than those observed at the beginning of the first day. It is important to note that the average size of the field in the lower FR direction decreased to below 15 cm by the 5^{th} day meaning that the majority of the fields, if analyzed separately for the two directions, fell below the field size threshold (17.5 cm) and thus were not included in the analysis. Notably, the within-session relative change in PF size became smaller with extended training, virtually disappearing by day 5. Moreover, the fluctuations in PF size were not coordinated with changes in illumination or visual context. Together, these results show that the multiday dynamics of PF size are opposite in sign for each of the two directions of running and are independent of visual cues.

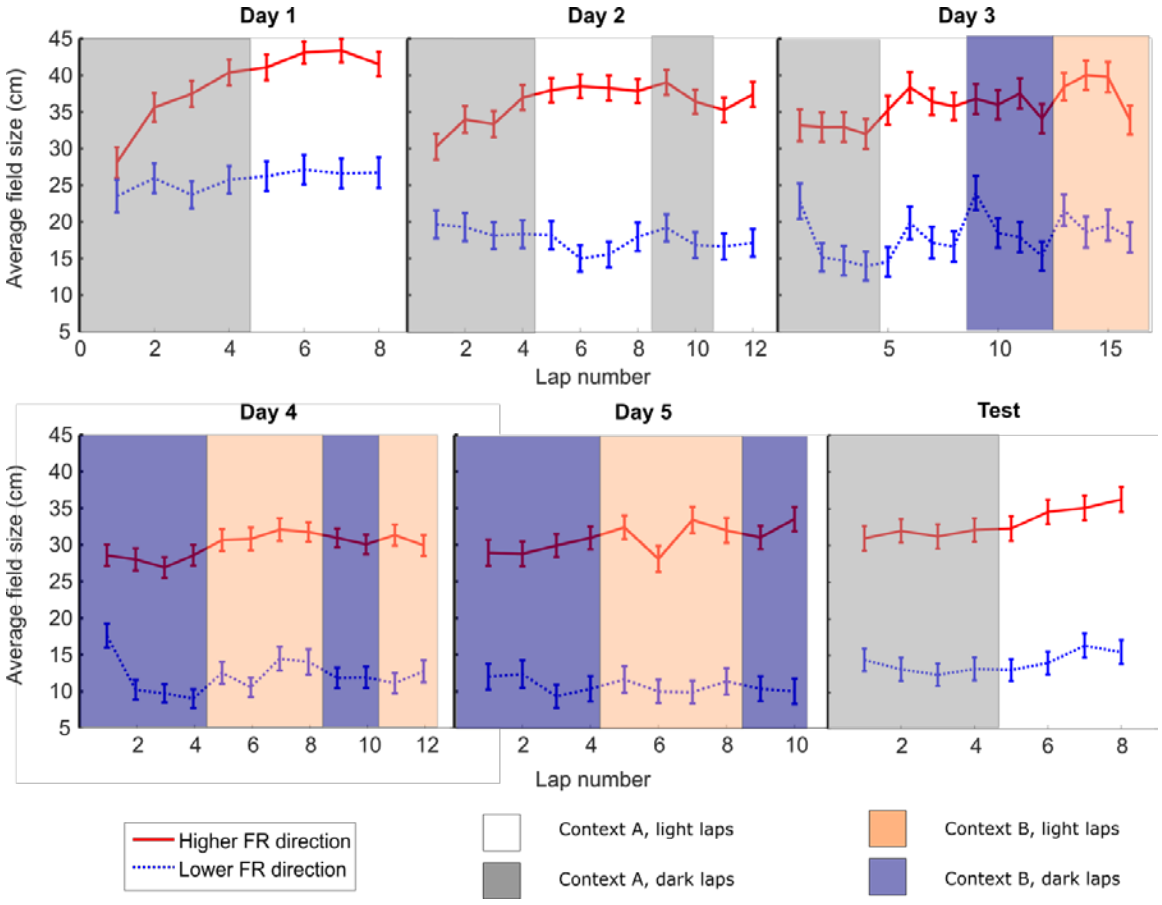

Figure 15. Average place field size by laps.

M±SEM. Note the familiarity of the environment results in a reduction of PF size, particularly in the lower FR direction. PF expansion is evident only for the higher FR direction on the 1st day of training.

The changes in PF position by laps over the 6 days of recording were analyzed only for the higher FR direction, because fields tended to have a very low number of spikes in the lower FR direction with extended experience and thus could be a confounding factor for the analysis. Figure 16 shows that the initial backward shift observed during dark laps became less prominent on subsequent days of training and the initial backward shift on the 5th lap was attenuated when the lights came on. Both shifts disappear by the 5th day of training.

Notably, on the 3rd day, the change of context and the introduction of the distant visual cues in the changed context also resulted in attenuation of the backward shift. Since the first 4 laps in the new context were ran in darkness, it is possible that the shift was not a result of the introduction of new cues but rather the disruptive manipulations associated with the context change (for example, covering the rat with the cardboard box).

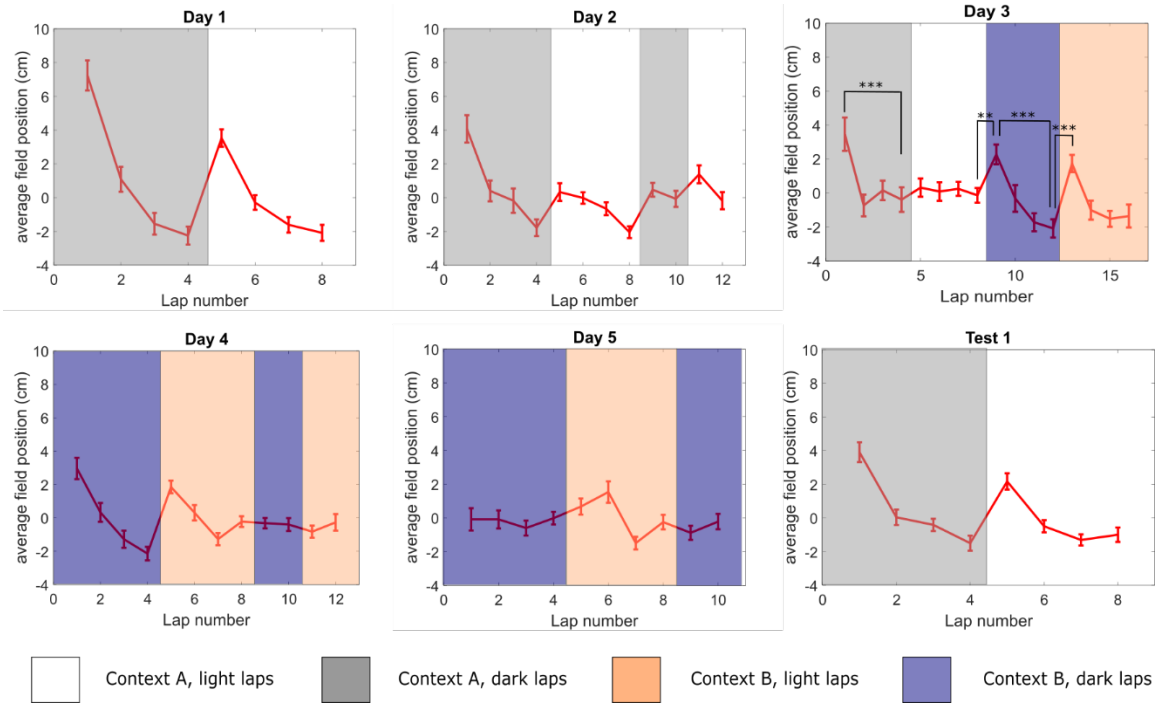

Figure 16. Place field position was affected by the introduction of visual cues and by the change of visual context.

Average field position for the higher FR direction during five days of training (cm), M±SEM. On the 3rd day of training, the 4 dark and 4 light laps in a familiar context were followed by a 30-minute break and a change of context, after which the rat made 4 dark and 4 light laps in the new context. The initial backward shift (lap 4 vs. lap 1: -3.9 cm, p=0.0002, d=-0.39) was abolished by the change of context on the 9th lap (lap 9 vs lap 8: 2.4 cm, p=0.0035, d=0.33). Dark laps in the new context were associated with a COM shift (lap 12 vs lap 9: -4.4 cm, p<0.00001, d=-0.61), which was eliminated when the lights came on (lap 13 vs lap 12: 3.8 cm, p<0.00001, d=0.46).

The backward shift of the place fields during dark laps and the forward shift on the 5th lap got progressively smaller as the rat became familiarized with the maze and surrounding cues (Fig. 16). The forward shift on the 5th lap disappeared by the 3rd day of training and then reappeared again on the 4th day, apparently reflecting the changes in distant cues introduced the day before. By the 5th day, PF position stabilized and was not affected by illumination conditions.

5.5 Discussion

Place field expansion and backward shift are well-known hippocampal phenomena (Mehta et al 1997, Mehta et al 2000, Navratilova et al 2012b). These phenomena are regarded as a consequence of establishing a spatiotemporal relationship between cells with adjacent field locations, enabling sequence learning in the hippocampus, as predicted by theoretical models (Blum & Abbott 1996, Hebb 1949, Levy 1989, Levy 1996). Such computational modeling suggests an asymmetrical redistribution of synaptic weights between two connected place cells representing adjacent locations on a track traveled in a stereotypical way(Hebb 1949). Repeated passes through the same sequences of place fields results in the formation of functional connections between the place cells, such that the preceding cell in a sequence acquires the ability to activate the next cell in the sequence. This causes the cells to start firing earlier on the track, extending their place fields to overlap the area of the previous place cells. This model assumes direct connections between the hippocampal cells; such connections are well known for the CA3 region and have also been described for the CA1 region (1% connectivity in CA1 and 4% connectivity in CA3)(Thomson & Radpour 1991). However, the sufficiency of these connections in

CA1 has been questioned and so many models emphasize the importance of the CA3 region (Levy 1996, Marr 1971, McNaughton & Morris 1987).

The data recorded on the 1st day of training showed a dissociation between the COM shift and PF field expansion (Fig. 13). Following the expansion over the course of a few laps, place field size remained stable but the backward COM shift diminished when visual cues around the maze became available. Further exploration of the maze in the illuminated room resulted in a new COM backward shift without any changes to field size. The dissociation between field expansion and backward shift has been noted before (Navratilova et al 2012b), where the fields in the lower FR direction produced a backward shift but did not expand as they did in the higher FR direction. The current study demonstrates an even larger dissociation, with the backward shift occurring and being attenuated independently of changes in PF size. This suggests that the two phenomena have different drivers which, according to the lack of dissociation in previous experiments (Mehta et al 1997, Mehta et al 2000), frequently coincide in time. According to our data, the plausible drivers should differ in their reliance on path integration data and visual stimuli. For these reasons, the foremost candidates for these drivers are projections from the MEC and LEC.

The MEC and LEC both send direct and indirect projections to CA1 neurons (Sewards & Sewards 2003). The MEC is known for having several types of spatially tuned cells, including the perfect machinery for path integration: grid cells (Hafting et al 2005b), HD cells (Sargolini et al 2006b), conjunctive head-direction + grid cells (Sargolini et al 2006b), and border cells (Savelli et al 2008, Solstad et al 2008). Lesions to the MEC impair an animal's performance in navigation tasks (Jacob et al 2017, Parron & Save 2004, Van

Cauter et al 2012), same as the lesions to medial performant path (Ferbinteanu et al 1999). The importance of the MEC for reliable place cell function in the hippocampus has also been shown in an inactivation experiment (Ormond & McNaughton 2015). Strong functional connectivity between these two regions has been demonstrated by coordinated global remapping of place fields and grid realignment in the entorhinal cortex (Fyhn et al 2007). Neurons in the LEC demonstrate non-spatial (Hargreaves et al 2005, Yoganarasimha et al 2011) object-specific firing (Deshmukh & Knierim 2011, Tsao et al 2013). Lesions to the LEC produce a deficit in non-spatial, but not spatial, latent learning (Stouffer & Klein 2013), impair object-context recognition (Wilson et al 2013) and learning complex features of the local environment (Kuruvilla & Ainge 2017). Non-spatial input from the LEC and spatial input from the MEC converge on hippocampal neurons, providing the necessary information for episodic memory formation (Knierim et al 2006). Therefore, the MEC and LEC provide the hippocampus with complimentary spatial and non-spatial projections.

We tested whether the backward and forward COM shifts on day 1 are the consequence of redistribution of the input weights on the same neurons, or if the oppositely directed COM shifts resulted from engagement of different neuronal populations. The data showed that the fields involved in the first backward shift (B1) in darkness on day 1 produced a greater forward shift after the switch to illuminated conditions (F1) than those not involved in the initial backward shift (Fig. 17). The fields involved in the forward shift on day 1, on average, showed a greater new backward shift than the fields that did not follow the general trend of the forward shift. The correlation between the first backward shift (lap 4 position – lap 1 position) and the forward shift (lap 5 – lap 4) was -0.36 (p=

3.19e-05); between the forward shift (lap 5 - lap 4) and second backward shift (lap 8 - lap 5) was -0.43 (p= 3.53e-08); and between the two backward shifts was -0.03 (p=0.77). The low correlation between the two backward shifts indicates that the magnitude of the second backward shift for a particular place field did not depend on the magnitude of the first backward shift. It also suggests different afferent projections were the drivers of the two backward shifts.

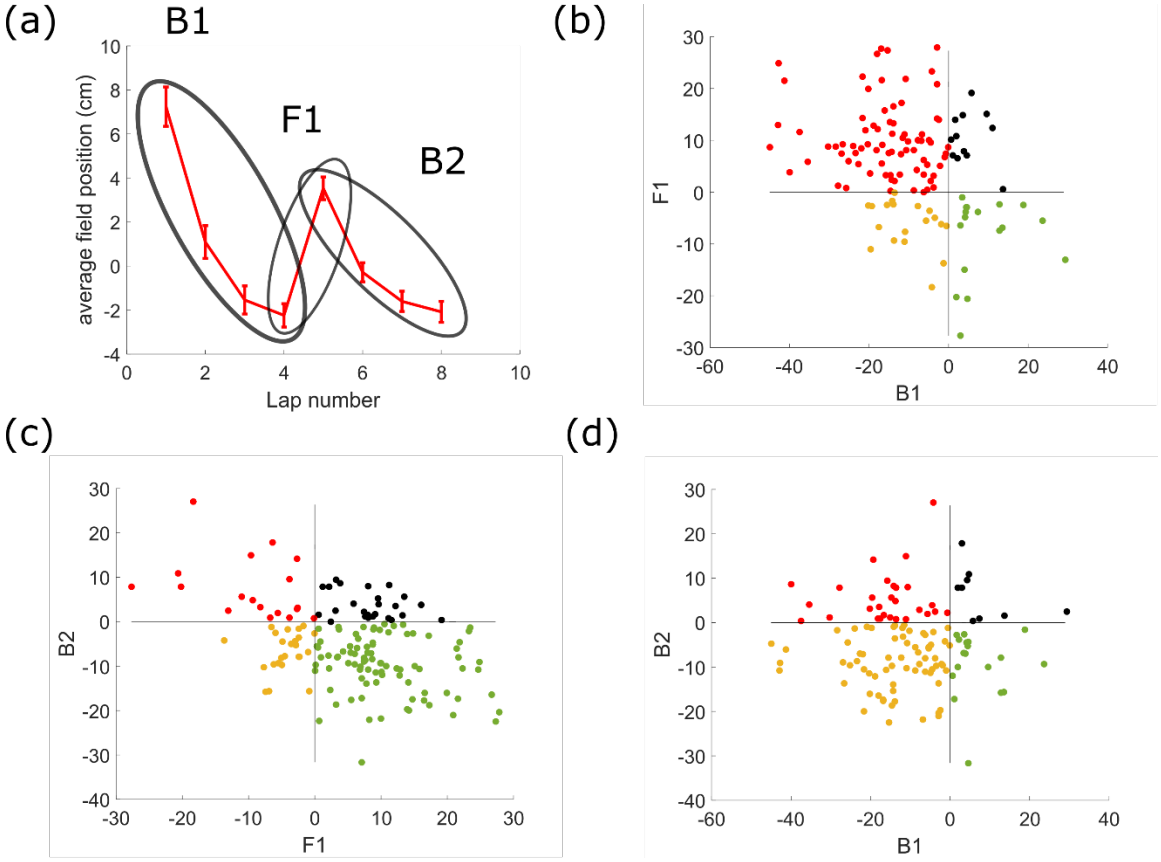

Figure 17. Backward and forward shifts are produced by the same subpopulations of place fields.

(a) Changes in place field positions in a new maze (the 1st day of recording) in the higher FR direction were divided into the following time frames: B1 – first backward shift (lap 4 – lap 1), F1 – forward shift (lap 5 – lap 4), and B2 – second backward shift (lap 8 – lap 5). (b-d) Scatter plots for the magnitudes of COM shifts associated with pairs of time frames.

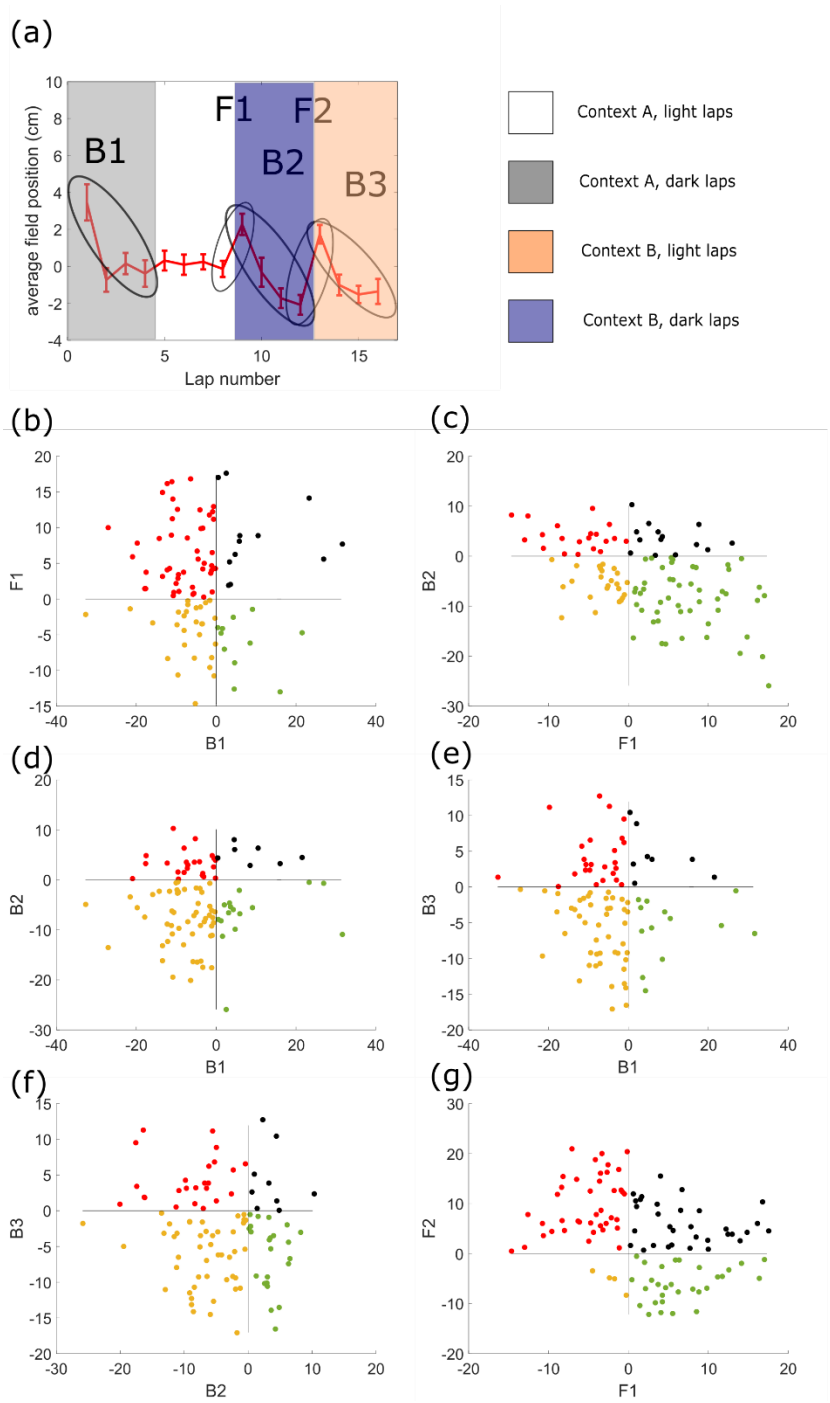

Figure 18. Two forward COM shifts on day 3 produced by different subsets of place fields.

(a) Changes in place field positions on the 3rd day in the higher FR direction in a familiar context and after the context change. (b-g) Scatter plots for COM shifts observed on day 3. Note: in the plots for any two backward shifts (d, e, f), most of the cells are contained

in the lower left quadrant, demonstrating similarities in the PF subsets involved in the two shifts. The plot for the two forward shifts (g) contains few fields in the upper right quadrant, demonstrating limited overlap between the subset of fields involved in the two forward shifts.

On the third day, two forward shifts were observed following the change of context: one occurred in darkness immediately after the context change while the other occurred when the lights were turned on to reveal the altered visual context. The latter is additional evidence of visual cue control over place field position. The first shift that occurred in darkness, however, likely has a different nature than the expected second shift. Similar to the shifts observed on the first day of training, the forward shifts on the 3rd day were followed by pronounced backward shifts. The fields involved in a forward shift actively participated in the immediately following backward shift (Fig. 18). However, the fields participating in the two forward shifts were different: the fields not involved in the first forward shift (in darkness) were the ones that produced the greatest second forward shift (Fig. 18). Correlation analyses (Table 3) demonstrated that the two forward COM shifts were negatively correlated. In sum, the backward shifts on day 3 follow the same pattern as on day 1: the correlation between individual backward shifts is close to zero, but the correlations with the preceding forward shift are significant and negative. The first forward shift, however, differs from the other forward shifts on day 1 and day 3: it is not negatively correlated with the preceding backward shift. In addition, it is negatively correlated with the second forward shift. Altogether, the origin of the forward shift on the 9th lap appears to differ from the origin of the second forward shift on the 13th lap and is not related to the altered visual context, because there was none at the time.

Table 3. Correlation between changes in place field positions on day 3.

	B1	F1	B2	F2
F1	*r*=-0.06 *p*=0.55			
B2	*r*=0.09 *p*=0.38	***r*=-0.47** ***p*=4.0e-07**		
F2	*r*=-0.035 0.74	***r*=-0.36** ***p*=0.0001**	***r*=-0.29** ***p*=0.002**	
B3	*r*=-0.08 *p*=0.44	*r*=0.03 *p*=0.75	*r*=-0.14 *p*=0.15	*r*=-0.12 *p*=0.23

5.6 Conclusions

Place field COM shift and expansion were characterized as two separate plasticity related processes each with different reliance on visual cues. Experience-dependent modifications of the hippocampal cognitive map reflect long-term modifications of connections within the path integration system and strengthening of associative connections between the path integrator and representations of visual cues.

6 Velocity and location effects on place field directionality, position and size

6.1 Background

There is an ongoing debate regarding the uniformity of the place cell code for neutral places and goal locations (Poucet & Hok 2017). Some studies found a lack of place cell activity modulation near a goal location (Duvelle et al 2019, Grieves et al 2016, Speakman & O'Keefe 1990). Other studies showed overrepresentation of place fields around a goal location (Hollup et al 2001, Mamad et al 2017, Tryon et al 2017), which can be due to the migration of fields towards the goal location (Dupret et al 2010, Kobayashi

et al 2003, Lee et al 2006b, McKenzie et al 2013). The maze we used in this study had two reward locations that could have affected experience-dependent changes in place field properties.

Place cell firing rate tends to increase with the speed of a freely moving animal (McNaughton et al 1983a, Muir & Bilkey 2003, Sharp et al 1990, Wiener et al 1989). In a novel maze, a rat hesitates to make long, fast runs, introducing a speed bias into the place field firing properties. The speed of animals in this experiment increased substantially during the first few laps on the first day of training. The same laps were associated with a marked increase in place field directionality, place field expansion in the higher FR direction, and a pronounced backward shift of the place field COM. This apparent correlation warranted a separate analysis to test velocity modulation of place field directionality, position, and size.

6.2 Study objectives

A separate analysis was aimed at determining the effects of speed and location on the evolution of place field properties such as directionality, position, and size. Speed and location control plots were made for the first day of training when the hippocampal map showed marked changes following exploration of a novel maze. The same controls were applied for the 3rd day of training when alterations to the hippocampal map were associated with the change of context.

6.3 Study design

For the location controls, the maze was binned into 10 cm bins. Mean values for place field directionality index, changes in COM, and changes in PF size were calculated

for each bin. For presentation purposes, the curves on the graphs were smoothed using 5-bin smoothing window. Note: the place fields around the feeding wells were excluded from analysis due to contamination with masticatory artifacts. Thus, the graphs represent changes in place field properties in the vicinity of the goal location but not at the goal location itself.

For speed controls, the order of laps was rearranged for each field according to the speed of animal travel through this particular field. Thus, the speed-ranked order of laps for each field was different. The first 3 laps of the behavioural session were excluded from analysis due to the high correlation between animal speed and the lap number.

6.4. Results

An increase in the DI was observed along the entire length of the maze (Fig. 19 a, b). The DI increments at the ends of the maze, however, were almost double the changes observed in the middle of the maze. The place field DI in close vicinity to the goal locations evolved from negative values on the first lap to close to maximal positive values on the last lap. This is indicative of strong rate remapping accompanying the change of preferred direction of firing.

We tested whether these drastic changes in the DI values were due to the fields that had zero spikes on the 1st lap in either direction (these fields have a DI of -1 or +1 for the first lap). When these zero-spike fields were excluded from the analysis, the distribution of the DI changes along the maze became flatter, without the larger peaks near the goal locations. Thus, the increase in direction specificity near the goal locations was largely due to rate remapping in the place fields with initially unidirectional firing (those that emitted spikes in only one direction on the 1st lap).

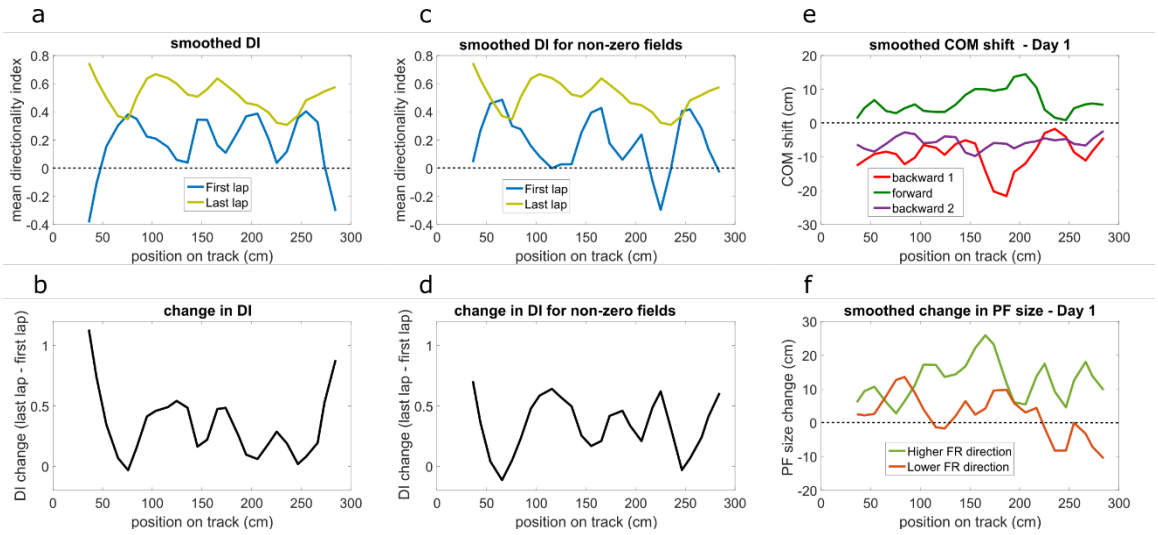

Figure 19. Changes in place field directionality, position and size as a function of field location on the maze on day 1.

a, b – the DI and changes in the DI as a function of field location on the maze; c, d – the DI and changes in the DI when the fields with zero spikes in either direction on the first lap were excluded; e – changes in place field COM during 3 major shifts: the backward shift during the 4 dark laps (backward 1), the forward shift when the lights were turned on (forward 1), and the backward shift during the 4 light laps (backward 2); f – changes in PF size during the first 4 laps on the maze.

The magnitude of COM shifts was influenced by the field location on the track. Both the first COM backward shift and the forward shift were more pronounced in the 150-200 cm section of the maze (Fig. 19, e). The magnitude of the second backward shift was uniformly distributed along the length of the maze. One can assume that the backward shift during the dark laps was driven by a set of spatially uniform internal stimuli and location-specific external stimuli located in the 150-200 cm section of the maze. When the lights were turned on, the previously relevant spatially uniform and location-specific cues lost their importance, hence the bounce back after the 150-200 cm section and the uniform forward shift along the maze. Under illuminated conditions, a new set of relevant cues

served as the driver for a new backward shift. According to the COM distribution along the maze, the salience of relevant visual cues did not vary with location on the track.

On the 3rd day of training, we observed 5 COM shifts. The most interesting is the relationship between the first backward shift (during the dark laps) and the first forward shift after the context was changed, which the rat was unaware of. Despite the opposite directions of the shifts, the correlations between the distributions of magnitudes along the length of the track was very similar (Fig. 20). Note how it differs from the distributions on the first day (Fig. 19, e) where the first two shifts along the maze were negatively correlated. These differences emphasize the existence of a unique driving factor for the COM shift that occurred on day 3 immediately after the context change but before the rat could notice it.

The spatial distributions of other COM shifts and of the DI changes on day 3 were unremarkable.

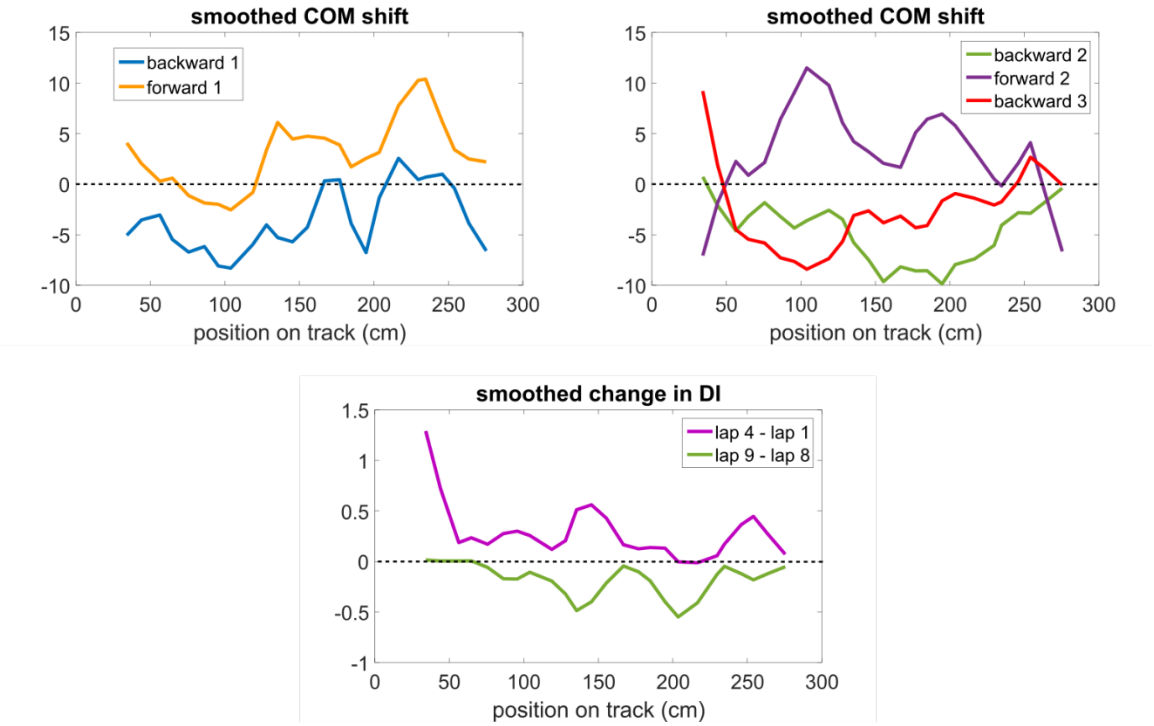

Figure 20. Changes in place field directionality, position and as a function of field location on the maze on day 3.
The distributions of COM shifts were separated into two different figures for clarity.

The speed controls showed that none of the changes in the DI, COM position, or PF size on day 1 and day 3 could be attributed to the difference in the speed of travel through the fields (Fig. 21).

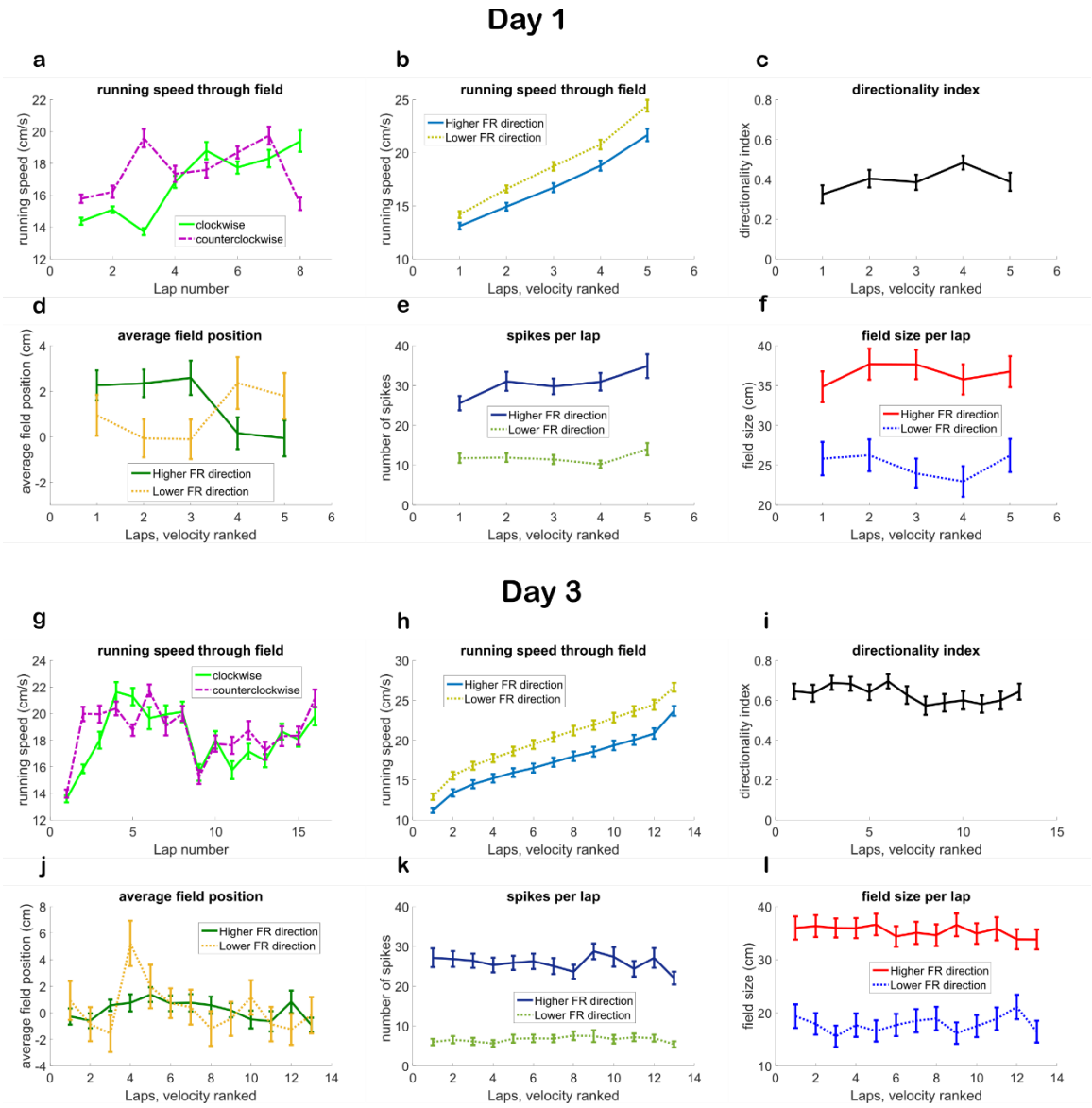

Figure 21. Place field directionality, position, and size as a function of speed of travel through the place field.

a, g – running speed for clockwise and counterclockwise directions on day 1. b, h – average speed through the field by laps, velocity ranked. The first 3 laps are excluded from the analysis. The order of laps was assigned individually for each field. All of the parameters were calculated according to this order of laps for each field.

6.5 Discussion and Conclusions

The MEC provides generally uniform input to the hippocampus via the firing of spatially tuned grid cells (Hafting et al 2005a). Input from the LEC, in contrast, can be very nonuniform due to the object-specific firing of cells (Deshmukh & Knierim 2011, Tsao et al 2013). All efforts were made to remove cues from the maze but the presence of extra-maze cues, particularly of a non-visual nature, was not fully controlled.

Changes in the DI are more pronounced in the vicinity of the goal locations. Some of the cells with place fields near the food wells underwent drastic rate remapping with the change of preferred direction of firing. These changes can be explained by increased influence of LEC input, likely from the cells tuned to the reward locations.

The backward COM shift in a new maze in darkness was observed for fields located along the entire length of the track but was more pronounced in a specific section of the maze. This section appeared unremarkable to the experimenter. Fields located in the same section then experienced the largest forward shift when the lights went on. An explanation for this preferred location of the COM shifts may lie in the presence of a salient non-visual cue that drove LEC activity in the darkness, but not under illuminated conditions, when multiple visual cues became available.

Changes in the DI, COM locations and the PF size were not influenced by the animal's running speed.

7 Possible origins of place field expansion and the COM shift

Place field expansion independent of distant visual cues is probably driven by egocentric cues and therefore by spatially tuned cells in the MEC. The backward COM shift occurs in darkness, then gets attenuated by half, and develops anew when the lights

are turned on. This suggests that the MEC can be the only driver of the COM shift in the absence of LEC input but, if the LEC also contributes, both the LEC and MEC can drive the COM shift. Both the PF expansion and the COM shift became less prominent on the following days, virtually disappearing by the 5th day of training, suggesting a gradual stabilization of plasticity changes. The COM shift, but not field size, was affected by the context shift on the 3rd day, emphasizing the effect of visual input on field position. A plausible mechanism for the place field COM shift is presented in Fig. 22.

Note that the activation/deactivation of the DG/CA3 cells in this model do not have to follow the all-or-nothing mechanism. More likely, it will appear as profound rate remapping where DG/CA3 place fields do not disappear/appear but rather their effects on postsynaptic cells will change substantially.

Another plausible mechanism explaining the backward shift of PFs does not require a layer of cells with recurrent connections. Instead, the asymmetric redistribution of synaptic weights occurs in the projections from one layer to another, for example, from CA3 to CA1 (Mehta et al 2000) (Fig. 23).

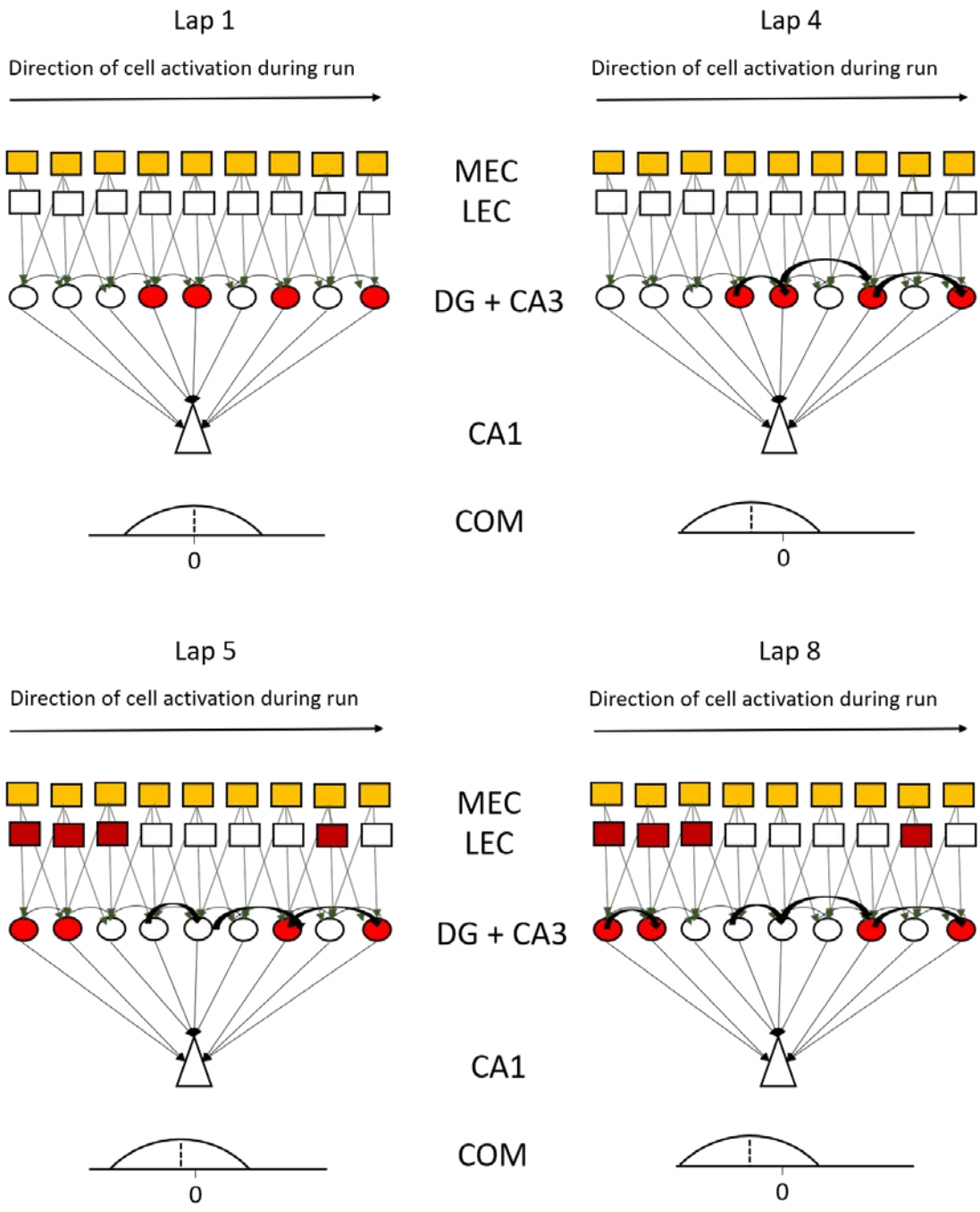

Figure 22. A plausible mechanism for place field COM shift on a restricted track with a change in the availability of distant visual cues.

The model incorporates several layers: MEC – providing allocentric information for navigation, LEC – providing information about visual cues, DG + CA3 – a structure with extensive recurrent connectivity, CA1 – target structure. COM – centre of mass of a CA1 place field. **Lap 1**: Only MEC cells are firing, activating a portion of DG+CA3 cells.

COM is at 0. **Lap 4**: The connections between the active cells in layer DG+CA3 get asymmetrically stronger. The cells with PF in the rat location are now capable of activating the cells with the PF farther along the track, effectively shifting their COM backward. **Lap 5**: The same amount of MEC cells and some of the LEC cells are active. The set of active DG+CA3 cells has changed. Some of the cells with strengthened connections are not active. The cells that have just become active are lacking strong connections. The COM shift is reduced. **Lap 8**: All active cells developed strong connections and the COM shift is maximized.

Previous results have called into question the reliability of the COM shift phenomenon (Lee et al 2004a). In (Lee et al 2004a), rats ran a familiar circular track with salient local and distant cues. Both sets of cues were rotated to the same extent: one set in the clockwise direction, another set of cues in the counter-clockwise direction. Following cue manipulation, the COM shifted backward in CA3 on day 1, but not on subsequent days. The COM shifted backward in CA1 on second day and on subsequent days, but not on the first day. A possible explanation for the contrast between the results of the present study and the results published by Mehta and Knierim may lie in the use of salient local cues. Additional analysis (Lee & Knierim 2007) showed the different sets of fields in CA1 and CA3 that followed either distant or local cues following the rotation.

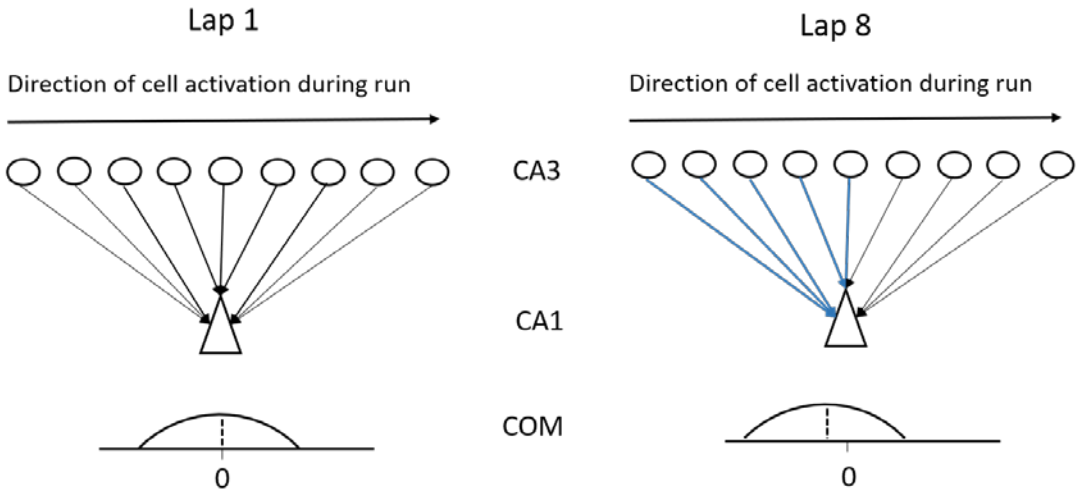

Figure 23. Another plausible mechanism for the experience-dependent backward shift of place fields.

The arrow shows sequential activation of the CA3 place cells during stereotypical running. The thickness of lines corresponds to the connection strength and grey lines indicate synapses with zero strength. On the first lap, the connectivity matrix is symmetrical (red lines). With experience, the synapses activated before a postsynaptic action potential undergo LTP and the synapses activated after a postsynaptic action potential undergo LTD, resulting in an asymmetric connectivity matrix (blue lines). (based on (Mehta et al 2000)).

The models shown in Fig. 22 and 23 differ in their predictions and explanatory power. The Mehta model (Fig. 23) does not require asymmetry in the recurrent CA3 connectivity matrix or any substantial role of recurrent connectivity at all. However, the phenomena associated with recurrent connectivity, if present, will result in even more of a pronounced shift. The model proposed in this thesis (Fig. 22) explicitly differentiates between MEC and LEC inputs to CA3 in order to explain the effects of visual cues on a backward shift. The Mehta model did not aim to explain the effects of visual cues but the addition of the MEC and LEC layers would probably produce a similar result. The major difference between the two models is the location of fast synaptic changes resulting in an asymmetric connectivity matrix: DG/CA3 terminals in CA1 or connections within the DG/CA3 region. Conceivably, the aforementioned synaptic modifications may occur at all these levels but their relative impact on the CA1 place field shift has yet to be established.

A plausible mechanism for place field expansion should involve positional and directional signals as CA1 place cell afferents. It should also lack an intermediate layer of recurrently interconnected cells because the positional signals projected to a layer of interconnected cells would lead to predictive coding as it does for place field COM shifts.

A plausible mechanism controlling place field size in both directions of running is presented in Fig. 24.

The model is comprised of just two layers. First is a layer of conjunctive cells with opposite preferred directions of firing (MEC), and second is a layer of target place cells (CA1). The MEC projections to CA1 alternate between two types of plasticity. The change of synaptic weights during the behavioural session follows the BCM rule (Bienenstock et al 1982). According to this rule, the synapses which activity closely precedes the spike of target neuron get stronger, the synapses which activity rarely associated with target neuron firing get weaker. This is the mechanism that explains acquisition of ocular dominance in the primary visual cortex. In the visual cortex, a stochastic, minuscule bias toward the cell being activated by the input from one of the eyes starts the process where slightly stronger synapses get stronger and slightly weaker synapses become weaker until the neuron becomes specific to inputs from only one eye. We suggest that something similar may be occurring in the hippocampus at the synaptic terminals of conjunctive cells.

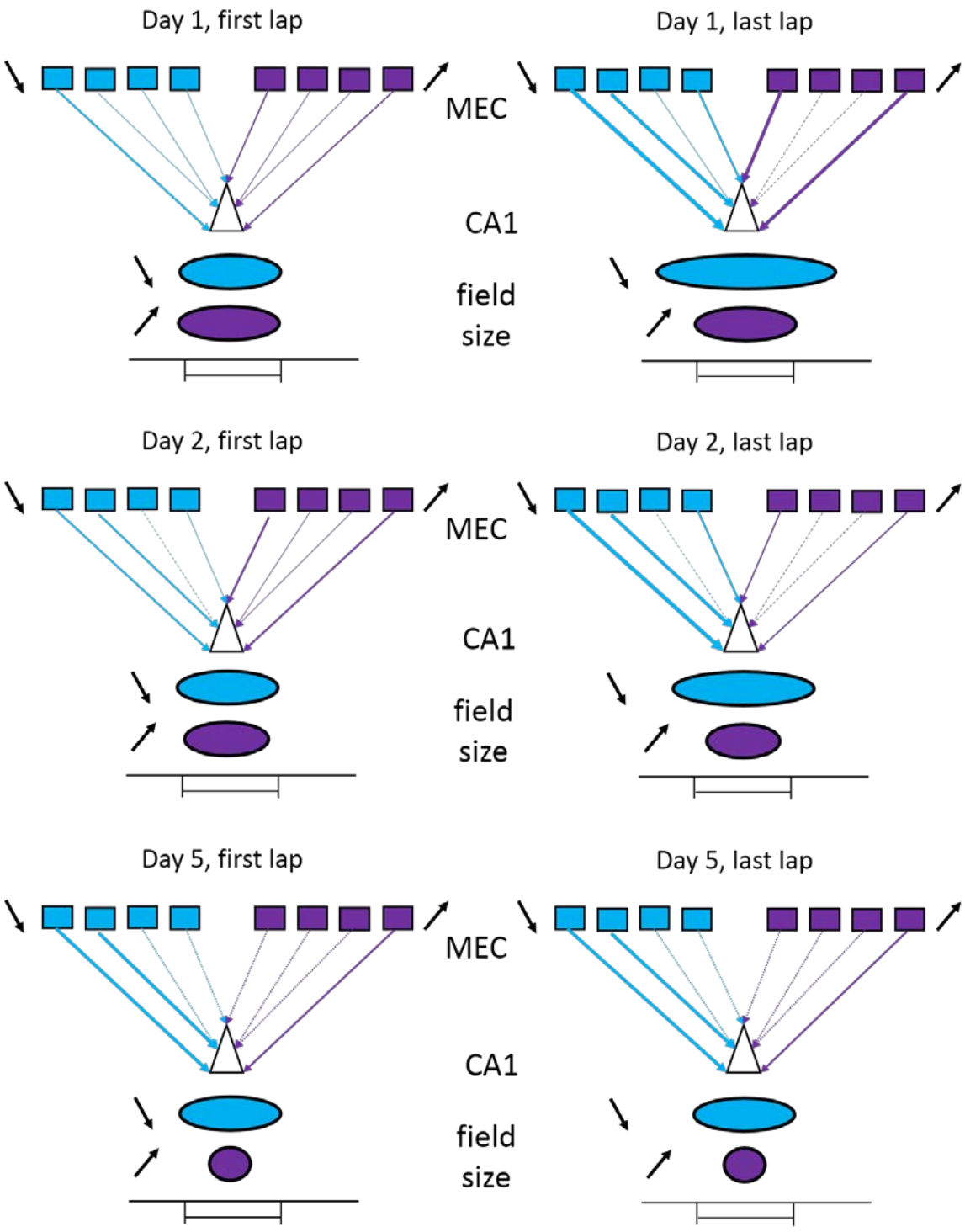

Figure 24. A plausible mechanism for the change of place field size with extended experience.

Black arrows show the direction in which the conjunctive cells fire. Initially, conjunctive cells for two directions of running have almost equally strong inputs to the CA1 cells, with a slight bias toward one direction (blue). During the running session, some of the inputs have slightly higher probability to precede the target neuron spikes. These inputs get stronger. Other synapses get weaker. Following the running session, the connections for both directions become stronger but the strengthening is more pronounced in the direction favoured by the initial bias (blue), creating a stronger bias for this direction. Homeostatic plasticity attenuates the strengthened connections overnight for both directions, reducing the PF size but preserving the bias. The next day, the same sequence of events occurs amid a stronger bias than the day before and continues until the cells for one of the directions virtually stop participating in the target cell firing.

The size of the field tends to change during the overnight break between the recording sessions, which warrants introduction of another plasticity mechanism into the model that attenuates synaptic changes produced during behaviour. This mechanism is known as homeostatic plasticity (Fernandes & Carvalho 2016, Turrigiano 1999). During rest, the strengthened synapses will become slightly weaker and the weakened synapses will regain some of their strength. This combination of synaptic changes following both the BCM rule and the homeostatic plasticity theory can explain the changes observed in place field size during training.

On the first day of training, the input from conjunctive cells in the MEC to CA1 neurons is virtually symmetrical for both directions of running but marginally stronger for one of the directions. This direction becomes the higher FR direction, more incoming action potentials coincide with the neuron's up-state, and more synapses for this direction get strengthened. The field becomes larger and contains more spikes. In the opposite direction of running, some synapses get stronger, some get weaker, and the average field size remains the same. Overnight, the elicited synaptic changes are attenuated. Empirical data suggests that the homeostatic regulation more strongly affects strengthened synapses than weakened. Thus, at the beginning of the next day, the field size is closer to naïve

values in the higher FR direction but only somewhat reduced in the lower FR direction. The changes in synaptic weights follow the same BCM rule throughout the second and subsequent training sessions. Synapses that are weakened by homeostatic plasticity have a smaller likelihood of being strengthened during the subsequent training session and eventually almost stop participating in signal transmission. By the 5^{th} day of training, the field size remains virtually constant in both running directions in the absence of further plasticity. The proportion of strong and weak synapses is very different between the two directions and the capacity for plasticity is limited because the strong synapses reach maximum strength whereas the weak synapses become virtually silent.

Origin of place cells

Several models were proposed to explain spatial selectivity of place cells. Most of them include input from grid cells as a major driving factor. The origin of periodic activity in grid cells is also a subject of extensive modelling initiatives. A vast majority of grid cell models can be attributed either to oscillator interference models or to continuous attractor models. Further discussion will be focused on these two major types.

Place cells and grid cells undoubtedly represent different parts of the same path integration system. The following features are the most characteristic for these two types of neurons. Firing of both place cells and grid cells is modulated by theta rhythm (Hafting et al 2008, O'Keefe & Recce 1993). The size of place fields / grids and spacing between grids increase from dorsal to ventral parts of hippocampus and MEC (Brun et al 2008, Jung et al 1994, Kjelstrup et al 2008, Royer et al 2010).

Oscillator interference models (OIMs) rest on a proposition that regular spiking pattern may arise in each cell from the interference of two subthreshold membrane oscillators with slightly different frequencies (Burgess 2008, Burgess et al 2007, Giocomo & Hasselmo 2008, Hasselmo et al 2007, O'Keefe & Burgess 2005). Summation of the frequencies at certain moments will produce a current above the action potential threshold and the cell will produce a burst of spikes.

Apparently, the frequency of the bursts and their duration can be controlled by the frequency of the main rhythm and the relative difference between the two oscillations. Higher main frequency and larger differences between the oscillators correspond to smaller spacing between the bursts and smaller durations of bursts. As a standard, the main oscillator in the models has fixed frequency and located in the soma of the neuron, whereas another oscillator in the dendrites may have variable frequency, which anyway is higher than the one of main oscillator.

Since the theta-frequency oscillations are the ones most commonly observed in spatially selective cells in both hippocampus and MEC, the computational models were built around this frequency range. Notably, the layer II of MEC, where majority of the grid cells reside, is especially rich in theta-modulated cells (Hafting et al 2008).

The OIM got support from experimental data showing prominent subthreshold membrane potential oscillations in layer II stellate cells of MEC (Alonso & Llinás 1989, Dickson et al 2000). These oscillations appeared to have higher mean frequency in dorsal MEC than in ventral MEC (Giocomo et al 2007), what corresponds to more frequent and sharper crossings of the threshold by the cells in dorsal part of MEC and subsequently to smaller firing fields with smaller spacing between them. This proposition of the model

completely complied with the pre-existent experimental data (Hafting et al 2005b). OIM is also capable of explaining the hexagonal pattern of grid cell firing fields. It requires several dendritic oscillators with different preferred orientations, so their crossings form a hexagonal pattern (Burgess et al 2007).

Meanwhile the OI models explain the firing properties by the electric events happening within one cell, continuous attractor models (Burak & Fiete 2009, Fuhs & Touretzky 2006, Guanella et al 2007, McNaughton et al 2006, Navratilova et al 2012a) use a population of mutually interconnected cells to explain their firing properties. In models of grid cells, the cell population is arranged in a virtual two-dimensional sheet, where neuronal connections are symmetric and local, so an excitatory input applied to the network results in a local bump of increased cellular activity.

Model of grid cells requires a two-dimensional attractor map with several hidden layers, one for each direction of movement. The probability or the strength of connections also reduces with the distance between cells, so the network's most probable state is a two-dimensional bump of activity. When the rat moves, vestibular input to one or few layers of conjunctive cells makes the bump move correspondingly (McNaughton et al 2006).

OIMs suggest the importance of theta oscillations to generate a grid-like pattern. In fact, the reduction of theta rhythm by temporal inactivation of septal input to the hippocampal formation does abolish periodic firing pattern in grid cells (Koenig et al 2011). Interestingly, some of the cells did not restore the firing pattern after the septal inactivation, suggesting that they do not produce the firing pattern independently, but probably inherit it from other grid cells. The OIM suggests the specific pattern of spiking generates independently in each cell. The fact that some cells regained the grid-like firing

pattern after temporal inactivation and others did not suggest the periodic spiking activity might be not inherent at least for some grid cells.

A critical testing colliding OIMs and CAMs in vivo was performed by means of whole-cell recordings of grid cells in mice running tracks in virtual reality (Domnisoru et al 2013). The whole-cell recording technique is capable of capturing small, subthreshold changes of membrane potential. Results showed the ramps (predicted by CAMs) and theta oscillations (predicted by OIMs) are both reflected in membrane potential dynamics of the grid cells. Nonetheless, the ramps appeared to be a more reliable predictor for spike generation and the cumulative data favoured the CAMs.

One more piece of evidence against OIMs is the presence of grid cells and other classical spatially tuned cells in bats, that have no theta oscillations (Yartsev et al 2011).

CAMs in their majority suggest the excitatory cells exhibiting grid cell properties should be densely interconnected. The in vitro experiment on slices from rat MEC and HPC showed virtually no recurrent excitatory connections for principal cells in MEC layer II (Dhillon & Jones 2000). Although the study found high degree of recurrent connectivity for pyramidal cells in layer V and layer III, stellate cells in layer II comprise the main pull of grid cells.

More detailed testing of the stellate cell connectivity in MEC layer II was performed using clustered whole-cell recordings *in vitro* (Couey et al 2013). Simultaneous recordings from sets of 3 to 4 cells confirmed the lack of excitatory interconnections between stellate cells but also revealed that the majority of stellate cells is embedded in the network with recurrent inhibitory connections. Notably, the connections followed “all-or-nothing” pattern, which is opposite to the graduate change in connectivity implemented in

most of CAMs. Nevertheless, authors proposed an attractor model capable of generating a grid-like pattern using a bimodal type of connectivity.

Considering the abovementioned experimental data, there is a small chance for OIMs to reflect processes crucial for periodic firing of the grid cells. The data favour CAMs by showing existence of interconnected networks of stellate cells in MEC, but “all-or-nothing” type of connectivity and exclusively inhibitory nature of connections argue against most of attractor models.

8 General Conclusions

Testing effects of visual cues was a useful tool to disentangle PF expansion and the backward COM shift as two different phenomena. The PF backward shift is still a good candidate mechanism for disambiguating storage of different sequences in the hippocampus. According to our data, the disambiguated sequences also incorporate information about landmarks such as distant visual cues. Place field expansion, or change in place field size, and place field directionality may be the consequences of the mechanisms underlying sparse coding in the hippocampus. PF directionality may reflect increasing sparseness within the first day of learning, whereas changes in PF size may represent the sparse code for an already consolidated hippocampal map.

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Appendix 1.

Supplementary Data

Place field directionality by days

Table S1. Place field directionality by days.

	N of PFs	Start directional			End directional		Start and end directional			Never directional	
		N	End not directional	Reverse directionality	N	start not directional but reverse	N	Reverse directionality	Increase directionality	N	Increase directionality
D1	171	86	21	4	123	19	63	20	33	23	12
%	**100**	**50**	**24**	**5**	**72**	**15**	**37**	**32**	**52**	**13**	**52**
D2	146	88	17	4	108	6	68	5	33	17	8
%	**100**	**60**	**19**	**5**	**74**	**6**	**47**	**7**	**49**	**12**	**47**
D3	112	71	21	5	77	10	47	3	26	11	6
%	**100**	**63**	**30**	**7**	**69**	**13**	**42**	**6**	**55**	**10**	**55**
D4	149	107	28	5	103	5	78	5	36	17	11
%	**100**	**72**	**26**	**5**	**69**	**5**	**52**	**6**	**46**	**11**	**65**
D5	108	74	10	2	83	3	62	1	17	13	5
%	**100**	**69**	**14**	**3**	**77**	**4**	**57**	**2**	**27**	**12**	**38**
T1	167	117	22	2	118	3	94	9	36	25	9
%	**100**	**70**	**19**	**2**	**71**	**3**	**56**	**10**	**38**	**15**	**5**

Place fields by the type of remapping

Place fields can be arranged into groups based on the way they remap: by increasing FR in both directions, decreasing FR in both directions, increasing FR in a preferred direction while decreasing FR in a nonpreferred direction, or decreasing FR in a preferred direction while increasing FR in a nonpreferred direction. These different types of remapping are illustrated in Fig. S1.

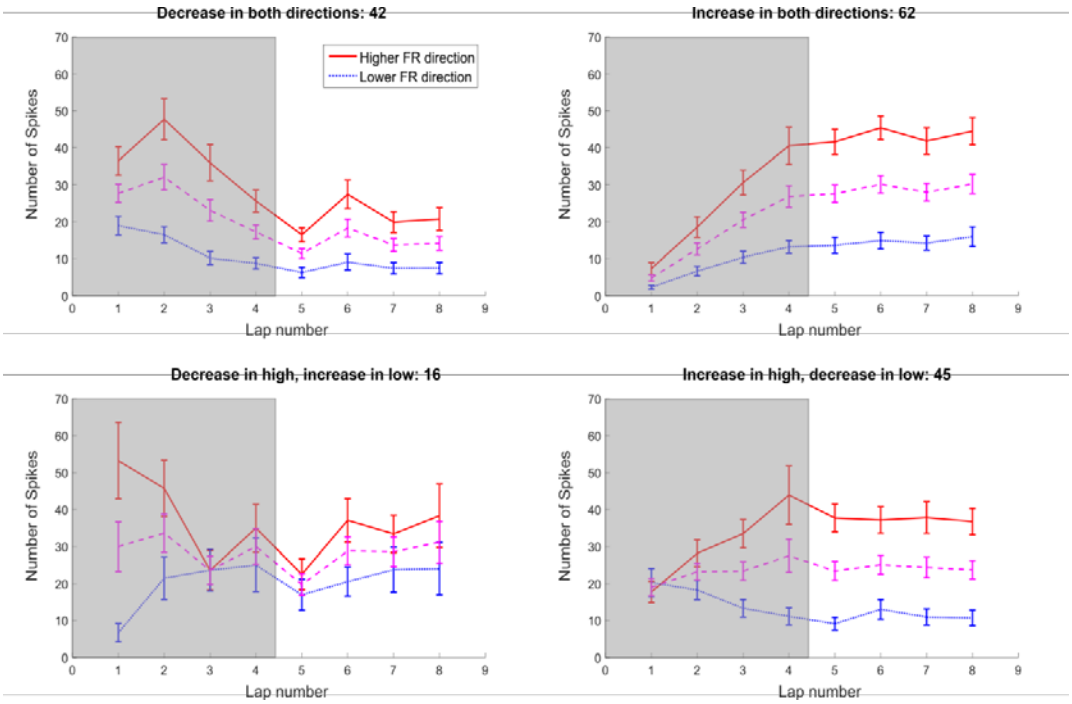

Figure S1. Different types of place fields arranged by type of rate remapping on the 1st day of training.

Number of spikes by laps for different types of place fields, M ± SEM. The number of fields is noted after the colon. No type of fields demonstrated a FR change following the introduction of salient visual cues.

Notably, not a single type of place field showed any changes in FR when the lights were turned on. The DI values for these four types of place fields also revealed no changes (Fig. S2).

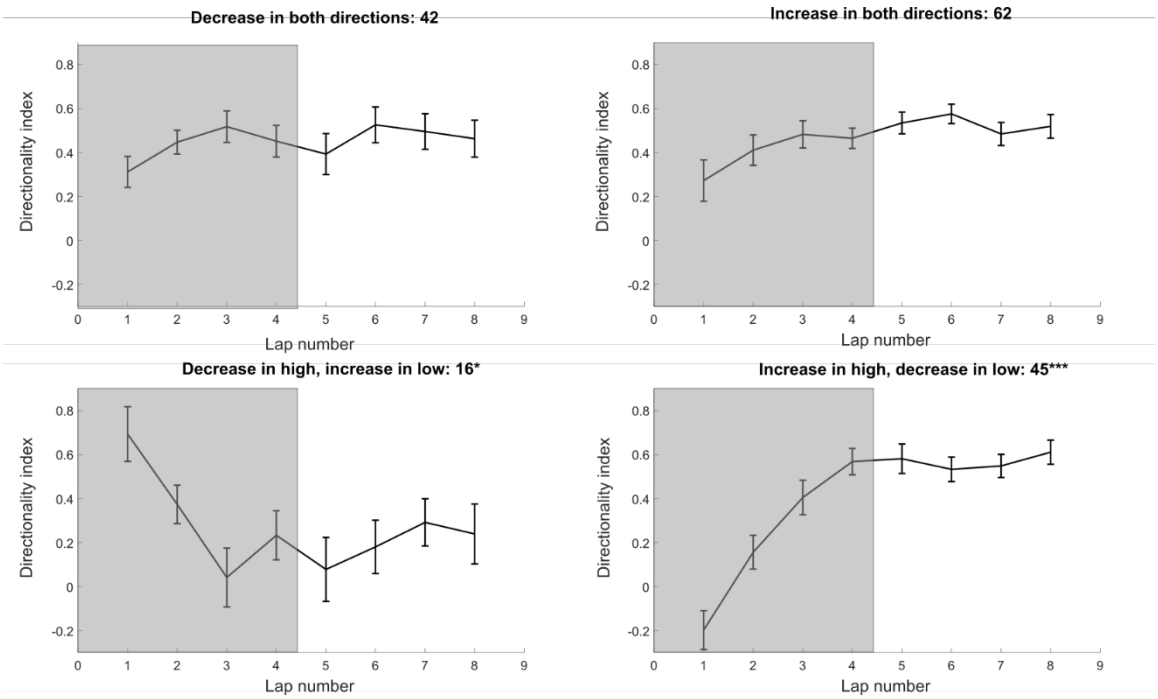

Figure S2. Acquisition of direction specificity by different types of place fields on the 1st day of training.

Directionality index, M ± SEM. Grey area corresponds to dark laps. The number of fields is stated after the colon. *p<0.05, ***p<0.001 for the DI difference between the first and the last laps.

The most populated group was the one where FR increased in both directions but the rate remapping in this group was not significant. The group where FR increased in the preferred direction but decreased in the nonpreferred direction was the only group with a significant increase in DI values. It is revealing that one group, though the least numerous, showed a significant decrease in DI values, opposite to the general trend.

Table S2. The mode of place field rate remapping by days

	N of fields	Increase in high, decrease in low	Increase in both directions	Decrease in high, increase in low	Decrease in both directions
D1	171	45	62	16	42
%	100	26	36	9	25
D2	146	42	34	16	40
%	100	29	23	11	27
D3	112	30	15	13	42
%	100	27	13	12	38
D4	149	31	28	20	37
%	100	21	19	13	25
D5	108	25	22	15	23
%	108	23	20	14	21
T1	167	31	34	33	40
%	100	19	20	20	24
T2	67	12	15	12	21
%	100	18	22	18	31

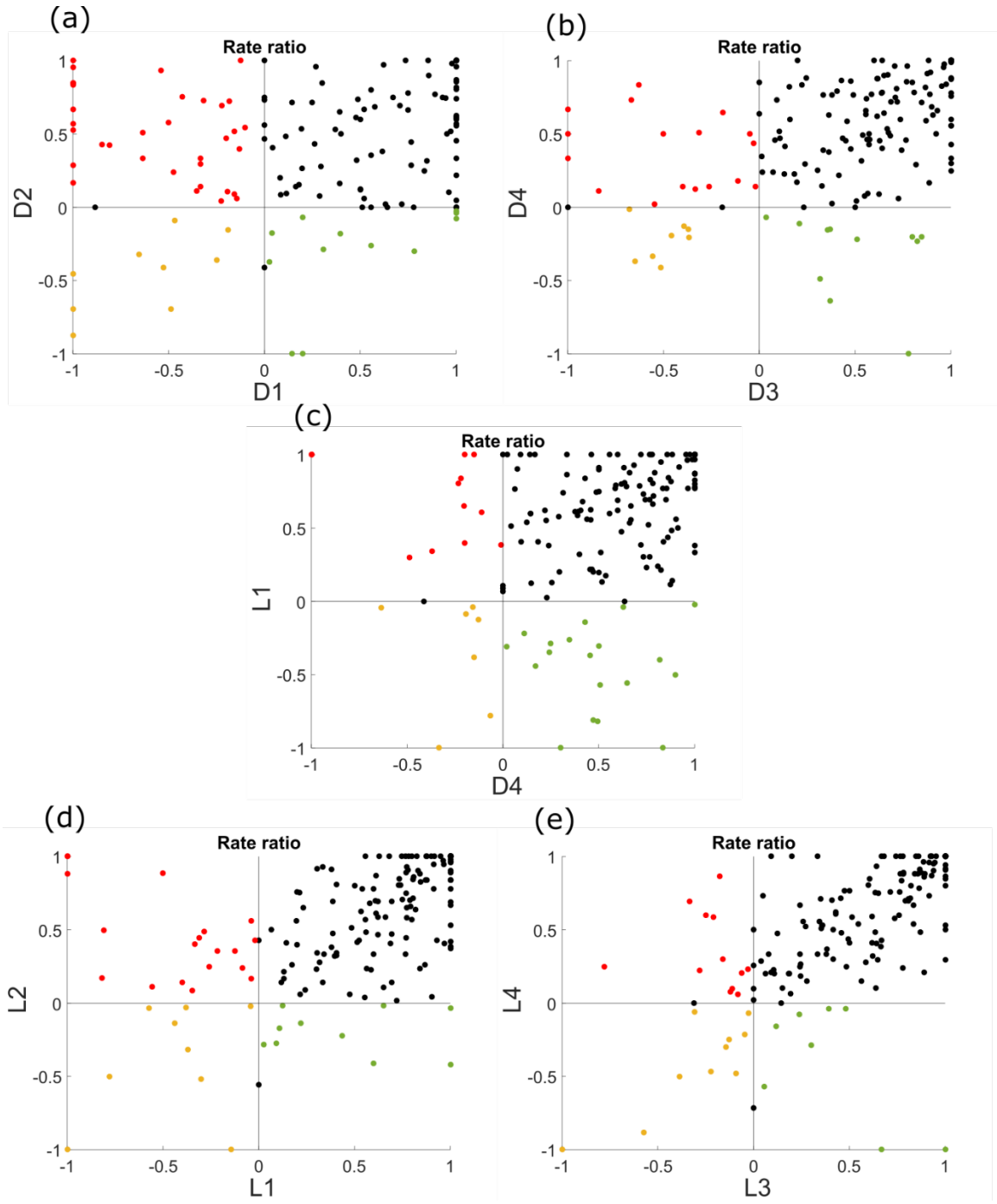

Figure S3. Scatter plots for PF directionality indices for day 1.

L - light laps (L1- the first light lap, and so on). D – dark lap (D1 – the first dark lap). Green and red dots indicate PFs that reversed their directionality. The introduction of visual cues (c, L1-D4) was associated with directionality reversal in certain proportion of PFs. Note that (c) has higher number of green dots (PFs that changed DI sign from positive to negative) than any other plot.

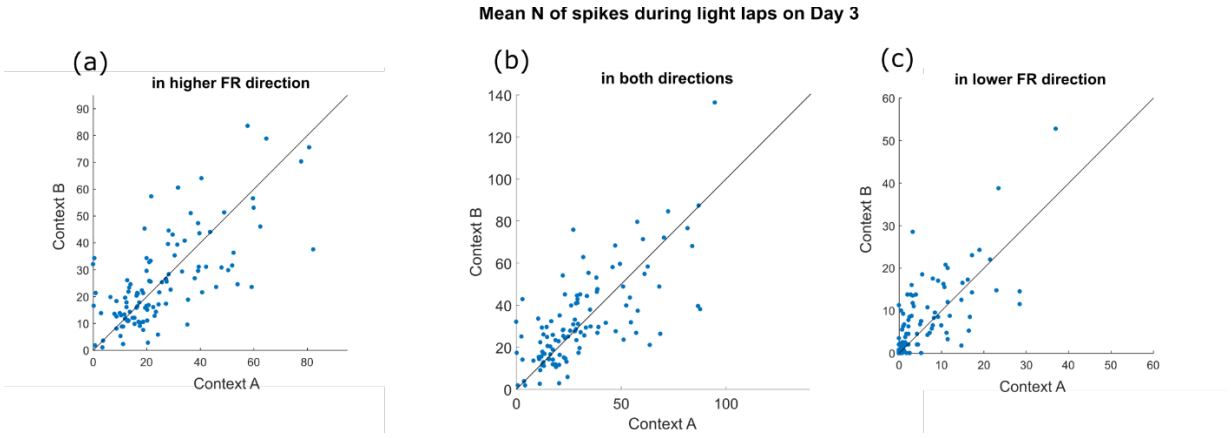

Figure S4. Scatter plots for mean numbers of spikes emitted during light laps in context A and context B.

(a) For both directions of running, most PFs are located along the diagonal, demonstrating relatively similar firing activity. However, a small proportion of field underwent rate remapping folowing the context change.

Place field size in left and right hippocampi by days

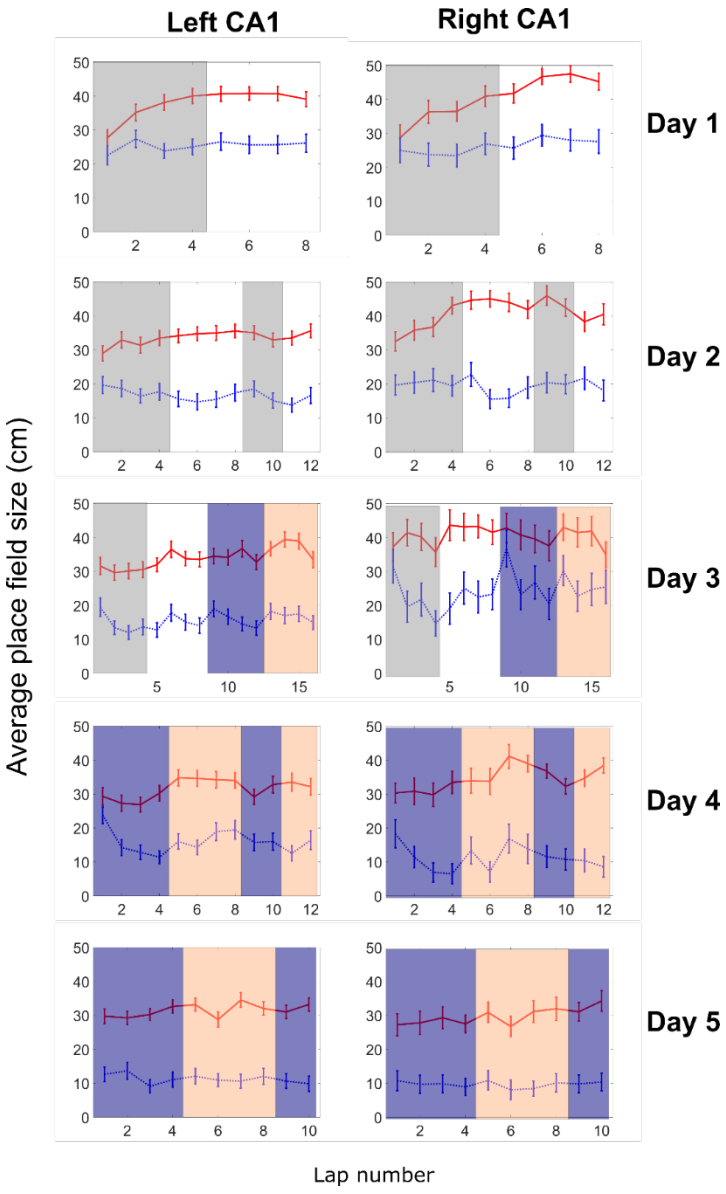

Figure S5. Average place field size in left and right CA1 over 5 days of training.

Red – higher FR direction, blue – lower FR direction, M ± SEM. Note: the place field expansion is slightly more pronounced in right CA1 during the first 2 days of training.

Changes in DI, place field size and COM location in left and right hippocampi by days

Changes in three parameters, the DI, PF size and COM location, were analyzed for each day and the delta values were plotted to compare the magnitude of changes on different days (Fig. S6).

The results revealed a modest increase (delta) in the DI values throughout the test session, comparable with the increase (delta) on the last day of training (Fig. S6, a). In summary, the DI dynamics during the test session resembled those on day 5 but not day 1. Thus, plasticity changes associated with place cell directionality were preserved 5 days after the last training session.

PF size showed a non-significant increase during the dark laps of the test session in contrast to the first day of training. The magnitude of changes throughout the test sessions were virtually the same as in the last training session (Fig. S6, b, c). The mean values for the higher FR direction were around 30 cm in the test session, which roughly corresponded to those on the 1st lap of day 1 and on laps 1-10 on day 5. The mean values for the lower FR direction were around 15 cm, which is lower than those on the first day but higher than those on the last day of training. Thus, plasticity associated with PF size was generally preserved.

Two COM shifts were analyzed: 1) the backward COM shift between the 1st and the 4th dark laps; and 2) the forward COM shift between the 4th and the 5th lap when the lights were turned on. Both COM shifts were analyzed separately for the higher FR direction and the lower FR direction. The magnitude of the backward COM shift (laps 1-4) during the test session differed from those on the 1st and the 5th days of training (Fig. S6, d), suggesting partial memory retention. The forward COM shift (laps 4-5) during the test

session differed from that on day 5, but not day 1 (Fig. S6, e), suggesting no preservation of plasticity changes. Therefore, the COM shift data on the test day is paradoxical: the lack of COM shifts in the lower FR direction, as opposed to significant backward and forward shifts on day 1, suggests preservation of the plasticity changes. However, the substantial COM shifts in the higher FR direction suggest a relearning process and therefore a lack of memory retention. Moreover, the memory associated with the backward COM shift (Fig. S6, d) appears to be more preserved than the memory reflected in the forward COM shift (Fig. S6, e). These discrepancies can be explained by the change of context between the last training day and the test session. Notably, the similar context shift on the 3rd day resulted in more pronounced COM shifts on the 4th day. However, the significant difference between the COM shifts during dark laps on the first day and the test session suggests at least the retention of the memory associated with the COM shift in the dark. Other parameters, the DI and PF size, were not affected by visual cues, including the change of context between the last training session and the test session.

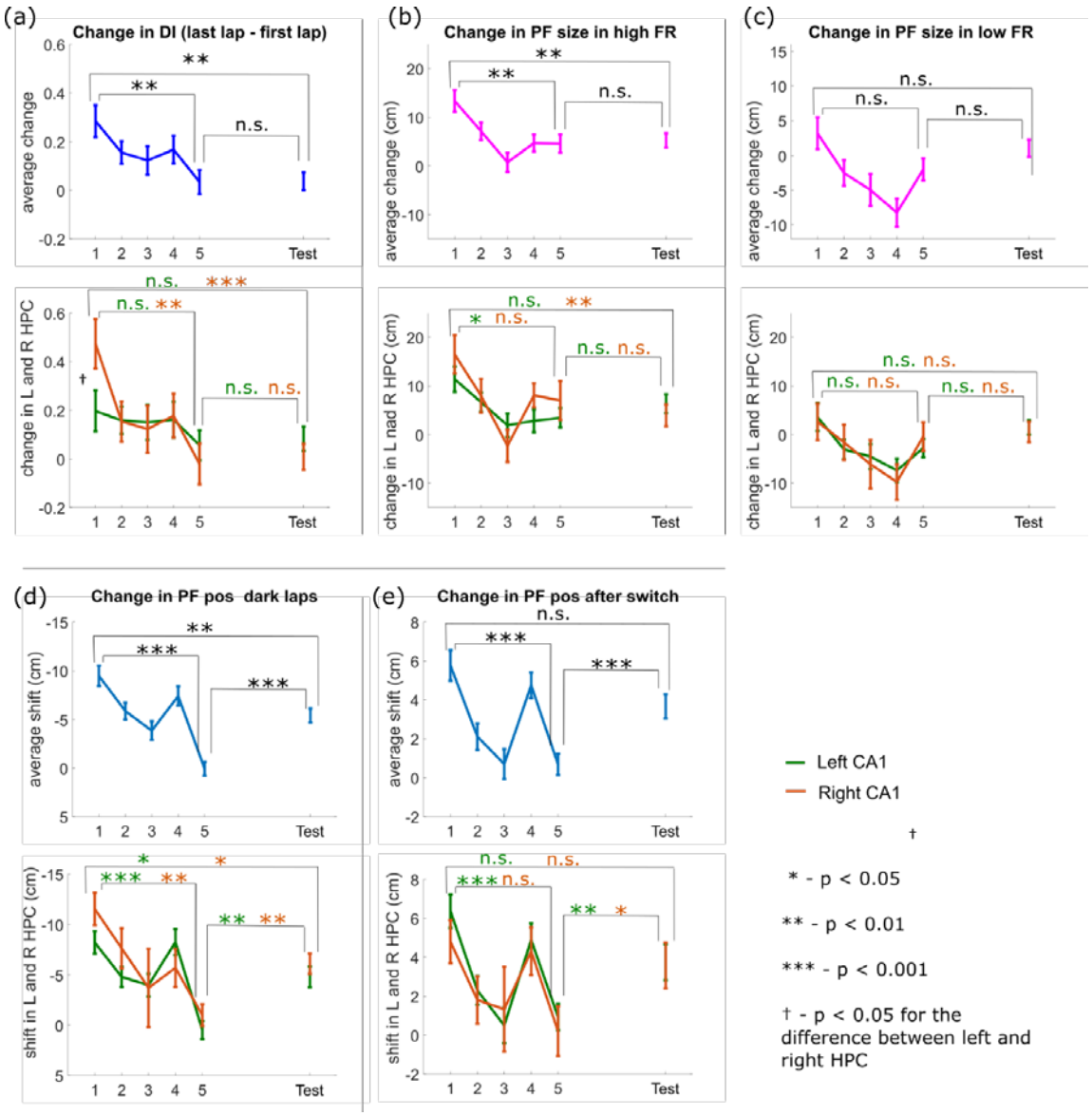

Figure S6. Dynamics of within recording session changes in place field properties.

Average values represent the magnitude of parameter changes during a recording session on a particular day. The magnitude of change declines by day 5, which allows use of the magnitude as a measure of memory retention on the test session.

(a) Increase in the directionality index during the test session was as subtle as on the last day of training and significantly smaller than on the first day (last lap – first lap). Day 1 vs. Test p=0.0017, d=0.36; day 1 vs. day 5: p=0.0088, d=0.34; day 5 vs. test: p=0.97, d=-0.005. Left and right HPC: day 1, left vs. right HPC: p=0.046, d=-0.34207; Left HPC: day 1 vs day 5: p=0.32, d=0.16; day 1 vs test: p=0.31, d=0.15; day 5 vs. test: p=0.92, d=-

0.017; right HPC: day 1 vs. day 5: p=0.0047, d=0.66; day 1 vs. test: p=0.00012, d=0.69; day 5 vs. test: p=0.82, d=-0.049.

(b) During the test session, the increase in place field size in the high FR direction was similar to that on the last day of training and significantly different from the increase on the first day of training (last lap – first lap). Day 1 vs. day 5: p=0.0057, d=0.34; day 1 vs. test: p=0.0023, d=0.33; day 5 vs. test: p=0.79, d=-0.033. Left HPC: day 1 vs. day 5: p=0.026, d=0.34; day 1 vs. test: p=0.13, d=0.22; day 5 vs. test: p=0.31, d=-0.16. Right HPC: day 1 vs. day 5: p=0.13, d=0.32; day 1 vs. test: p=0.0048, d=0.48; day 5 vs. test: p=0.48, d=0.14

(c) Change in place field size in the lower FR direction was similar to the first day of training, last day of training and to the test session (last lap – first lap). Day 1 vs. day 5: p=0.099, d=0.20; day 1 vs. test: p=0.41, d=0.089; day 5 vs. test: p=0.13, d=-0.19. Left HPC: day 1 vs. day 5: p=0.096, d=0.26; day 1 vs. test: p=0.54, d=0.089; day 5 vs. test: p=0.076, d=-0.28. Right HPC: day 1 vs. day 5: p=0.59, d=0.11; day 1 vs. test: p=0.62, d=0.082; day 5 vs. test: p=0.79, d=-0.053.

(d) During the test session, the change in PF position during the dark laps (4^{th} lap – 1^{st} lap) significantly differed from both the first and the last days of training. Day 1 vs day 5: p=4.9e-09, d=-0.79, day 1 vs. test: p=0.0034, d=-0.35; day 5 vs. test: p=3.8e-06, d=0.61; left HPC vs. right HPC on day 1: p=0.22, d=0.22. Left HPC: day 1 vs. day 5: p=5.01e-07, d=-0.84; day 1 vs. test: p=0.026; d=-0.35; day 5 vs. test: p=0.0013, d=-0.051. Right HPC: day 1 vs. day 5: p=0.0021, d=-0.73; day 1 vs. test: p=0.032, d=-0.39; day 5 vs. test: p=0.0033, d=0.31.

(e) During the test session, the change in PF position after the lights were turned on (5^{th} lap – 4^{th} lap) was similar to that on the first day of training and differed from that on the last day of training. Day 1 vs. day 5: p=4.51e-06, d=-0.58; day 1 vs. test: p=0.081, d=-0.20; day 5 vs. test: p=0.00029, d=0.46. Left HPC: day 1 vs. day 5: p=3.92e-06, d=-0.74; day 1 vs. test: p=0.083, d=-0.26; day 5 vs. test: p=0.005, d=0.45. Right HPC: day 1 vs. day 5: p=0.06, d=-0.4; day 1 vs. test: p=0.59, d=-0.095; day 5 vs. test: p=0.018, d=0.12.

The Hyperdrive

The files used to 3D print the hyperdrive are stored at a public domain and can be found at https://github.com/chekhov-sergey/bilateral-hyperdrive