Hippocampal reactivations after aversive or rewarding experience : classical and deep learning approaches

Dmitri Bryzgalov

PSL UNIVERSITÉ PARIS

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Hippocampal reactivations after aversive or rewarding experience: classical and deep learning approaches

(Réactivations hippocampiques après une expérience aversive ou récompensante: approches classiques et d'apprentissage profond)

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Dmitri Bryzgalov
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Composition du jury :

Daniela POPA École Normale Supérieure	*Président*
Stéphanie TROUCHE L'Institut de Génomique Fonctionnelle	*Rapporteur*
Adrien PEYRACHE McGill University	*Rapporteur*
Lisa ROUX Université de Bordeaux	*Examinateur*
Caswell BARRY University College London	*Examinateur*
Karim BENCHENANE ESPCI Paris – Université PSL	*Directeur de thèse*

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Table of abbreviations

AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANN – artificial neural network
BLA – basolateral amygdala
CeA – central amygdala
ChR2 – channelrhodopsin type 2 (excitatory light-gated opsin)
CNN – convolutional neural network
CPU – central processing unit
CS – conditioned stimulus
EV – explained variance
GPU – graphics processing unit
HD – head direction
ICA – independent component analysis
KDE – kernel density estimator
LC – locus coeruleus
LFP – local field potential
LSTM – long short-term network
LTP – long-term potentiation
MFB – medial forebrain bundle
NAc – nucleus accumbens
NREM – non-rapid eye movement (sleep)
PAG – periaqueductal gray matter (prefix 'dl' – dorsolateral, prefix 'v' - ventral)
(m)PFC – (medial) prefrontal cortex
PCA – principal component analysis
PTSD – post-traumatic stress disorder
REV – reversed explained variance
REM – rapid eye movement (sleep)
rVLM - rostral ventrolateral medulla
rVMM - rostral ventromedial medulla
S-R – stimulus-response
SN – substantia nigra
STD – standard deviation
SWRs – sharp-wave ripples
TTL – transistor-transistor logic (referred as a type of digital pulse in the text)
US – unconditioned stimulus
VTA – ventral tegmental area

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Chapter 1. Hippocampus-dependent memories and place cells

Introduction

Large degree of adaptability is one of the most striking properties of living organisms. Naturally changing environmental conditions demand from the living systems constant fitting to altered circumstances in order to survive. Adaptations could span generations, ensuring survival of populations, but also any living being possesses at least minimal means for adaptions that increase the chance of a single organism for longer life.

One of the types of such adaptations, **adaptations to past events and experiences** is one of the most studied phenomena in neuroscience, and it is widely known under the term **"memory". The most common framework to think about memory postulates that memory processes constitute the ability to encode, store and retrieve information**. Present-day neuroscience perspective sees encoding of memories as a process of converting information from perceptual event to a form of neuronal code. Storage includes all operations within neural tissue that conserve this information for some period of time. Retrieval is accessing stored memories resulting in the formation of new memory and/or motor reaction.

Hippocampus-dependent memories

Hippocampus is important for memorization

Hippocampus became a center of memory research after the case study published by William Scoville and Branda Miller (Scoville and Miller, 1957). The patient Henry Gustav Molaison (referred later as HM) has had a surgery ablating bilaterally hippocampal formation, amygdala, parts of entorhinal and temporal cortices as an attempt to cure severe pharmacoresistant epilepsy. Epilepsy was placed under control; however, the procedure had a profound effect on the memory of HM. He partially forgot information memorized before the surgery but more strikingly, he was completely unable to form new memories – a phenomenon that was labeled *'anterograde amnesia'.* Interestingly, HM had perfect capability to form new motor skills, but any verbalizable information could not be memorized by him.

This case and the extensive research that HM and other amnesic patients had undergone have led to several important insights:

- Medial temporal zone including hippocampus and its surrounding structures are crucial for the process of memorization;
- Memory is not a homogeneous entity – it consists of several domains that could be independently affected by lesions and/or experimental procedures;
- There is a neural substrate for the process of memory consolidation: converting labile just-encoded memory into its "solid" form for future storage and retrieval.

Classification of memory

Memory can be classified by the time after which it can be retrieved (*Fig. 1-1*). HM had untouched capacity to form short-term memories and impaired ability to form long-term

memories. Long-term memory splits in two very different domains: declarative memories and non-declarative memories. Notably, as Milner's research has shown, patient HM was able to form non-declarative forms of memory. Non-declarative type includes pavlovian conditioning, priming, any non-associative memory and procedural memory (Milner et al., 1998; Squire, 2004). Forms of memory that were impaired in HM were named declarative memories. Declarative memories can use language to be retrieved. Semantic memories are a subtype of declarative memories that represent facts and concepts. Episodic memories were introduced earlier by Endel Tulving (Tulving and Schacter, 1990), and were defined as the memory of events in the precise spatial and temporal context (subjective memories of agent in particular circumstances).

This classification is based mostly on neuropsychological data recorded from humans and requires care when we transfer it into animal's research. Without entering intricate debates on what constitutes declarative memories in animals, we will accept here an operational definition of episodic-like memories: a unified memory about place, time and contents of the event (Eacott and Easton, 2010). Notably, episodic memories in humans and episodic-like memories in animals share neural substrates: medial temporal lobe and hippocampus (Eichenbaum, 2017).

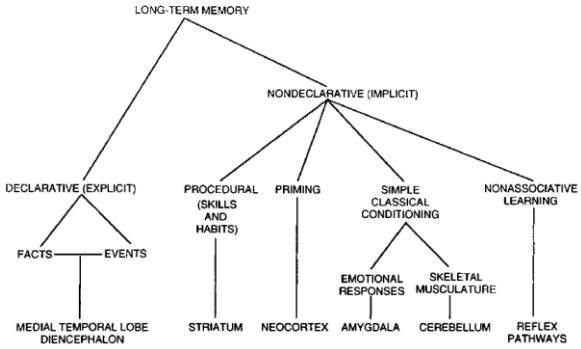

Figure 1-1. Classification of long-term memory. Adapted from Milner et al., 1998.

Spatial memories are also hippocampus-dependent memories

Richard Morris proved a crucial role of hippocampus in spatial memory (Morris et al., 1982). In his experiment, rats had to navigate through a swimming pool with opaque water to a platform (a Morris maze), which is naturally attractive to the animals who do not like to swim. In one condition, a platform was visible to the swimming rat, and in the other condition it was hidden in opaque water. In both conditions, animals took little time to master the task, i.e. to find the shortest way to the platform regardless of starting point. However, while animals with lesions in the hippocampus kept solving the condition with visible platform with equal ease, in the condition with hidden platform lesioned animals demonstrated large deficits in memorizing location of the platform.

This experiment allows for distinction between hippocampus-dependent and hippocampus-independent memory which also bears close resemblance to memory classification

in humans. Without overstating that spatial memory is a special case of episodic-like memories are the same phenomena, it would be rigorous to say that their neural correlates vastly overlap. Hippocampus-independent behaviors can be triggered by any sort of external cue that informs agent to act a certain way, and they do not require intact hippocampus to be manifested (Morris et al., 1982; Kim and Fanselow, 1992). On the other hand, hippocampus-dependent behaviors usually demand to form a certain representation of a situation, and they are impaired upon lesions in hippocampus.

Cognitive map

Term "cognitive map" was introduced by Edward Tolman who aimed to prove that animals can learn in the flexible manner rather than using only 'stimulus-response' (S-R) mechanism as prevailing behaviorism theories stated at the time. Tolman built complex mazes, and rats were allowed to explore freely the environment for several trials (Tolman et al., 1946). After exploration phase, a reward was placed in certain location of the maze and an original path was blocked; a large proportion of animals were able to use newly available shortcut, which it could not be predicted by the S-R theory. **Tolman suggested that instead of using S-R mechanism, animals had built systematically organized sets of knowledge that function as a map that represents environmental relationships and possible paths in the psychological space** (Tolman, 1948).

Neural correlates of spatial memories

Place cells and their properties

This idea became very influential in later cognitive sciences but probably its most popular reappearance happened after John O'Keefe and Jonathan Dostrovsky has discovered that **population of neurons in subfield CA1 of dorsal hippocampus fired action potentials in the location-specific manner** (O'Keefe and Dostrovsky, 1971). These neurons were called "place cells", and their existence was confirmed in many species including bats (Yartsev and Ulanovsky, 2013), primates (Courellis et al., 2019), humans (Ekstrom et al., 2003), and even birds (Payne et al., 2020). Moreover, place cells were also found in the subfields CA2 and CA3 of hippocampus (Kay et al., 2016; O'Keefe, 1979), dentate gyrus (Leutgeb et al., 2007), ventral hippocampus (Poucet et al., 1994), subiculum and parasubiculum (Sharp and Green, 1994; Taube, 1995). However, the vast majority of research that concentrates on place cells focus on pyramidal neurons of dorsal CA1; this manuscript will follow the convention and will center itself on them too.

Box 1-1. Anatomy of hippocampus and trisynaptic loop

Hippocampal formation consists of hippocampus itself and surrounding regions that functionally support hippocampus. Hippocampus is a layered structure that is phylogenetically older than neocortex ('archicortex') – it comprises only of three layers. Hippocampus consists of 3 subfields, or areas, numbered serially: CA1, CA2 and CA3 and the dentate gyrus. Cell bodies of principal cells, named pyramidal cells, could be found in thin *stratum pyramidale*, or pyramidal layer. Basal dendrites and axons of pyramidal cells are located dorsally from pyramidal layer in the layer called *stratum oriens.* Apical dendrites of pyramidal cells projects ventrally in the *stratum radiatum* and further in *stratum lacunosum-moleculare*. Another subtype of neurons found in hippocampus includes various GABA-ergic interneurons (for detailed review on interneurons, please see Pelkey et al., 2017).

Dentate gyrus is a three-layered structure that can be found below field CA1, and it consists mostly of glutamatergic granule cells that lay in *stratum granulare.* Outer *stratum moleculare* includes mostly fibers projecting from entorhinal cortex, and inner *stratum multiforme,* or hilus, consists of excitatory mossy cells.

Entorhinal cortex is situated laterally from hippocampus. It is a neocortical 6-layered structure. Entorhinal cortex is the main hub that connects the rest of the neocortex with hippocampal archicortical networks. Pyramids of layer III projects into hippocampus and conveys sensory information in the hippocampus, whereas pyramidal cells of layer V receives projections from the hippocampus and send the downstream to other cortical and subcortical areas.

Trisynaptic loop is the main functional connectivity pattern in the hippocampal formation. In its most simplified form (for more detailed version, please see *Fig. 1-2b*), the connections follow this schema: pyramidal cells of layer III in entorhinal cortex project to the dentate gyrus via perforant path. Granular cells of DG send their axons to the subfield CA3 of hippocampus; these axons are called mossy fibers. Pyramidal cells of CA3, in turn, project to apical dendrites of CA1 pyramidal cells and send collaterals to lesser known area CA2. CA2 in turn projects to basal dendrites of CA1 pyramidal neurons. Pyramidal cells of CA1 send their projections to deep layers of entorhinal cortex and outside of hippocampus. It is important to note that entorhinal cortex projects also directly to CA3 and CA1 neurons.

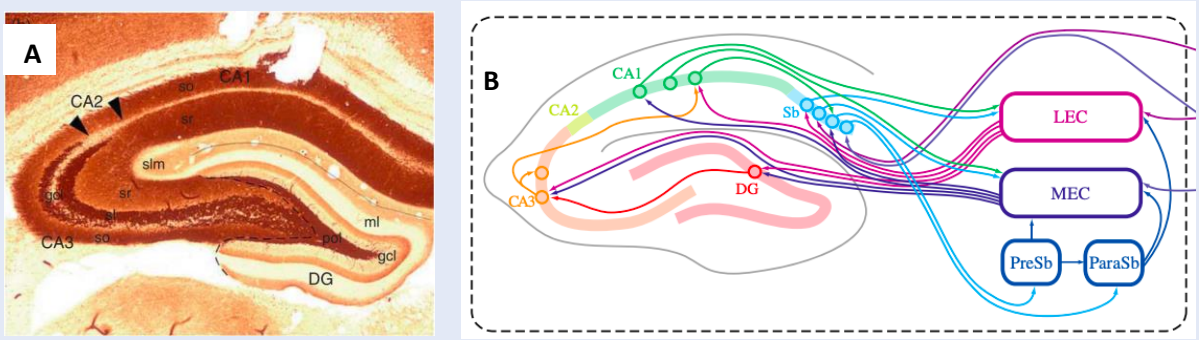

Figure 1-2. Anatomy of hippocampus and its connectivity. ***A.*** *Coronal section of hippocampus stained with Timm's method. Adapted from Burwell and Agster, 2007.* ***B.*** *Schematic depiction of trisynaptic loop. Adapted from Hartley et al., 2014.*

Locations of neuronal firing, or place fields, typically appear in the first minutes after an animal is placed in the novel environment (Wilson and McNaugton, 1993), and hippocampus robustly keeps place coding of a certain environment for long periods of time (Thompson and Best, 1990). Importantly, if an animal is exposed to the new environment, place field of a particular neuron does not depend on its place field in the previous environment (O'Keefe and Conway, 1978). However, certain changes in the environment can trigger alterations in place coding. Substantial reorganization of place fields is called 'global remapping', or 'complete remapping' (Muller and Kubie, 1987; Leutgeb et al., 2005). Global remapping occurs if the majority of distant landmarks have moved, for example, if one changes the room where the environment is located. In contrast to that, if only proximal cues are changed, rate remapping can be observed (Leutgeb et al., 2005; Fyhn et al., 2007). Rate remapping involves changes in firing rate of place cells without altering their place fields. It has been suggested that phenomena of global and rate remapping demonstrate different axes of encoded information. If global remapping points at the existence of navigational system in the brain, which can build maps of physical space, rate remapping indicates that non-spatial features of environment are also represented on top of place cells population code (Colgin et al., 2008). Indeed, rate remapping was observed in the experiments where place coding could be affected by task parameters (Wood et al., 1999; Anderson and Jeffery, 2003), or even factors related to motivation and emotions. I will describe in detail how emotional stimuli affect place cells activity in *chapter 4*.

Phase-coding in place cell system

Place cells do not use solely firing rates to represent position of the animal. It has been shown that time of spikes fired by a particular place cell depend on the phase of ongoing theta oscillations present during any exploratory activity in rodents (O'Keefe and Recce, 1993; Skaggs et al., 1996). When an animal enters the place field of a place cell, neuron starts to fire spike in the late phase of theta cycle; however, while the animal traverses the place field spikes shift to the earlier phases of theta cycle. This phenomenon of theta phase precession has an important implication (*Fig. 1-3*).

Let's we assume slightly overlapping place fields of three place cells and a place cell system with phase precession. When an animal is in the center of neuron B's place field, it is in the beginning and the end of the place fields of neuron A and C correspondingly. According to the rules of theta precession, it would mean that neuron C would be firing action potentials in the early phases of theta, neuron B – at the trough of a cycle and neurons A at the late phase in a cycle. Therefore, these three neurons fire in close succession after each other. Such **co-activity of place cells with adjacent place fields creates a temporal opportunity for long-term potentiation of their synapses, and therefore, organization of place cells into sequences (so called 'theta sequences').** Theta sequences are considered an evidence of cell

assemblies' existence and point to the fact that spatial memories could be stored in hippocampus as an auto-associative network (Buzsáki, 2006).

Place cells can drive location-specific behaviors

Activity of hippocampal neurons can be used to drive spatial behavior. In the seminal study, improving technology first used by Garner et al., 2012, used immediate early gene-driven strategy of expressing ChR2 was used, an excitatory light-gated opsin, in the neurons that were active in a specific environment 1 (Ramirez et al., 2013). After that, authors have transferred animals into the environment 2 where they have performed fear conditioning using activation of neurons tagged in the environment 1 as a conditioned stimulus. However, when mice where placed back to environment 1 they exhibited elevated levels of freezing despite the fact that they never had formed aversive association there. These results suggest that firing of dentate gyrus neurons is required and sufficient to retrieve the behavior that was learnt in pavlovian paradigm. However, it was not clear what was the role of place cells in this experiment and what is physiological meaning of simultaneous firing of neurons that are usually fire separately. Later it was demonstrated that pairing intracranial rewarding stimulation with reactivation (see below) of a particular place cell during sleep results in increased preference for the reinforced location (de Lavilléon et al., 2015). It confirmed that rewarding an animal at the time when place cell firing is completely detached from behavior is sufficient to create positive association with place. Another study used targeted activation of place cells that code for rewarded zone (Robinson et al., 2020). Animals have learnt to lick at the specific location on a virtual track to receive reward. If place cells that represented reward zone were optogenetically activated outside of reward zone, lick rate was increasing two-fold suggesting causal role of place cells in driving behaviors associated with spatial memory.

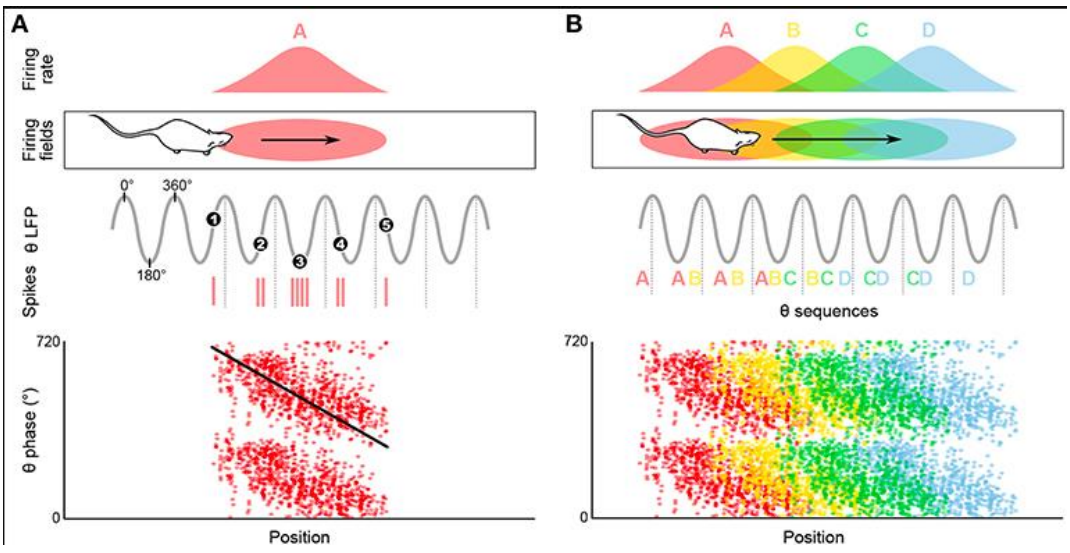

Figure 1-3. Schematics illustrating theta phase precession. Adapted from Drieu and Zugaro, 2019. ***A.*** *Theta phase precession in one place cell. Spike of the place cells progressively moves earlier in the phase of theta cycle while the mouse traverses place the field.* ***B.*** *Theta*

phase precession with four neurons. When an animal traverses overlapping place fields, their place cells fire in different phases of theta cycle, which creates time-compressed theta sequences of place cells (see also text).

Head direction cells

Place cells are not the only cells in the brain that contribute to navigation. Probably, the most basic feature, the direction of the animal's head is coded by head direction (HD) cells (Taube et al., 1990). They could be recorded in subiculum and entorhinal cortex of hippocampal formation but most of them are studied in anterior dorsal nucleus of thalamus and retrosplenial cortex (Taube, 2007). HD cells are considered to provide basis for the allocentric direction coding and are often described by the metaphor of compass. Importantly, selective lesions in HD system can also damage place fields of place cells (Calton et al., 2003).

Grid cells

Grid cells were described by the laboratory of May-Britt and Edvard Mosers (Hafting et al., 2005). Grid cells could be recorded in the medial entorhinal cortex, and their receptive field tesselate environment with a regular pattern. First reports suggested that grid system could participate in the place coding by providing information about path integration. However, relationships between grid cells and place cells go beyond simple fact that grid system could give a reference frame for place cell system. Inactivation of medial septum leaves place coding intact but completely disrupts grid cell receptive fields (Koenig et al., 2011), and inactivation of hippocampus itself damages grid code completely (Bonnevie et al., 2013). There is still no consensus on how exactly grid cells contribute to navigational system in the brain.

Boundary cells

Boundary cells were first predicted theoretically (Hartley et al., 2000; Burgess et al., 2000), and only recently they have been recorded in an experiment. Boundary cells have receptive field that stretches along one or is at the angle of two natural boundaries in the environment. They have been recorded in subiculum (Barry et al., 2006; Lever et al., 2009), medial entorhinal cortex (Solstad et al., 2008; Savelli et al., 2008), and also in pre- and para-subiculum (Boccara et al., 2010).

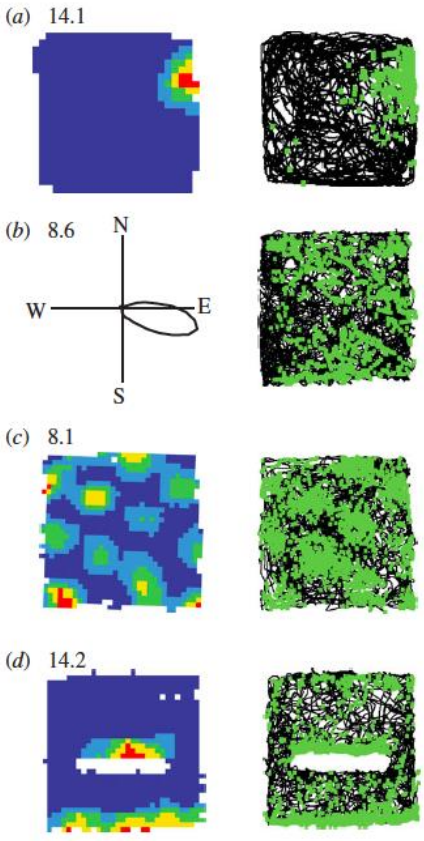

Figure 1-4. Fundamental types of spatial cells. Adapted from Hartley et al., 2014. ***Left:*** *tuning maps of each cell.* ***Right****: trajectories with spike points (in green) superimposed.* ***A****. Place cell.* ***B****. Head direction cell.* ***C****. Grid cell.* ***D****. Boundary cell.*

Models of memory. Consolidation

There are several ideas suggested in the past that are relevant to understand modern hippocampal-dependent memory research and its subfield of spatial memory. They focus on different aspects of memory and different levels of brain organization and serve as a reminder that memory is complex and dynamical process.

Hebbian synapse

Donald Hebb suggested a model of how new memories can be formed at the neuronal level (Hebb, 1949). Hebb proposed that short reverberatory neuronal activity constitutes short-term memory. Given that, he assumed that **if the activity persists long enough, it adds to the stability of connection between neurons and induces long-term changes.** Summarized by the short motto "neurons that fire together wire together", his model describes two hypothetical neurons that are synaptically linked to each other. If neuron A persistently activates another neuron B, synaptic link between them is strengthened, and it takes less excitation from neuron A to induce action potential in neuron B. This idea will be called *Hebbian synapse* in the further text.

Long-term potentiation

The Hebb's model remained theoretical possibility until Timothy Bliss and Terje Lømo discovered in hippocampal slices phenomenon of long-term potentiation (LTP) (Bliss and Lømo, 1973). They have stimulated perforant path, a bunch of projections of entorhinal cortex to hippocampus (*see Box 1*), and recorded its downstream target, neurons of dentate gyrus (DG). They have demonstrated that certain type of stimulation – very fast ('tetanic') trains of short pulses (100-400 Hz) – increased a response of post-synaptic neuron to a single stimulus. Later, researchers have shown that this effect is caused by increased conductance of ionotropic AMPA receptors on the post-synaptic membrane and *de novo* synthesis of more glutamate receptors. These structural changes are long-lasting – thus, Hebb's theory obtained its first experimental prove.

Later, it was demonstrated that tight temporal coordination between spikes of pre- and post-synaptical neurons is required to induce LTP. Action potential of the neuron A should occur less than 20 ms before neuron B for the LTP to occur in neuron B (Markram et al., 1997; Bi and Poo, 1998).

Systemic consolidation models

David Marr kept Hebbian synapse in mind when he suggested the first mechanistic model of memory consolidation (Marr, 1971). **He noticed that hippocampus has internal connectivity different than that of neocortex and proposed that hippocampus act as a temporary storage for sensory information, whereas neocortical networks are well-suited for long-term storage.** Marr suggested that new memories are encoded continuously during active

periods, and due to presence of recurrent connections in hippocampus, a full memory can be retrieved upon activation of one neuron participating in storing a memory. However, potentially limited capacity to store information in hippocampal networks can lead, according to Marr, to the fact that hippocampus progressively sends newly encoded memories to neocortex, a storage of long-term memories. This transfer would constitute the process of consolidation. Strikingly, this purely theoretical work generated a huge number of predictions that were validated years later by experimental neuroscientists.

Most notably, György Buzsáki has proposed **two-stage memory consolidation model** that was built mostly on the foundations laid by Donald Hebb and David Marr (Buzsáki, 1989). Trained as a physiologist, Buzsáki have unified several experimental observations into coherent model of systems consolidation. He has stressed the fact that hippocampal activity is very different during active exploratory behaviors and calm consummatory behaviors, immobility or sleep. During active exploration, hippocampal neurons oscillates in theta band (5-10 Hz); during such theta state, dominantly active subpopulation of neurons is granule cells of dentate gyrus. Calm state is characterized mostly by large-amplitude irregular activity that is rarely interrupted by fast oscillatory events called 'sharp-wave ripples' - SWRs (120-200 Hz). During ripples, CA3 neurons are massively and synchronously discharge and excite mostly their downstream CA1 neurons that, in turn, activate their neocortical targets. During this state, pyramidal cells in CA1 are the most active neurons.

Two-stage model of memory consolidation postulates that during theta exploratory behavior information is encoded in labile form in the structures downstream to the dentate gyrus. Information is transferred into a long-term storage during population bursts in the CA3 of hippocampus. It is important to stress that in this model, hippocampal neurons that were potentiated during active behavior have higher probabilities to be excited during SWRs and thus be converted into long-lasting memory.

Reactivations of place cells: neural correlates of spatial memory

As it was mentioned above, place cells are organized in the sequences during active exploratory behavior, and temporal relationship between spikes within these sequences allow for long-term potentiation, which in turn results in the increased possibility of co-firing of neurons that were active together in the past. There are several studies which show that blocking or manipulating with long-term potentiation, potentially key player for binding theta sequences together, in hippocampus results in severe impairments in spatial-dependent behavior and place cell activity (Shapiro, 2001; Robbe and Buzsáki, 2009). Moreover, theta sequences disrupted during passive transportation of an animal result in degraded post-sleep co-firing, suggesting necessity of theta sequences formation for reactivations (Drieu et al., 2018).

Discovery of reactivations

During exploration of the environment, place cells with overlapping place fields demonstrated significantly larger mean pairwise cross-correlations than place cells whose place fields did not overlap, which confirmed that place cells with adjacent place fields fire together (Wilson & McNaughton, 1994). More strikingly, in post-exploration sleep period authors also detected increased cross-correlations of neuron pairs with overlapping place fields – this effect was comparable to the effect during exploration. Cross-correlations during rest period, which preceded explorations, did not show any difference for two types of place cell pairs. This effect suggested that place cell pairs, which had on average higher co-firing rate than other place cell pairs, maintain their temporal relationships during subsequent rest period. This effect was named **'reactivations'**.

In this manuscript, I will follow the consensus nomenclature proposed by a group of researchers in the special issue dedicated to reactivations (Genzel et al., 2020). They suggested that **reactivation is an umbrella term that define reinstatement of the pattern of neuronal activity that represents a prior experience significantly stronger than it is observed before the experience took place. A replay would be a special type of reactivation that includes sequential information.**

Reactivations happen during NREM sleep

Later, pairwise correlations method allowed researchers to show that temporal relationships between co-firing neurons are stable across exploration and post-exploration sessions. Using 'temporal bias' measure, it was shown that place cells with overlapping place fields on average have significant bias to fire in the specific order (Skaggs & McNaughton, 1996). It is important to mention that this result was obtained in unidirectional exploration paradigms, so there was only one way to approach any given place field. Later, similar results were obtained in the parietal cortex neurons (which have multiple place fields) but no temporal bias was detected between hippocampal and neocortical neurons (Qin et al., 1997).

Improving on correlational methods described above, the group of Bruce McNaughton suggested to assess similarity of cross-correlation matrices during exploration and rest periods, introducing *'explained variance'* measure (Kudrimoti et al., 1999). Using this new measure, authors confirmed previous results and also demonstrated that almost no cross-correlation variance during REM episodes of post-exploration sleep could be explained by previous exploration experience, ruling out potential role of REM reactivations in memory consolidation process.

Compressed place cells sequences are replayed during NREM sleep

Detection of specific place cell activation sequences, which represent trajectories taken by the animal, opened a possibility to match these sequences to any given spike train. Matching a template epoch on the running epoch using a sliding window yielded significantly

Box 1-2. Methods to detect neuronal reactivations

The main idea behind any method to detect reactivations is to look for the neuronal activity that was present in active state of an animal in the subsequent period of time (mostly, researchers are interested in sleep or calm consummatory behaviors). For detailed review on the methods, please see Peyrache and Tingley, 2020.

The first big group of strategies is designed to search for pairwise correlations between neurons. Indeed, if two cells have fired together they would develop strengthened connections according to Hebb's rules and they would continue to fire together after conditions that have driven their co-firing have gone. Such effect could be detected using pairwise correlations of spike trains (Wilson and McNaughton, 1994; Skaggs and McNaugthon, 1996). However, it is very indirect method to assess reactivations, and it does not account for possible correlations present already before the experience. To overcome these limitations, explained variance measure was introduced (Kudrimoti et al., 1999). Authors proposed that if co-firing of neurons during active period translates to co-firing of neurons in later sleep, similarity between matrices of pairwise correlations in active behavior and in subsequent sleep would be higher than between matrices in active behavior and in the sleep that precedes it. To assess similarity, correlation coefficients of pairwise correlation matrices was used, and an explained measure would be

$$EV = \left(\frac{R_{task,post} - R_{task,pre} * R_{pre,post}}{\sqrt{\left(1 - R^2_{task,pre}\right) * \left(1 - R^2_{pre,post}\right)}} \right)^2$$

where R correspond to correlation coefficient of pairwise correlation matrices between periods of time indicated in lower index. Note that explained variance accounts for correlations already present during sleep that precedes active task behavior and isolate only correlations in post-sleep that were inherited from active behavior.

Another group of methods takes the templates of neuronal activity that was observed during exploratory period and matches them to other epochs of interest (Nádasdy et al., 1999). To account for possible replays that dynamically adjust their compression factor, template matching methods could use rank order correlations (Lee and Wilson, 2002). Template matching methods work great in the linear track tasks, however in more complex environments and with increasing number of place cells with multiple place fields, construction of templates becomes very difficult.

Another strategy to detect reactivations comes as a hybrid between correlational methods and template matching (Peyrache et al., 2009; Peyrache et al., 2010). The idea is to extract meaningful components from the correlation matrix of neuronal activity by means of dimensionality reduction techniques (usually, PCA or ICA). Obtained signals (or 'templates') could be projected to the spike trains in the epoch of interest to obtain reactivation strength measure. High reactivation strength measure at the particular period of time is interpreted as an instance of reactivation of a particular template that is, in turn, interpreted as cell assembly

of neurons that fired together during active behavior. This strategy keeps temporal resolution and flexibility of classical template matching combining it with plain logic of correlational methods.

Despite the fact that formally decoding falls in the template matching group of methods, we will stop at it separately as it is the golden standard of reactivation methods nowadays. If one knows unambiguous relationships between firing of a neuron and location of an animal, one could decode animal's position from neuronal activity only (Zhang et al., 1998). The most used decoding framework uses Bayesian relationships, calculating posterior probabilities of finding the animal in the specific spatial location from the population vectors of neuronal firing at any given time point. This approach could be successfully applied to decode the positions replayed during reactivation sequences (and would be described in more detail in chapter 4).

In recent review on reactivation methods by Peyrache and Tingley (2020), authors have pointed out that assumption of different strategies to detect reactivations to show similar result on the same dataset is largely wrong. After using correlational methods, template matching and Bayesian approach on the same real or simulated datasets of candidate events, they have observed large disagreement between all methods used except linear correlation and Bayesian approach. This could have deep impact on the joint interpretations of body of literature, in which different research groups use different methodology.

more repetitions than it would be expected by chance (Nádasdy et al., 1999), and confirmed that sequences of neuronal activity are compressed in time approximately 10-fold. Later, template matching method was brought to its full power in the thorough study in which authors divided neuronal activation sequences with two, three and more than three neurons (Lee and Wilson, 2002). Obviously, probability of exact sequence matching to any spike train is inversely proportional to the sequence length. The study demonstrated that the sequences detected during post-behavior NREM sleep had significantly stronger matching rate to the spike trains recorded during active behavior than it could be predicted by the random occurrence of neuronal activations. It is important to notice that random in this context implies possibility of each neuron to excite each neuron within a neural network with equal probability. (It does not correspond to reality, neurons are always confined by connectivity patterns and synaptic weights).

By early 2000s, there was an ample evidence suggesting that place cells that were active during specific behaviors could be found re-activated during subsequent sleep period with higher probability than cells that were silent during behavior (Kudrimoti et al., 1999). Moreover, these re-activations that happen mostly during periods of SWRs in NREM sleep tend to preserve temporal structure of their activity (Louie & Wilson, 2001; Lee & Wilson, 2002). It is important to note that reports on REM sleep reactivations are still very rare (Louie & Wilson, 2001; Zielinski et al., 2021), and the consensus here has not been reached.

Sleep reactivations and replays were suggested for the role of neural substrate of memory consolidation. Indeed, their occurrence mostly coincides with SWRs that were identified by two-stage memory consolidation model as events that promote memory consolidation. In addition, due to their repetitive nature, reactivations of hippocampal could induce long-term synaptic changes in their downstream cortical targets. I will review causal evidence that hippocampal reactivations are important for memory consolidation later in this chapter.

Place cells are also replayed during calm wakefulness

Place cells reactivations were also found during calm periods during wakefulness (Foster and Wilson, 2006). **Interestingly, these periods were also characterized by high probability of SWRs occurrence**. Reactivations observed unfolded mostly in the order opposite to the order in which animal traversed place fields. These events were termed ‘reverse replays’. Later, it was demonstrated by means of template matching approach that reverse replays occur after behavioral sequence was completed, and forward replays during wakefulness were observed before start of a behavioral sequence (Diba and Buzhaki, 2007).

Forward and reverse replays during the task were observed in the linear track tasks. In the linear track, place cells are activated in strict succession. Each place field could be approached only in two ways. In the two-dimensional environments, each location has several paths to be approached by. This significantly reduces number of neuronal reactivation sequences that could be detected due to random neuronal firing. Despite that fact, very strong

bias towards sequences activated during running epochs was found in re-activated sequences detected during transient halt periods with high density of SWRs (Csicsvari et al., 2007). These results further confirm existence of awake replay phenomenon.

Advances in decoding and recording techniques allowed researchers to determine which locations are reactivated during different types of replay events. Thus, it was shown in the linear environment that reactivations could begin not only with the place cell which was active the last (and the place field which animal currently occupies) but also from remote locations in the environment (Davidson et al., 2009). Other group has shown that animal could replay trajectories associated with the environment explored before the environment it is currently in (Karlsson and Frank, 2009). Very important findings were described in the work by Pfeiffer and Foster (2013). Authors showed that during the goad-directed behavior in two-dimensional open field animals replays future trajectories during transient stops in the behavior. These replayed sequences were biased towards the goal location and, more interestingly, they reflected trajectories that animals chose to take after a replay event. Close relation of awake reactivation events to the task requirements were later confirmed in the study that used a linear track with two stopping points where an animal was receiving reward (Ólafsdóttir et al., 2017). Authors demonstrated that during the periods of immobility directly following and directly preceding locomotion, animal reactivated mostly trajectories that it has just traversed or it will traverse in the immediate future. On the other hand, during immobility periods that were not flanked by locomotion probability to detect remote replay was significantly higher.

Replays that happen during wakefulness are thought to have a role beyond memory consolidation**. Since awake replay often is not mere repetition of explored trajectories but simulation of future paths or even of trajectories that were never and will not be taken, they are considered to play a role in planning and decision making** (Pfeiffer and Foster, 2013; Ólafsdóttir et al., 2018; Pfeiffer, 2020). In an attempt to show causal role of awake replay in spatial decision making, researchers interrupted SWRs while rats where performing W-maze alternation task (Jadhav et al., 2012). Performance of these animals were significantly worse than in control ones. However, it is hard to say whether manipulation with SWRs also truncated replays and whether deficits in behavior were caused by problems in decision making rather than memory consolidation (without going into a debate about interconnections between those two processes).

Preplays of preconfigured sequences

Surprisingly, one group of researchers have found that temporal sequences that are active during subsequent exploratory period could be observed even in sleep that precedes the experience (Dragoi and Tonegawa, 2011). In their seminal paper, they used template matching methods to show that place cell sequences of a certain part of environment were present in the previous sleep despite the fact that animal had never seen this enclosure. These

results were criticized due to methodological issues (Silva et al., 2015). However, the group of George Dragoi has managed to reply to the criticism and has published a paper confirming existence of preplays (Farooq et al., 2019). These results are interpreted as the **evidence that sequential motifs of neuronal activations are selected from the limited pool of pre-connected cell assemblies** (Dragoi, 2020; Buzsáki, 2019). These pre-defined sequences are developing in ontogenesis and they acquire meaning during the experience.

Causal evidence for involvement of hippocampal reactivations in memory consolidation

There is a widespread consensus in the literature that sleep is beneficial for memory performance (see *Box 3* for more details). Moreover, it is widely accepted nowadays that reactivations and replays that occur during NREM sleep are the neural substrate for memory consolidation. Indeed, they mostly occur during calm (offline) states and they do not interfere with the ongoing experience in accordance with two-stage memory consolidation theory of David Marr and György Buzsáki (Marr, 1971; Buzsáki, 1989). In addition, reactivations are powerful bursts of neural activity that are repeated multiple times throughout sleep, which create conditions for long-term potentiation of downstream synapses, and therefore preservation of correlational structure of neuronal activity.

There are few studies that show causal role of place cell reactivation in memory consolidation processes. In the seminal study, rats that have performed hippocampus-dependent multiple-choice task in the radiant maze after which they have been placed for sleep (Girardeau et al., 2009). Control rats have received either intracranial stimulation outside SWRs or no stimulation, whereas one group of animals have undergone closed-loop protocol that have interrupted SWRs each time they occurred. Performance in the task tested after sleep has been significantly lower for the rats in which SWRs were truncated compared to other experimental group. These results were independently confirmed later (Ego-Stengel and Wilson, 2010).

Furthermore, implication of hippocampal reactivations in memory consolidation was proved in the study by the group of Joseph Csivari (Grydchin et al., 2020). Two cheeseboard mazes task were used. Using closed-loop system, authors selectively perturbed specific cell assemblies that correspond to trajectories in the particular environment. They were able to demonstrate that animals spend significantly more time around the reward zone in the control environment, reactivations of which were not interrupted, than in the target environment, suggesting absence of goal-directed behavior in mice with interrupted reactivations. Importantly, it has been shown that place fields of target environment were destabilized after sleep, however they have quickly come back to normal behavior during subsequent relearning.

The question of causality was further approached by our research group: authors have used rewarding medial forebrain bundle stimulation triggered on sleep reactivation of a

Box 1-3. Evidence for the role of NREM sleep in memory consolidation

Idea that sleep is beneficial for subsequent memory performance seems to be present in folk psychology for the whole period of human culture existence. First experimental evidence confirming this view came in 1920s (Jenkins and Dallenbach, 1924), but it takes many decades and the discovery that sleep consists of cyclic alternation between two stages (NREM and REM sleep) to start accumulating modern experimental results. Initially, dominant line of research linked REM sleep to memory consolidation function due to the fact that dreaming occurs mostly in REM stage, however, at the moment there is a large body literature that demonstrates indispensable role of NREM sleep in consolidation of hippocampus-dependent memories (for more detailed reviews, see Diekelmann and Born, 2010; Born and Wilhelm, 2012).

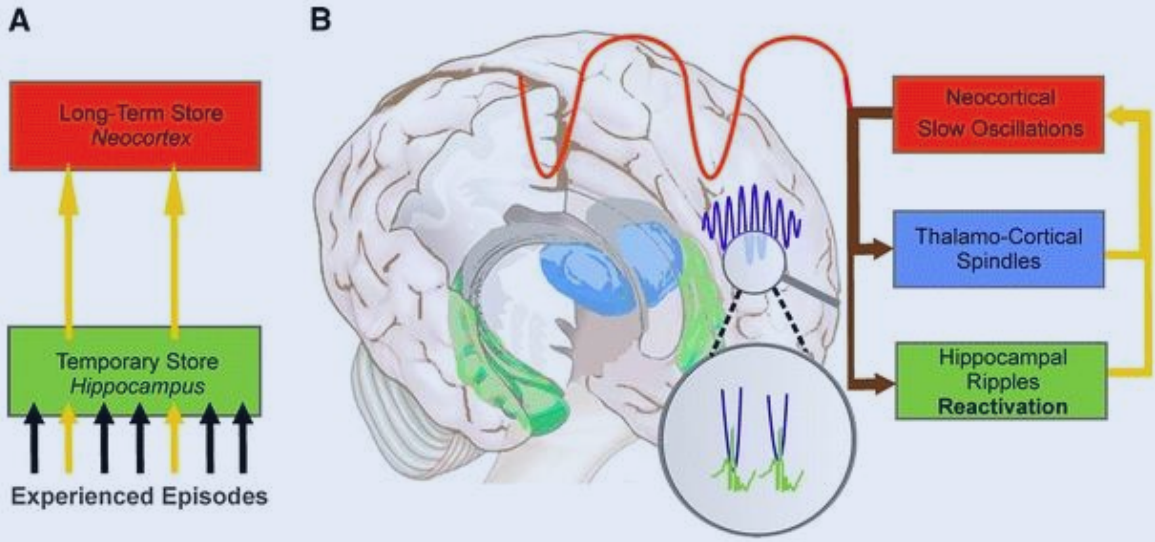

Figure 1-5. Schematic vision on modern version of two-stage model of memory consolidation. Adapted from Born and Wilhelm, 2012. *New memories are encoded in hippocampus during active theta state (not shown), and are consolidated during calm periods. The process of consolidation involves tight coordination between hippocampus in neocortex: neuronal activity at the time of hippocampal sharp-wave ripples happen in very close temporal proximity to neocortical slow oscillations and corresponding to them 'UP' and 'DOWN' states as well as to thalamo-cortical spindles. Perturbations of any of these oscillatory signatures alter memory performance.*

Thus, after training in declarative memory tasks, human subjects that were asked to retrieve their memory after the first part of the night, in which NREM is the dominant sleep stage, performed better than subjects who were tested after the second part of sleep, which has much larger density of REM sleep (Plihal and Born, 1997; Plihal and Born, 1999). In different study, participants have learnt card-pair location in the presence of a particular odor (Rasch et al., 2007). In later sleep, this odor was re-exposed to participants during different

stages of sleep or during wakefulness. Memory performance during test was significantly better in the group where odor was presented during NREM sleep compared to two other groups.

NREM sleep is characterized by several neocortical oscillatory signatures: namely, slow oscillations (< 1 Hz), delta waves (1-4 Hz) and spindles, transient oscillatory activity in the range of 12-16 Hz. Moreover, delta waves orchestrate 'UP' periods in neocortex when neurons extensively fire action potentials and 'DOWN' periods when neurons are silent. Interestingly, neocortical NREM sleep signatures are tightly coordinated with hippocampal SWRs. Thus, hippocampal SWRs occur mostly at the transitions between 'DOWN' and 'UP' states in neocortex (Sirota et al., 2003; Battaglia et al., 2004). In the similar manner, spindles closely follow delta waves and could be observed at the transition to 'UP' states (Peyrache et al., 2011).

Manipulations with oscillatory signatures during NREM sleep confirm their important role in memory consolidation and close functional relationship with hippocampal SWRs and, possibly, reactivations. Delta waves and spindles were electrically induced in the neocortex after detection of SWRs in hippocampus (Maingret et al., 2016). This procedure of reinforcing natural coordination between hippocampus and neocortex during NREM sleep improved performance of animals in hippocampus-dependent memory task. In other study, authors used mice that have undergone contextual and cued fear conditioning protocol (Latchoumane et al., 2017). In subsequent sleep, spindles were induced by stimulation of thalamic nucleus reticularis in phase with neocortical rising phase of slow oscillations (Latchoumane et al., 2017). This procedure resulted in increased freezing rate only in contextual fear conditioning protocol, a task which is thought to depend on hippocampal function, but not in the cued version, which is hippocampus-independent.

Taken together, these studies (and many more, reviewed elsewhere) provide compelling evidence that NREM sleep is beneficial for consolidation of hippocampus-dependent memories. Moreover, results reviewed suggest that memory consolidation heavily rely on the precise coordination between hippocampal and neocortical networks (see *Fig.* 1-*5*).

particular place cell during sleep (de Lavilléon et al., 2015). In the subsequent wake period, animals increased five-fold the time spent in the place field of the reinforced place cell compared with pre-sleep exploration. It is important to mention that animals also demonstrated signatures of goal-directed behavior towards the place field area (decreased latency to reach it).

Up to date, these report remains the only ones that directly demonstrate that reactivations of place cells play causal role in memory consolidation during NREM sleep. Due to technical difficulties of manipulating place cells, further studies are still to be performed, and there are still questions that remain without answer.

Résumé

Hippocampus is required for encoding and consolidation of declarative memories in humans. In animals, hippocampus-dependent memories are often studied in the paradigm of spatial navigation.

Neurons in hippocampal formation demonstrate tuning to different aspects of spatial navigation system. Most known of them are place cells that are recorded mostly from the CA1 of hippocampus and exhibit location-specific firing. There are also grid cells the firing pattern of which represents a grid-like structure overlaid on the explored environment, head direction cells which are tuned to the angular direction of the animal's head and boundary cells that are active at the natural boundaries within the environment.

Memory is a complex dynamical process that occur at several levels of brain organization. Encoding of spatial memories is thought to happen during active exploratory period when theta oscillations organize neurons in sequences via long-term potentiation mechanisms. Encoded memories are consolidated during sleep, consummatory or immobility periods that are characterized by irregular oscillatory patterns called sharp-wave ripples. Repetitive and massive activation of neurons during sharp-wave ripples is considered a mechanism of memory consolidation.

Sequences of place cells explored during active behavior are reactivated both during calm periods during wakefulness and sleep suggesting their potential role in consolidation of spatial memories. There are three main groups of methods that researchers used to detect reactivations: correlational methods, template matching methods and decoding methods.

Reactivations detected during wake could be reverse or forward in respect to the primary direction of exploration. Moreover, they have been found to represent not only explored trajectories but trajectories that will be taken in the future or trajectories that will never be explored. For that reason, the community agrees that wake reactivations play a role in planning and decision-making (via potential mental simulations).

Reactivation during sleep were detected mostly during NREM sleep but there are few reports showing REM sleep reactivations. NREM sleep reactivations mostly occur during ripples, and most of them in the early parts of post-exploration sleep. Their role in supporting

memory consolidation has found solid evidence in several causal studies, in which authors either impaired memory retrieval by truncating sleep reactivations, or changed emotional valence of previously neutral memory during NREM sleep proving the fact that memory was still in labile form at the time when sleep reactivation occur.

Questions:

- What are the mechanisms in the brain that select information to be re-played? Which conditions affect contents of any specific replay?

- Does hippocampus code only spatial information or does it include other non-spatial parameters in its rate and/or phase coding? – chapters 4, 5, 7

- How do emotions and motivation change rate and contents of reactivations? – chapters 4, 5

Chapter 2. Decoding of animal's position from neuronal activity in hippocampus

Introduction

Decoding algorithms attempt to reconstruct the variable potentially encoded in the neural activity from this activity. There are multiple fields in neuroscience and medicine where decoding from neural signals plays a prominent role, including decoding of various aspects of movement from motor cortex (Ethier et al., 2012), or decoding decisions from activity in prefrontal and parietal cortices (Baeg et al., 2003; Ibos and Freedman, 2017). Historically, decoding was first applied as a passive tool to understand properties of the neural code and for analysis of neural activity that was detached from active behavior, for example, during sleep. However, in recent years decoding became an active part of closed-loop experimental systems and brain-computer interfaces, allowing answers to questions on causality within neural circuits. In this chapter, we will discuss decoding of animal's position from the activity of hippocampal neurons.

Decoding of animal's position from hippocampal place cells is relatively easy task due to unambiguous relationships between their firing pattern and the variable to decode. Indeed, one needs only few sharply tuned place cells to build robust and accurate decoder. Situation appears more difficult when reactivations are attempted to be decoded – in these circumstances decoding is one of the few ways to discover actual contents of a particular replay event. However, we do not have any ground truth during reactivations, which means that the decoding interpretation critically depends on the assumptions embedded in the decoding algorithm.

In addition, use of position decoder for **closed-loop experiments** places more demands: **decoder should be computationally fast and require minimal manual curation.** In this chapter, I will review existing strategies to decode animal's position from hippocampal signal in light of these aspects of their functioning.

Bayesian decoding

Bayesian framework seems to be the natural choice for decoding problems. It explicitly asks the question of interest**: given the neuronal firing at the time t, what is the most probable value of the variable we want to decode**? Bayesian decoder (and – almost – all other decoders that we will discuss in this chapter) aims to predict the most probable location of an animal in spatially binned environment based on the firing rates of neurons from a certain time window (also called bin).

If we apply Bayes formula to the problem of position decoding, it will look as follows:

$$P(pos|spikes) = \frac{P(spikes|pos)\,P(pos)}{P(spikes)}, \qquad (1)$$

where *P(pos)* is the prior, or the probability of the animal to be found in certain spatial bin of environment, *P(spikes)* is the probability of certain number of spikes to occur in the temporal bin, *P(spikes|pos)* is the likelihood, or the conditional probability of certain number

of spikes given certain position, and *P(pos|spikes)* is the probability of an animal to be found in certain position – or the posterior probability we would like to find.

From the usual experimental dataset with multiple simultaneously recorded place cells and behavioral tracking, one has instantaneous position of the animal and the spike trains. Thus, we can easily calculate *P(pos)*. P(spikes) could be obtained using marginalization of conditional probability *P(spikes|pos)* over all spatial bins, and therefore one does not need to measure it directly. There is one term needed to calculate posterior probability in the equation 1: conditional probability *P(spikes|pos),* or likelihood.

There are two key assumption in Bayesian approach to position decoding: spiking of any given neuron is drawn from Poisson distribution, and all neurons are statistically independent from each other (Zhang et al., 1998). Given Poisson statistics of spike trains, likelihood to find n spikes fired by one neuron in the time window *t* is

$$P(n|pos) = \frac{(tf(pos))^n}{n!} * e^{-tf(pos)}, \qquad (2)$$

and, given statistical independency of all neurons,

$$P(spikes|pos) = \prod_{i=1}^{N} P(n|pos) = \prod_{i=1}^{N} \frac{(tf(pos))^{n_i}}{n_i!} * e^{-tf(pos)}. \qquad (3)$$

When inserting equation 3 into equation 1, we get

$$P(pos|spikes) = C(t, spikes)P(pos)(\prod_{i=1}^{N} \frac{(tf(pos))^{n_i}}{n_i!}) * e^{-t\sum_{i=1}^{N} f_i(pos)}, \quad (4)$$

where *C(t, spikes)* is the normalization constant such as sum of *P(pos|spikes)* across all spatial bins equals 1.

Zhang and colleagues further discussed validity of those assumptions. In fact, it is hard to say that pyramidal cells in hippocampus fire action potential according to Poisson statistics. Their firing is largely skewed due to their low basal firing rate and with peaks of activity strongly dependent on the position of the animal. Also, on short time scales (<10 ms) hippocampal pyramidal cells tend to fire bursts, called complex spikes, which is not grasped by Poisson distribution. However, as we can see later in this chapter, Poisson process is the good enough starting point to model neuronal firing, and Bayesian model with this assumption yields fairly accurate results in decoding tasks (Zhang et al., 1998; Brown et al., 1988).

Second assumption about statistical independency of neuronal firing contradicts what we know about physiological properties of hippocampal network: once an animal explores the environment, correlations between spike trains are developing rapidly (Wilson and McNaughton, 1994; Kudrimoti et al., 1999). If in the recorded dataset one does not have overlapping place cells, neurons rarely fire together but, even given that, independence assumption should be considered as simplification.

Despite this, Bayesian framework demonstrate good decoding accuracy (*Fig. 2-1*). When calculating distance between predicted and true positions, Bayesian decoder outperformed linear methods by the factor of three (Zhang et al., 1998). Decoder was trained on the datasets containing 25-30 place cells, and authors have shown that decoding accuracy is proportional to the number of cells in the analysis. Importantly, authors were able to theoretically demonstrate that Bayesian decoder performs close to the theoretically optimal decoder, defined on the basis of Fisher information.

Interestingly, once validated on the awake data where population vectors of hippocampal neurons codes precisely for the actual position of an animal, Bayesian decoder could be used to decode replays. **For fine-grained decoding of compressed contents of hippocampal sequences, researchers usually use small (20-50 ms) time windows and apply various assumptions constraining sequences to be continuous** (Zhang et al., 1998; Davidson et al., 2009; Wu and Foster, 2014). Using this method, it is possible to decode replayed trajectories in calm wake (Johnson and Redish, 2007; Davidson et al., 2009; Pfeiffer and Foster, 2013), and sleep (Dragoi and Tonegawa, 2011; Farooq et al., 2019). It is important to mention that instead of point estimates, Bayesian decoders gives the user probability distribution of inferred positions, which could be used as the handle of prediction confidence.

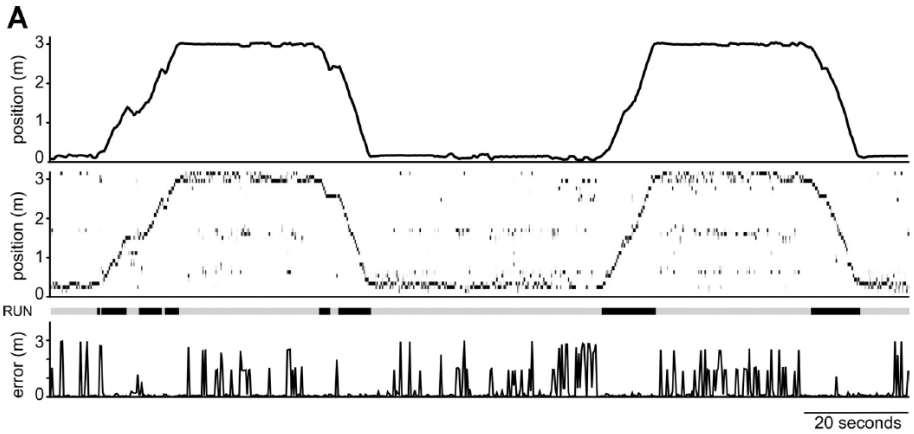

Figure 2-1. Example of the Bayesian decoding. Adapted from Kloostreman et al., 2014. ***Top.*** *Real position of the animal.* ***Middle****. Inferred position of the animal.* ***Bottom****. Speed of the animal. The ribbon is colored in black for active periods of the animal, grey – for calm periods.*

Application of Bayesian decoder to closed-loop experiments

In essence, online decoding of the position is not different from offline one. The only additional requirement is that the decoding computations in each time window should run as fast (or faster) than an update of each of those windows. Therefore, the main requirement for online decoding algorithm is an optimal code on the fast machine.

The first report where bayesian decoder was successfully applied to decoding animal position in real time was published in 2011 (Guger et al., 2011). Position was decoded from the dataset of hippocampal neurons recorded from rats performing random foraging in the open field. Authors have manually sorted spike data on pre-existing dataset, and they used resulting waveforms as templates for incoming spikes during the experiment, achieving real-time spike sorting. Fairly accurate online reconstruction of animal position was demonstrated but this strategy (although important as a proof of concept) suffers from several imperfections. First, to estimate firing rate of the neurons, spiking data over long periods (several seconds) need to be estimated, which makes this algorithm not usable for decoding of replays that happen on the fast scale. This could be overcome by using firing rates pre-recorded over long enough periods. More importantly, this method relies on the manual spike sorting step which is very time-consuming, if a researcher has more than 15-20 units to spike sort.

This latter constraint was overcome by Fabian Kloosterman and colleagues (Kloosterman et al., 2014). Instead of fully sorted spikes, authors used waveform features obtained from principal component analysis (PCA) performed on prerecorded spikes. They have developed the version of Bayesian decoder that, instead of using firing rates of individual units, assesses *P(pos|spikes)* based on waveforms features. To assess probability distribution in real time, kernel density estimators (KDE) were used. This algorithm was able to decode position of the animal with accuracy that was comparable to the classical Bayesian decoder with sorted spikes. However, once quality of spike sorting was artificially decreased, feature-based decoder demonstrated better performance. Summing up, fully functioning online decoding algorithm based on Bayesian approach was validated, and it was shown that it requires minimal manual curation. One group of parameters that needs to be tuned is the bandwidth for kernel density estimators: as it is pointed out in the paper, bandwidth should have been found separately for each dataset.

Another sensitive part of online Bayesian decoding algorithm is that KDE used for constructing probability distribution is computationally slow, and could significantly slow down performance, which is undesirable if one wants to decode replay events happening at the timescales of tens of milliseconds. On one hand, kernel density compression techniques were suggested to speed up KDE (Sodkomkham et al., 2016), which only marginally degraded decoding accuracy. On the other hand, parallelization proposed by graphics processing units (GPUs) was utilized to significantly accelerate decoding classically built for central processing unit computations (Hu et al., 2018).

As a result, this technique was successfully applied to decoding contents of replays in hippocampal CA1 in real time (Ciliberti et al., 2018). In this study, population bursts were identified using manually selected threshold as candidate events that could harbor replays. Using Bayesian framework with unsorted spikes described above, replays were detected in the time windows of 50 ms with 70% sensitivity and specificity and with 95% accuracy compared with offline Bayesian decoding, which allows using this algorithm to perform closed-loop experiments.

Even such impressive technological advance in closed-loop neuroscience has its drawbacks. First, **the whole pipeline is very heavy, and either requires a lot of expensive equipment or extremely good quality of recording, which is not always achievable, especially in experiments with mice.** Second, as it was mentioned above, Bayesian decoders place two heavy assumptions on the statistics of firing units: their Poisson distribution and statistical independency. These assumptions are not satisfied for neuronal firing, and this fact could hide certain aspects of location-related activity in hippocampus. Other machine learning techniques of position decoding were proposed to promote more out-of-box (out-of-Bayes) thinking about hippocampal replays.

Artificial neural networks to decode position

Artificial neural networks (ANNs) are the natural choice for the tasks where mapping from high-dimensional data to low dimensional outputs is required (Richards et al., 2019). Importantly, **ANNs make few assumptions about input data**, and nowadays ANN algorithms are highly optimized to run both on CPUs and GPUs. These factors make ANNs good candidates for the backbone of the position decoders.

Long short term memory (LSTM) neural network is the type of recurrent neural network that was designed to detect dependencies unrolling in time. LSTMs were largely used in the time series prediction and recognition of speech. Sequences of hippocampal activity have structural resemblance to language because they have distinct words (place cells) that are formed into sentences (theta sequences). It has been demonstrated that LSTMs could be successfully applied to the problem of position decoding using manually pre-sorted spikes as inputs (Tampuu et al., 2019). Decoder based on the LSTMs significantly outperformed Bayesian decoder, and it was slightly more robust to number of single units used for training than Bayesian decoder.

Another approach was developed to avoid spike sorting step, which inflates time needed for a full cycle of training and, more importantly, introduces human-related biases in the algorithm (Frey et al., 2019). Instead of spiking activity, inputs are derived from wavelet transform of raw electrophysiological signals that are fed into consecutive layers of 2D convolutional network (CNN). CNNs are widely used in computer vision tasks and are known for

their ability to learn representations meaningful for the current tasks from raw pictures. Indeed, if we treat wavelet decomposition as two-dimensional picture, we can build the algorithm that can infer position of the animal from those pictures. This approach yielded excellent results: CNN-based decoder demonstrated 2 times smaller error than Bayesian decoder. This algorithm was robust to downsampling and more robust to decreasing number of inputs than Bayesian decoder.

It is important to stress here that these two very different takes on position decoding performed better than conventional Bayesian decoder, albeit the fact that they all used (preferentially) spiking activity. This is indirect evidence that assumptions used in Bayesian approach actually constrain performance of the decoder, and ANN-based decoder could offer different view on position coding in hippocampus and, more interestingly, on hippocampal reactivations.

In addition, **ANN-based decoders have a huge potential to be used in closed-loop experiments: computational cost of using trained ANN is small (unlike Bayesian decoder), and variety of different backend solutions make deep learning more convenient candidate for fast and accurate decoding than Bayesian approach**. In this manuscript, we will demonstrate a solution that combines advantages of strategies without spike sorting with recurrent ANN models.

Résumé

Decoding of the position from the activity of hippocampal neurons is a powerful tool for studying reactivations and replays of past experience that happen during offline states, and a tool for asking questions about causality during closed-loop experiments. Therefore, it is important to realize strengths and limitations of the decoding tools in order to interpret results correctly. There are two additional demands that are placed on the real-time decoders: computational speed and minimal manual curation of data.

Bayesian decoders were golden standard for position decoders for two decades. Bayesian approach is essentially calculating conditional probability of animal's position given population vector of neuronal firing. Within this framework, we assume that firing of the neurons in hippocampus follows Poisson distribution and that different neurons are statistically independent from each other. These assumptions are not confirmed by recording of real neurons. However, Bayesian decoders demonstrate accurate performance when attempting to decode a position of active animal, and they can be applied to decode offline replays. Importantly, instead of point estimates, Bayesian decoders gives the user probability distribution of inferred position, which could be used as the handle of prediction confidence.

Bayesian decoders could be optimized for the use in closed-loop experiments. There is series of report that present impressive technological development that allow to avoid time-consuming and error-prone spike sorting and to significantly speed up the inference. This set-up was successfully applied to online decoding of hippocampal replays.

Another branch of research centered on position decoders utilizes artificial neural networks. In addition to being fast, ANNs are free of assumptions incorporated in Bayesian approach. Two algorithms were proposed in the literature: one based on the use of recurrent neural networks, another based on convolutional neural networks. Both of them outperformed Bayesian decoder. However, there is still little evidence on the success of using ANNs to decode replay contents or on the use of ANNs in the closed-loop system for position decoding.

Questions:

- Can we design position decoder based on artificial neural networks that would not require spike sorting? – chapter 6

- If yes, can we use such network as a tool to study neuronal reactivations? – chapter 6

Chapter 3. Emotional behaviors and their correlates in the brain

Introduction

Learning recruits a large number of structures in the brain, and the big share of them is directly involved in processing of reinforcement (either positive or negative). In the current thesis, we employ both reward and defensive systems of the brain, and it is crucial to understand main architecture and principles of their functioning and interaction.

Both reward-based and aversive learning have stereotypical sets of behavioral correlates: for instance, seeking behavior after reward exposure, or freezing behavior after aversive event. In natural conditions, these behaviors intermingle within each other, further complicating the picture of affective learning.

One interesting example is changing the emotional valence of an object, or counter-conditioning. Can we use it to change behaviors related to maladaptive association?

Organization of reward system

Reward-related behaviors

Reward, also known as positive reinforcement, is any object or event that generates approach behavior and/or consumption, produces learning of this behavior, and/or is an outcome of decision-making process (Schultz, 2007). Rewards produce positive changes in behavior**. Positivity in this case refers to the fact that primary rewards (such as food or liquid) induce reaction of consumption as well as approach behavior as opposed to the reaction of avoidance and flight** (Schultz, 2006). The most obvious example of reward-related behaviors could be seen in the operant conditioning – behavioral paradigm, in which an animal should take action to receive the reward. In such conditions, animals will repeat the behaviors that lead to the rewarding outcome as long as the contingency between reward and action is kept. Learning of the contingency between reward and action and approach behavior characterized by physical movement towards rewarding stimuli satisfy the definition of goal-directed behavior (Dickinson and Balleine, 1994). Thus, in case of operant conditioning rewards prompt goal-directed behaviors. Importantly, rewards not only result in goal-directed behavior towards them but also in general arousal and proactive efforts to increase reward probability (Wise, 2006).

Interestingly, many theorists consider rewards to be a function that organisms maximize in order to survive and multiply their offspring. In that sense, distal rewards call for constant seeking behaviors: in order to pass genes to the next generation one has to find food, water and mates. Thus, rewards exist in a constant positive feedback loop, in which they drive, attract and amplify behaviors.

Reward system in the brain

Processing of reward is tightly linked to the functioning of dopaminergic structures of the brain.

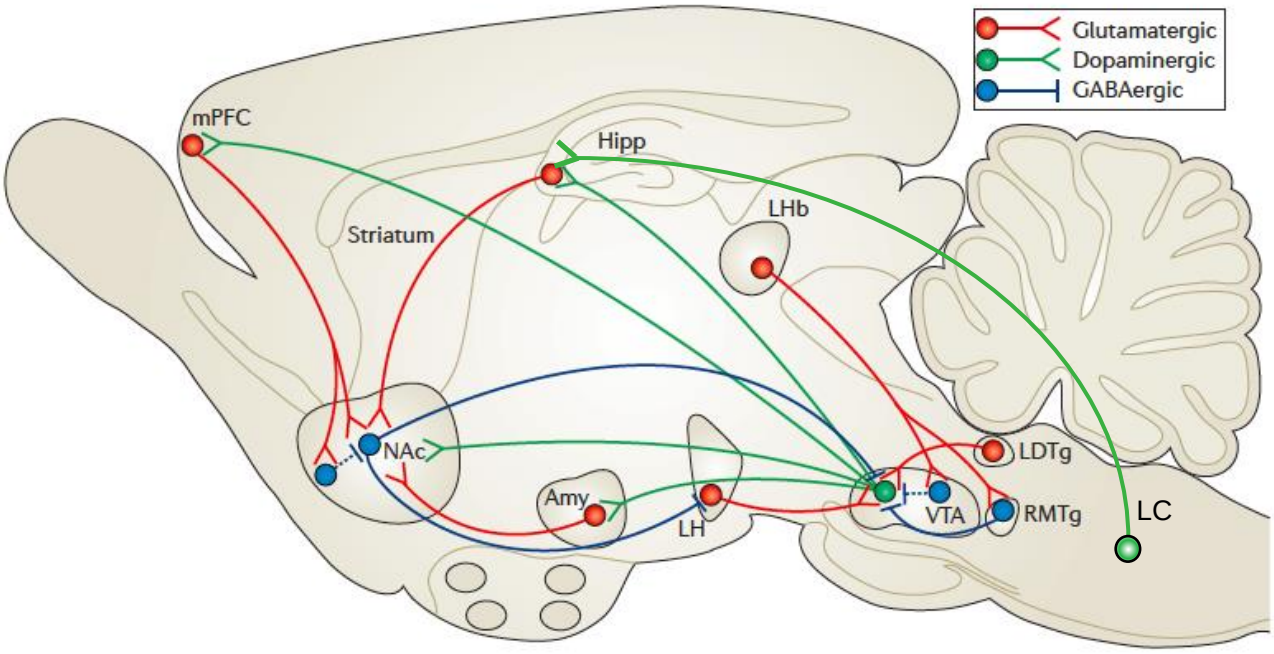

Figure 3-1. Simplified schematics of major reward-related systems in rodent's brain. Adapted from Russo and Nestler, 2013 with modifications. *The primary reward circuit includes dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), which release dopamine in response to reward-related stimuli. The NAc receives dense innervation from glutamatergic monosynaptic circuits from the medial prefrontal cortex (mPFC), hippocampus (Hipp) and amygdala (Amy), as well as other regions. The VTA receives such inputs from the lateral dorsal tegmentum (LDTg), lateral habenula (LHb) and lateral hypothalamus (LH). Important for spatial learning connections comes from locus coeruleus (LC) to Hipp. These various glutamatergic inputs control aspects of reward-related perception and memory. The dashed lines indicate internal inhibitory projections*.

Dopaminergic neurons are mostly localized in the ventral part of mesencephalon: in substantia nigra compacta (SNc), retrorubral field and ventral tegmental area (VTA). Axons originating from SNc form nigrostriatal tract that plays an important role in regulation of voluntary movements (Arrias-Carrión et al., 2010). Two other dopaminergic tracts – mesolimbic and mesocortical – are initiated from VTA neurons and are considered to be largely implicated in emotional behavior.

Baseline activity of dopaminergic VTA neurons (under anesthesia or in calm wakefulness) is composed of short bursts of action potentials and regularly spaced impulses (Schultz, 2007). Upon any reward-predicting event, primary reward or just physically salient stimulus dopamine-producing VTA neurons change their pattern of activity. Sign of modulation codes for prediction error (unpredicted reward leads to elevated firing rate, a fully predicted reward does not change baseline activity, and reward omission suppresses neurons at the time of the

predicted reward). Magnitude of response codes for the degree of surprise: partially predicted reward would elicit lower response compared to fully unexpected one.

VTA dopaminergic neurons projects to nucleus accumbens (nAc), a part of ventral striatum system, which receives dense glutamatergic projections from hippocampus, medial prefrontal cortex (mPFC) and amygdala (*Fig. 3-1*). VTA neurons also project to amygdala, hippocampus, and directly to mPFC, which are reciprocally interconnected between each other.

Whereas most of the structures shown on *Fig. 3-1* provide support in processing of reward-related information and decision-making, VTA and nAc respond to rewards-related events almost exclusively. For example, optogenetic activation of dopaminergic VTA neurons produce direct rewarding effect in the place preference paradigm (Tsai et al., 2009; Witten et al, 2011). Activation of neurons that bear D1/D5 dopamine receptors in nAc enhances cocaine reward, but activation of D2-type neurons suppresses rewarding effect of cocaine (Lobo et al., 2010).

Medial forebrain bundle

Direct rewarding (and self-stimulating) effect could be also produced by electrical stimulation of medial forebrain bundle (MFB), a large tract of fibers that passes through lateral hypothalamic continuum (Olds and Milner, 1954; Wise, 2005). Interestingly, stimulation of MFB provides not only the rewarding effect but it also induces seeking and consummatory responses (Margules and Olds, 1962). Exact anatomical outlines of MFB is hard to define largely because it comprises around 50 ascending and descending components mediated by at least 13 neurotransmitters (Nieuwenhuys et al., 1982). Certain areas are heavily connected through MFB (such as dopaminergic system of mesencephalon, hypothalamus and ventral striatum), others are connected lightly (such as dorsal striatum or hippocampus). Most of the fibers do not fully traverse the hypothalamic region from one structure to another but also give rise to many collaterals along the way. Among MFB components, there are massive ascending dopaminergic projections from VTA and SNc, descending axons originating in nAc and fibers connecting various hypothalamic nucleus with other brain structures. Thus, **MFB is a fiber tract of enormous complexity, and its precise composition and function are still an open question as of today.**

Originally, it was believed that rewarding effect of MFB stimulation was mediated directly by dopamine fibers. Indeed, there is a massive dopamine release in nAc upon stimulation of MFB, which mimics VTA-mediated phasic dopamine release upon presentation of reward-related information (Freels et al., 2020; Vajari et al., 2020). However, it turned out that only few dopaminergic fibers in MFB were directly activated in self-stimulation due to their high activation threshold (Yeomans et al., 1988). During experiments that involve rewarding MFB stimulation, two types of fibers were reliably recruited: cholinergic axons with ultrafast response and the second slow subpopulation with unidentified neurotransmitter (Gratton and Wise, 1985). There were many attempts to explain rewarding properties of MFB stimulation:

some suggested that stimulation directly or indirectly excites VTA neurons, others thought that the final target of MFB stimulation is mPFC; however, no definitive study has shown what type of fibers the rewarding effect depend on (Wise, 2005).

MFB stimulation was successfully used in the spatial learning paradigms: applied in a certain location, it can create place preference either during wakefulness (Kobayashi et al., 1997; Talwar et al., 2002; Kobayashi et al., 2003; Mamad et al., 2017), **or during sleep** (de Lavilléon et al., 2015).

Organization of defensive system

Defensive behaviors

Contrary to the relative straightforwardness of reward-related behaviors, **defensive behavioral strategies are complex and highly context- and species-specific.** In this thesis, we will focus on defensive strategies of rodents, as they are the animal model used in our study as well as the most used animal model in neuroscience nowadays (Manger et al., 2008).

To the large extent, behavior of rats and mice is defined by the fact that they are prey for larger species. In order to survive, they have rich repertoire of natural defensive behaviors that was studied in detail by Blanchard and Blanchard (2001). They have suggested to use visible burrow system, a behavioral paradigm that assesses different forms of animals' response to predatory threat. In visible burrow system, rodents live in the colony that contain both tunnels and open space. At the beginning of the experiment, animals are allowed to explore the environment freely, and this behavior recorded as a baseline. After this long period of habituation, animals are exposed to a natural predator, a cat. Interestingly, observed reaction to the predator was similar among most of studied rodents and temporally stereotyped. After cat entered the environment, all animals retreated to the tunnels where they spent long periods of time freezing. After the period of freezing, movements within the hiding places resumed and rodents started to probe the environment again. This period of risk assessing exploration is characterized by careful and slow motion and presence of stretch-attend postures. If predator did not return, pre-cat behavior gradually replaced threat-induced strategies.

If animal is placed at one side of the long hallway and then it is approached progressively by an experimenter (here imitating a predator), animals would freeze first in response to approaching human. After the experimenter will make contact or will move too close, rodents would jump or attack (Blanchard et al., 1986; Blanchard et al., 1993). However, if rodents are exposed to the area containing only cat's odor without presence of the actual predator, their defensive behaviors are very different (Blanchard et al., 1993). No attack or flight is observed, and the amount of freezing is minimal. Instead, animals would avoid the area where cat's odor was presented, demonstrate risk assessment behavior and suppress eating and drinking.

Thus, rodents never flight or attack when predator is not standing right in front of them as well as no risk assessment behaviors can be detected when the predator approaches. **It suggests that distance to the predator and/or its potential presence heavily influence the choice of defensive strategy in response to a particular threat event**. Moreover, this choice is also defined by properties of the environment. If the shelter is accessible, rodents would flee in its direction upon presentation of aversive stimulus; however, if it's not freezing is observed (Vale et al., 2017).

Defensive strategies projects to predatory imminence

The concept of 'predatory imminence' (or 'threat imminence') was proposed to move the attention from the characteristics of the environment to an internal psychological construct that defines the topography of defensive behaviors. **Predatory imminence is a psychological distance from the predator that is determined by physical, temporal and probabilistic closeness to contact with a threat** (Perusini and Fanselow, 2015). Predatory imminence incorporates distance to the predator, certainty of its presence, accessibility of shelter, etc.

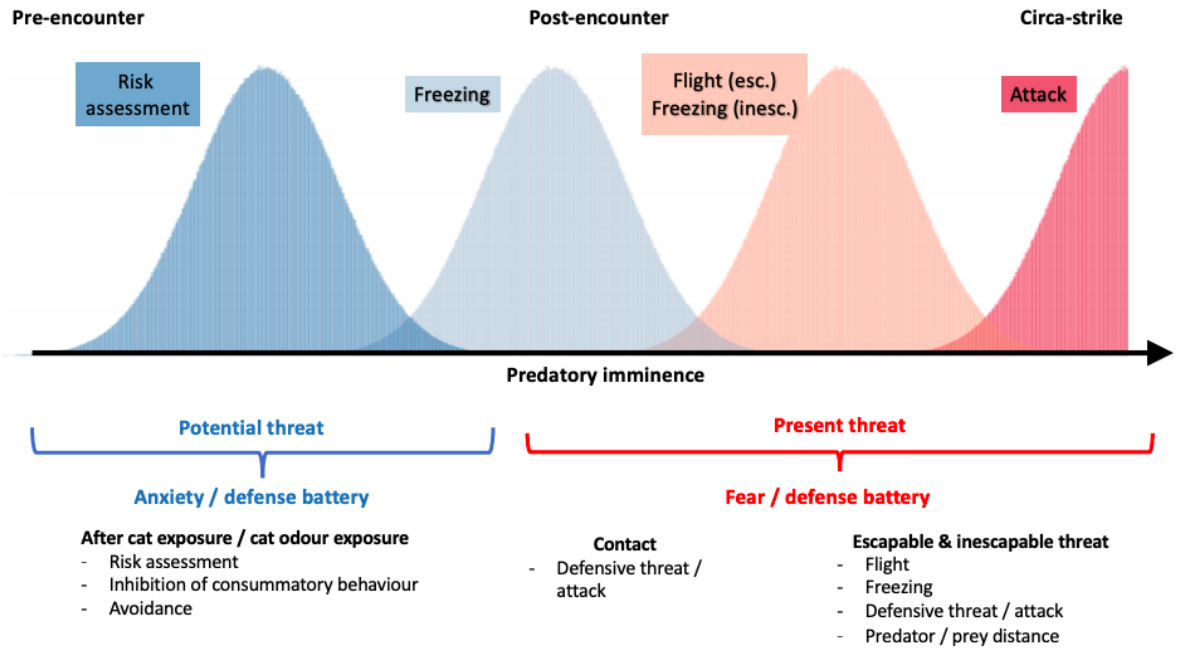

Figure 3-2. The repertoire of defensive behavior ranged along the predatory imminence axis. Adapted from Bagur, 2019. *Parameters that are studied in each particular situation is shown at the bottom.*

Three groups of defensive strategies can be distinguished according to the predatory imminence: pre-encounter, post-encounter and circa-strike (*Fig. 3-2*). Pre-encounter strategies are not defensive in the strict sense of the word. They restrict animal's behavior by minimizing probability to meet the predator: avoiding open areas, foraging at night etc. Post-encounter starts after enough evidence about predator's closeness has accumulated. If the predator is perceived to be close enough, flight behavior is elicited; when the predator is perceived

further away or if the threat is inescapable, freezing behavior is observed. Most of fear-related behavioral paradigms study post-encounter defensive strategies. Circa-strike responses are employed if the predator makes an attack or the avoidance strategies fail. In this case, rodents could fiercely attack the predator back.

Natural behaviors, most likely, combine drives for reward-related and defensive behaviors – for example, if food is located close to the predator, hungry animal faces a hard choice. This situation is studied in the set behavioral tasks using approach/avoidance conflict (McNaughton, 2010): when drinking or eating is punished by shock or in shock-probe burying test when aversive objects tend to be buried under the litter. Remarkably, anxiolytic drugs (medicine that reduce subjective feeling of anxiety in humans) reduce defensive behaviors in such tasks, which allows the authors to place the battery of defensive behaviors on the anxiety/fear axis.

Defensive system in the brain

Full repertoire of defensive strategies is not possible without two structures: periaqueductal gray matter (PAG) and amygdala. PAG is known as the common output structure for all kinds of defensive behaviors – it will be discussed in detail later in the chapter due to its particular importance for the present study. Amygdala serves as an interface that relay and select defensive behaviors to respond to incoming threats.

In amygdala, there are two subregions whose involvement in fear-related behaviors was intensely studied. **Basolateral amygdala** (BLA) is crucial for fear learning. It **gathers sensory information receiving projections from both thalamic relay nuclei and cortical sensory cortices as well as information about spatial context directly from ventral hippocampus**. Blockade of LTP in BLA or BLA legions prevent learning in feat conditioning paradigm (Fanselow and LeDoux, 1999). Importantly, neurons in BLA respond both to conditioned stimulus (Johansen et al., 2010) and unconditioned stimulus (Quirk et al., 1997; Goosens et al., 2003).

Basolateral amygdala projects to **central amygdala** (CeA) both directly and relaying at the neurons of intercalated mass. CeA is **considered to be the main output organizers of threat-induced behaviors – it projects to important brain areas that are recruited during fear-related behaviors and stress such as hypothalamic nuclei and ventral PAG**. Thus, the roles of CeA include active coordination of defensive behaviors as well as a site that supports extinction of fear-related memories (LeDoux, 2000; Fadok et al., 2017). CeA projects down to ventral PAG and bed nucleus of stria terminalis that drive execution of fear behaviors (Perusini and Fanselow, 2015).

Ventral hippocampus is reciprocally connected both to BLA and medial prefrontal cortex (mPFC**). It provides information about context, most probably, via dorsal hippocampus but it also directly participates in fear-related behaviors** (*see box 4-1 in chapter 4*). Most of the results concerning these aspects of ventral hippocampus functioning were obtained in the paradigms designed to assess levels of stress and anxiety. For example, inactivation of ventral

hippocampus produced deficits in elevated plus maze and in social interaction tests (McHugh et al., 2004). Moreover, neurons in ventral hippocampus that fire exclusively in anxiogenic situation of open elevated plus maze arms were identified (Ciocchi et al., 2015). Connections of ventral hippocampus to the mPFC and amygdala seem to be particularly important: manipulations with these projects can mediate anxiety-related behaviors (Felix-Ortiz et al., 2013; Padilla-Coreano et al., 2016).

Hippocampal formation has been suggested to play a role in resolving approach/avoidance conflicts (McNaughton and Corr, 2004). After the detection of the mismatch between the current goal and aversive environment, hippocampus is thought to shift the focus towards one kind of behavior. This view is based on the fact that anxiolytic drugs, injected both systemically and locally in hippocampus, reduce fear-related behaviors and change hippocampal activity. Indeed, offline replays during wakefulness as well as place cell activity during vicarious trial and error could be explained in the light of simulating and reorganizing the space of future decisions (Johnson and Redish, 2007; Pfeiffer and Foster, 2013).

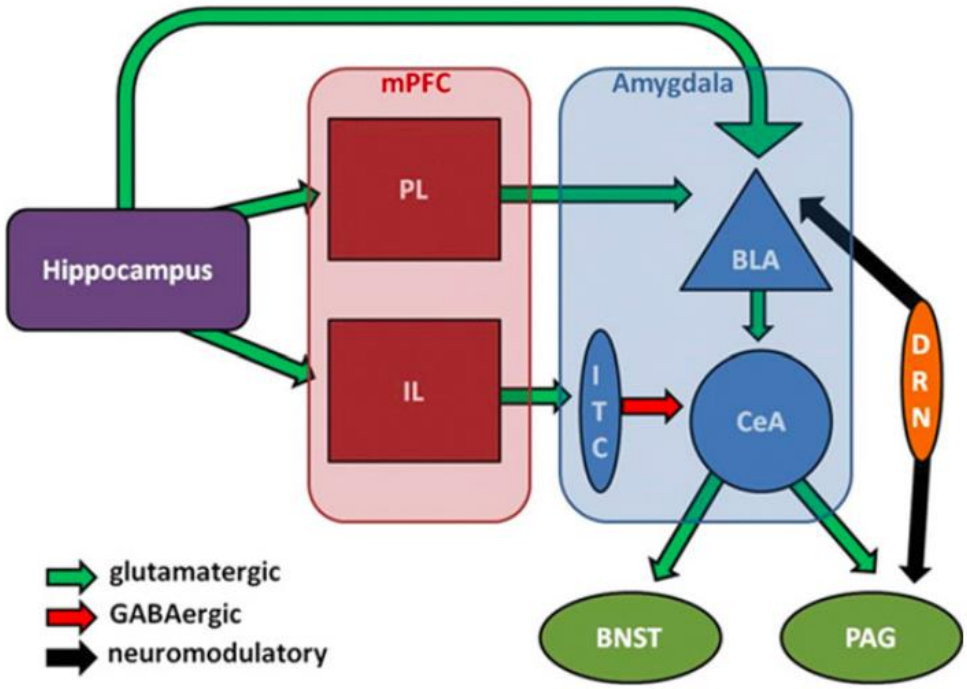

Figure 3-3. Simplified schematics of main brain structures that are recruited during fear-related behaviors. Adapted from Perusini and Fanselow, 2015. *The basolateral amygdala (BLA) receives sensory information from both thalamic and cortical regions as well as information about spatial context from the hippocampus. The BLA projects to the central nucleus (CeA) both directly and indirectly, via the GABAergic intercalated cell (ITC) masses that lie between these two regions. The CeA output to the periaqueductal gray (PAG) and bed nuclei of the stria terminalis (BNST) drive fear responding. Ascending projections from the brainstem and midbrain to the amygdala, such as from the dorsal raphe nucleus (DRN) projects to the dorsal PAG and to the amygdala in a manner that modulates defensive behaviors. Descending*

projections from the medial prefrontal cortex also differentially modulate the behavioral outputs of this circuit—the prelimbic (PL) cortex projects to the BLA modulating freezing response while the infralimbic cortex (IL) indirectly projects to the CeM via ITC to mediate extinction.

mPFC can be seen as a modulator of defensive behaviors that exerts top-down control on other structures described above. There are two areas in mPFC of rodents that play non-overlapping roles in fear-related behaviors: prelimbic and infralimbic PFC. Prelimbic PFC clearly supports freezing behavior: its inactivation during acquisition or extinction of fear conditioning associations decreased the amount of freezing (Corcoran and Quick, 2007; Sierra-Mercado et al., 2011), disinhibition of prelimbic PFC induced freezing in naïve animals (Courtin et al., 2014), and bidirectional optogenetic manipulation of prelimbic projections to ventral PAG has been shown to modulate amount of freezing (Rozeske et al., 2018). However, it's important to understand that prelimbic PFC is not necessary for freezing. Thus, freezing can be observed in animals with lesion in prelimbic PFC (Corcoran and Quick, 2007; Bravo-Riviera et al., 2014).

Infralimbic portion of PFC is crucial for active suppression of fear behaviors during extinction of fear association. Indeed, lesions in infralimbic PFC impairs extinction (Milad and Quirk, 2002; Bravo-Riviera et al., 2014). Infralimbic PFC projects to CeA via interneurons intercalated mass; this connection is thought to mediate behavior during extinction by blocking fear-related motor response that originates from CeA (Courtin et al., 2014; Perusini and Fanselow, 2015).

Periaqueductal gray matter

Periaqueductal gray matter (PAG) is the central output hub controlling defensive behaviors. PAG has columnar organization: according to different authors, there are 3-5 columns of gray matter in PAG. In this text, we will use the smallest possible distinction – PAG comprises of three distinct columns: dorsomedial, dorsolateral and ventral (*Fig. 3-4*), functional role of them will be discussed below. In addition, to columnar divisions PAG can be functionally split into a rostral third and caudal two-thirds: activation of rostral PAG induces aggressive attacking behavior, either defensive or predatory (Mota-Ortiz et al., 2009; Mota-Ortiz et al., 2012), whereas activation of caudal PAG evokes other defensive strategies.

Upon direct stimulation of dorsolateral PAG (dlPAG), animals demonstrate active flight or escape behavior, resembling escape from the predator or an activity burst observed as a reaction to the US in Pavlovian fear conditioning protocol (Carrive et al., 1989; Zhang et al., 1990; Schenberg et al., 1990; Kim et al., 2013; Deng et al., 2016). Such active defensive reactions are accompanied by vegetative reactions typical for sympathetic nervous system: elevated blood pressure, tachycardia, vasodilation in limbs, etc. In contrast, **direct stimulation of ventral PAG (vPAG) results in motor suppression or freezing together with hypotension and bradycardia** (Zhang et al., 1990; Carrive, 1993; Kim et al., 2013).

Reflecting their different functional roles, dlPAG and vPAG has different connectivity patterns. vPAG projects densely to the presympathetic neurons in rostral ventrolateral medulla (rVLM) and rostral ventromedial medulla (rVMM), which in turn projects to preganglion cells in the spinal cord, controlling vegetative state of the organism and pain perception, whereas dlPAG does not have direct projections there (most likely, it exerts influence on vegetative nervous system through hypothalamic nuclei). vPAG has a direct connection to lateral

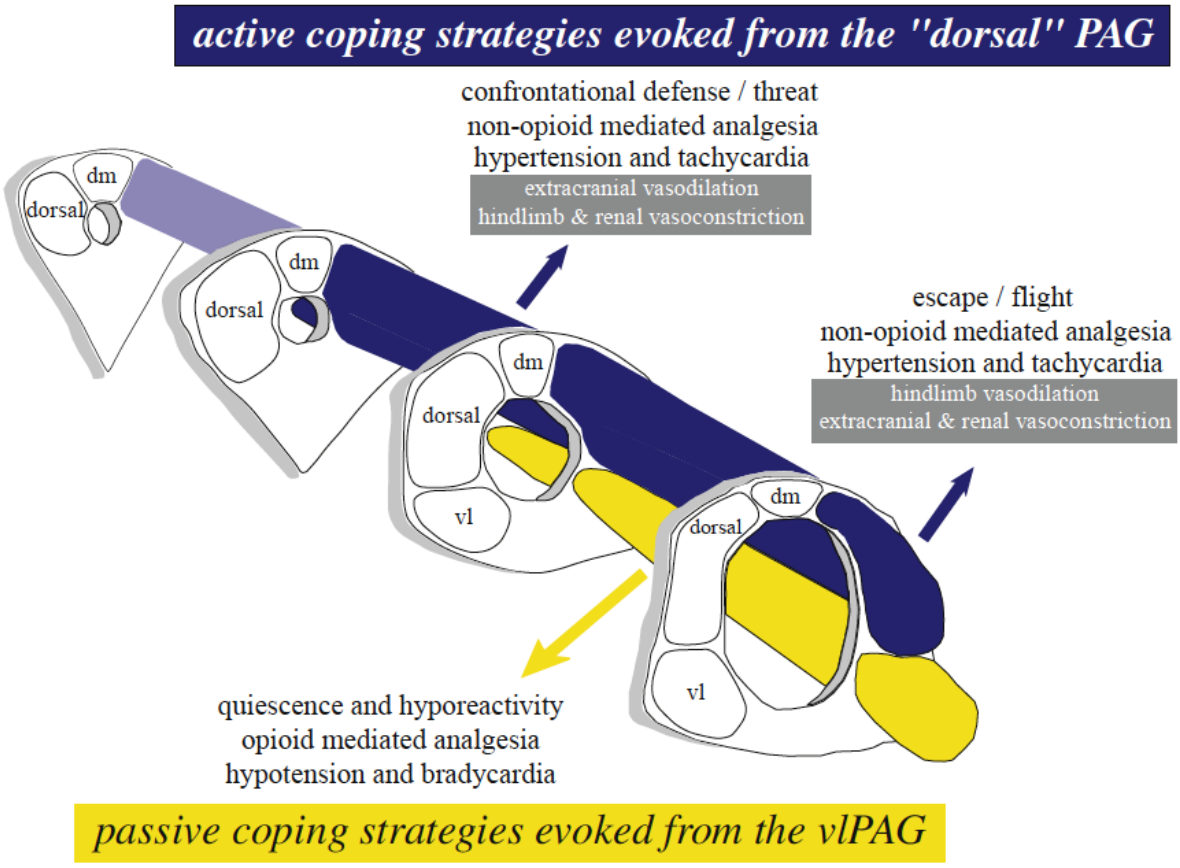

Figure 3-4. Organization and functional significance of PAG divisions. Adapted from Keay and Bandler, 2015. *Activation of the dorsolateral (blue) vs. ventral (vPAG) (yellow) columns evoke different defensive strategies. Activation of rostral portions of dlPAG evokes a confrontational defense/threat reaction, tachycardia, and hypertension (associated with decreased blood flow to limbs and viscera and increased blood flow to extracranial vascular beds). Activation of the caudal portions of the dlPAG induces escape/flight, tachycardia and hypertension (associated with decreased blood flow to visceral and extracranial vascular beds and increased blood flow to limbs). In contrast, activation of the vlPAG evoke cessation of all spontaneous activity (quiescence), a decreased responsiveness to the environment (hyporeactivity), hypotension and bradycardia. Non-opioid-mediated and opioid-mediated analgesia are evoked respectively from the dorsolateral and the vPAG.*

hypothalamic nuclei that control parasympathetic vegetative reactions (Allen and Cechetto, 1992), while dlPAG projects to dorsal and medial hypothalamic nuclei that are also implicated in flight and avoidance behavior (Kunwar et al., 2015; Wang et al., 2015; Wang et al., 2021). vPAG is strongly connected to CeA, whereas dlPAG is much closer connected to superior colliculi.

These two disctinctions of PAG are also very different in their ability to support learning. **Only dlPAG could be used as the US in the fear conditioning protocols and avoidance protocols** (Kim et al., 2013; Deng et al., 2016), which is the reason we use it in the present study.

Counter-conditioning

Persistent associations sometimes become maladaptive, especially if they continue to influence behavior after conditions that supported such association have gone. This topic is mostly discussed in human psychiatric literature in relation to post-traumatic stress disorder (PTSD), anxiety disorder, addictions, etc. However, years of research on learning and memory performed on animal models could offer important insights in possible solutions and treatments of above-mentioned conditions. One of the ideas, that initially was proposed by neuroscientists (Wolpe and Plaud, 1997), is counterconditioning, which was mostly investigated by human researchers over the last years.

During counterconditioning experiments, instead of 'unlearning' the association using extinction procedure it is suggested to use the US of opposite valence in hope that prominent motivational salience of the new US will speed up and will be more robust than using other methods (Keller et al., 2020). In typical counterconditioning experiment, after acquisition of association of certain valence, the CS is paired with the new US that is of the opposite valence. Indeed, counterconditioning has been shown to accelerate the reduction of the original conditioned response (Dickinson and Pearse, 1977; Rishardson et al., 1987) compared to extinction protocol, in which the CS becomes unpaired (or one can say 'becomes paired with nothing'). Yet, similar to extinguished behaviors, countercondtioned behaviors tend to return easily (Bouton, 2004).

The main motivation for the development of counterconditioning protocols is that they will outperforms extinction protocols in effectiveness and robustness. Extinction is thought to rely on the active learning to inhibit previously conditioned response after the CS lost its predictive nature (Dunsmoor et al., 2015). It has been demonstrated that extinction is mediated by dopaminergic system of the brain: optogenetic manipulation with dopamine-projecting VTA neurons can modulate the speed of extinction (Salinas-Hernández et al., 2018), and blocking dopamine activity in nAc impairs extinction (Holtzman-Assif et al., 2010). **Would naturally rewarding stimuli be more effective (i.e. provide larger dopaminergic response) in learning new association than during extinction?**

Substitution of the aversive US with food or intracranial rewarding stimulation decreased defensive behaviors more effectively compared to extinction (Richardson et al., 1982; Richardson et al., 1987; Reid, 1973). Counterconditioning was also more successful if the second US was just a novel object rather than innately rewarding stimulus (Anderson et al., 2013). Interestingly, if in aversive-to-appetitive counterconditioning an animal has to produce an action to receive the second rewarding US, less renewal of first association response was observed compared to the situation in which the rewarding US was presented freely (Thomas et al., 2012). It has been also shown that classical counterconditioning could be effective in reducing the first conditioned response for at least two months (Correia et al., 2016).

In contrast, a series of experiments have demonstrated that the association that was established after counterconditioning procedure was very unstable. Thus, if the CS was presented in the same context (Peck and Bouton, 1990), or if the first US is presented again (Brooks et al., 1995), or if enough time has passed (Bouton and Peck, 1992), the conditioned response to the first US comes back.

The critical factor of stability of newly learnt association appears to be the context where the learning takes place. Indeed, testing outside of the context where counterconditioning took place could result in lower effectiveness of the procedure even compared to the extinction (Holmes et al., 2016). To account for context dependence and to strengthen the associations with the second US, several approaches were suggested (Keller et al., 2020).

If counterconditioning is performed in several different contexts, it could prevent relapse of the counterconditioned response. This approach was successfully tested in extinction protocols (Gunther et al., 1998; Shiban et al., 2013) but not applied to counterconditioning studies. Another way to deal with unwanted renewals is to mix the second US into the learning procedure, progressively substituting the first US. This idea is based on the insight according to which if the first and the second USs are presented in very different context, the learning agent orthogonalize them and fails to generalize.

Presenting the second US during sensitive period of memory reinstatement could give a great boost to the effectiveness and robustness of counterconditioning procedure. During memory reinstatement, or reconsolidation, memories are thought to be brought back to their labile state. Reconsolidation is already used to ease the symptoms of PTSD or phobias (Guistino et al., 2016; Monfils et al., 2009). Several promising results were already obtained in studies that harness such mixed (reconsolidation and counterconditioning) approach, both in aversive-to-appetitive and appetitive-to-aversive settings (Haubrich et al., 2015; Pedraza et al., 2018; Goltseker et al., 2017).

Recently, appetitive association with a previously neutral location was created during sleep by pairing hippocampal place cell reactivations with intracranial rewarding stimulations suggesting that sleep reinstatement of neural patterns that were active during wakefulness can be used for learning (de Lavilléon et al., 2015**). Could one use the same technique but**

applied on an aversive association to perform counterconditioning during sleep? At this moment of technological development, it is hard to do but such method potentially has considerable advantages compared to classical extinction and counterconditioning studies. First, most likely it would also utilize reconsolidation mechanisms as it is thought that hippocampal reactivations could be the moments of labile memory reinstatement. Second, it provides completely different definition of learning context: it could be that learning during sleep is similar to learning in many different contexts as psychological distance between locations and environments may be warped. Third, sleep learning does not involve strong emotional reactions as during learning in wakefulness, which could be very unpleasant (in case of strong phobias) or too strong (in case of drug addiction) to allow for counterconditioning.

In the present manuscript, we will outline the main technological developments necessary to test the validity of sleep counterconditioning approach.

Résumé

Reward and aversive stimuli are similar in their ability to reinforce associations. However, they are mostly opposite in the behavioral patterns driven by them, and their neural substrates do not have a large overlap despite being tightly connected to each other.

Rewards induce approach and seeking behaviors which are supported by dopaminergic system in the brain. Key player is the ventral tegmental area that contains dopaminergic neurons, which respond to reward prediction error. Ventral tegmental area sends its projections to nucleus accumbens, ventral striatum area, that is important for structure for reward-related plasticity. Dopaminergic fibers are indirectly recruited during stimulation of medial forebrain bundle, which could serve as intracranial rewarding site.

Defensive behaviors are more diverse than reward-related ones. According to predatory imminence (psychological distance to the predator), defensive strategies could be ranged from pre-encounter (open space avoidance) through post-encounter (flight and freezing) to circa-strike (aggressive attack).

Periaqueductal gray matter serves as an output controller of fear behaviors. It consists of three columns of neurons. Activation of one of them, dorsolateral, could be used as a negative reinforcer to drive avoidance or conditioned freezing behaviors. Such stimulation evokes flight behavior that is followed by freezing.

Other regions implicated in aversive behaviors are amygdala and ventral hippocampus. Amygdala is crucial for fear learning and extinction. Ventral hippocampus provides context information for aversive learning as well as it is involved in processing of anxiogenic situations.

Counterconditioning could be used to change reward-related behavior to aversive and vice versa. In certain studies, it demonstrated more efficiency in ‘unlearning’ the first association than extinction. Counterconditioning could be used to reverse maladaptive associations.

Context management could crucial for temporal robustness of the second association during counterconditioning.

In this thesis, we propose to employ counterconditioning techniques during sleep to reverse aversive association to appetite one and discuss validity of such strategy in light of our results.

Questions:

- How reward and defensive systems of the brain interact with hippocampus during affective spatial learning? – chapter 5

- Can we counterconditioning during sleep be effective? – general conclusion

Chapter 4. Affective spatial learning: place coding after reward or aversive stimuli

Introduction

Historically, hippocampal reactivations were studied in the behavioral paradigm of 'free foraging' that uses food scattered randomly in the environment as an incentive to explore. With time, researchers started to use behavioral paradigms that imply learning and that include reward (food, water or intracranial stimulation) or punishment in a certain place. Naturally, a set of questions was raised in the community. **During affective learning, do place cell represent purely spatial information?** If they do, reactivation of this information during NREM sleep (obviously, in absence of any physical reward) should lead to extinction of the learned association. However, there is widely accepted consensus in the community supported by the large body of literature that NREM sleep (and hippocampal reactivations observed in NREM sleep) are beneficial for memory consolidation. Such discrepancy demands for better understanding of how motivationally and emotionally relevant information integrated in hippocampal place coding.

Are hippocampus and the system of place cells involved in the processing of emotional stimuli? Do rewarded or punished locations alter location-specific coding, and are they reactivated differently from other location representation in the environment? Are there neurons in hippocampus that selectively respond to appetitive or aversive stimuli? Or, in contrast, do subcortical structures (such as ventral striatum or amygdala) play an important role in spatial learning?

Cognitive map during reward-based spatial learning

Place cells research moved with years from free foraging behavioral paradigm to paradigms that employ goal-directed behavior, usually including reward to motivate the performance. Are rewarded zones have different representation than other location of the environment?

Several studies report that place fields tend to accumulate around rewarded locations. Overrepresentation of the goal location was observed in the version of Morris water maze (Hollup et al., 2001), in the continuous T-maze (Mamad et al., 2017), in the cheeseboard maze (Dupret et al., 2010), in the 8-arm radial maze (Xu et al., 20129), or in simple goal-oriented behavior in the virtual reality (Danielson et al., 2016; Kaufman et al., 2020). However, in the protocol that was designed to dissociate goal and reward locations, authors failed to reproduce the effect (Hok et al., 2007). In their task, animals had to stay in the goal location for two seconds to receive food pellet later in random place of the environment. In such conditions, goal zone was not represented by higher number of place fields compared to other locations; however, most of recorded cells slightly elevated their firing rate when an animal was at the goal location (Poucet and Hok, 2017). Early study where 4-arm plus maze, which varied considerably reward location and thus encouraged different trajectories, also did not found goal overrepresentation (O'Keefe and Speakman, 1987). Series of papers that used intracranial rewarding stimulation to induce place learning communicated that a proportion of

place fields was shifting towards the rewarded zone with increasingly stereotypical goal-directed behavior towards this zone (Kobayashi et al., 1997; Kobayashi et al., 2003).

Moreover, rewarded locations are overrepresented in hippocampal reactivations during the learning and in the NREM sleep that follow the learning (Singer and Frank, 2009; Dupret et al., 2010; Michon et al., 2019). It is possible that overrepresentation of the goal locations found in some studies could be explained by the properties of behavioral tasks that prompt stereotypic trajectories towards constant goal locations. Animals spend more time using reward-related trajectories and within the goal zone consuming the reward, which can naturally bias cognitive map and favor long-term potentiation for synapses of goal-related neurons. Indeed, more visited places during wakefulness have been shown to be reactivated more often during sleep (O'Neill et al., 2008).

Dopaminergic structures are strongly involved in reward-based spatial learning

Most likely, increased occupancy of goal trajectories and goal locations is not the only cause of changes in the cognitive map. In the task dissociating goal zone and reward locations even silent neurons exhibited slight increase in firing rate within the goal zone (Hok et al., 2007; Poucet and Hok, 2017). Presence of reward also increased firing rate of place cells in 8-arm radial maze (Hölscher et al., 2003). Hypothetically, **structures outside of hippocampus could also modulate firing in hippocampus and influence internal representation of space**. Dopaminergic activity is the apparent candidate to affect place cell activity at goal locations given its tight relationship with reinforcement, reward and goal-directed behavior (Schultz et al., 1997; Schultz, 2007). Indeed, it has been shown that antagonists of D1/5 receptors can prevent shifting of place fields towards reward location (Retailleau and Morris, 2018).

There is a high density of dopamine receptors in hippocampus (Devoto and Flore, 2006), but dopaminergic VTA projections to dorsal hippocampus (but not ventral hippocampus) are very sparse and constitute only 10% of all VTA projections there (Gasbarri et al., 1997; McNamara et al., 2014). Massive projections of dopaminergic neurons come from locus coeruleus (LC), a tiny, mostly norepinephrinergic nucleus in the brainstem (Takeuchi et al., 2016). Interestingly, optogenetic activation of dopaminergic projections from LC to dorsal CA1 has been shown to promote overrepresentation of goal location during reward-based learning (Kaufman et al., 2020). Activation of LC axons in hippocampus also enhanced spatial learning (Kempadoo et al., 2016; Takeuchi et al., 2016), but without providing direct rewarding effect as upon stimulation of VTA neurons (Tsai et al., 2009). Authors speculated that dopaminergic axons of LC support mechanisms of selective attention that biases exploration towards novel or rewarded zones.

It has been also demonstrated that optogenetic activation of ventral tegmental area (VTA) dopaminergic neurons or their synapses in the CA1 of dorsal hippocampus during spatial learning enhanced reinstatement of new spatial maps reorganized by the presence of reward

(McNamara et al., 2014). Indeed, neurons in VTA are significantly modulated by SWRs during quite wakefulness (Gompers et al., 2015). Moreover, units with place fields at rewarded locations were replayed in the coordinated fashion with VTA neurons during wakefulness but this effect disappeared during following sleep reactivations. Sleep reactivation of VTA neurons were found in different study, however their relationships with hippocampal activity was not addressed (Valdes et al., 2015).

In contrast to weak (at best) direct influence of VTA sleep reactivations on hippocampal circuits, activity in ventral striatum, direct downstream target of VTA (Russo and Nestler, 2013), seems to be tightly coupled to hippocampal replays. Neurons in dorsal hippocampus project directly to nucleus accumbens (Trouche et al., 2019), and synaptic strength between neurons that represent rewarded locations and nucleus accumbens (nAcc) increases (Sjulson et al., 2018; LeGates et al., 2018). Reactivations of neurons in nAcc were detected both in calm wakefulness and NREM sleep after reward-based task (Lansnik et al., 2008). Moreover, coordinated reactivations between dorsal hippocampus and nAcc were observed during NREM sleep (Pennartz et al., 2004; Lansnik et al., 2009; Trouche et al., 2019). Interestingly, in such reactivations hippocampal neurons fired earlier than neurons in nAcc, and stronger interregional reactivations were detected when place cells with place fields in rewarded locations were replayed (Lansnik et al., 2009).

Results reviewed above strongly suggest **that structures related to dopamine function exert strong influence on hippocampal circuits during reward-based spatial learning**. While dopaminergic release from VTA and LC seems to be an important mechanism that support spatial learning and selective attention towards reward, activity in ventral striatum is considered to code for the motivational and emotional aspects of spatial learning in already potentiated network. That is the possible reason why most studies of VTA report effects during wakefulness where raw reward signal modulates spatial representation in hippocampus and potentiates reward-related circuits in nAcc, binding these two aspects together in coherent representation. Whereas neurons in nAcc are observed to participate in replays and reactivations, probably representing reward in already formed spatial representation of rewarded location.

Reward cells in hippocampus

In addition to a corpus of research suggesting that emotional contents of spatial representation are stored outside of hippocampus, one study has found neurons that respond selectively to reward within dorsal hippocampus (Gauthier and Tank, 2018). Authors recorded massive population of neurons using Ca^{2+}-imaging in the virtual reality task and observed only very tiny proportion (1-5%) of neurons that consistently respond to reward. However, at the moment it is unclear to which extent hippocampal reward cells can affect circuits during spatial learning.

Aversive learning in spatial navigation paradigms

In this thesis, we both contrapose and underline similarities between aversive and reward-based spatial learning (Bromberg-Martin et al., 2010). Reward-based learning induces active seeking of reward as well as stimuli and contexts related to it; in contrast, aversive learning leads to avoidance behavior. In that sense, these two types of learning have opposite motivational value. However, both aversive and reward-based learning trigger elevated motivational level, attentional reactions of similar magnitude, and they both demand behavioral adaptation with similar strength. In this other sense, aversive and reward-based learning are analogous in their motivational salience. This distinction gives rise to interesting question: how is place cell coding different between reward-based and aversive spatial learning given that they are different in motivational value (i.e. in behavioral consequences) but similar in their motivational salience (i.e relevance for future behavior)?

Hippocampus during Pavlovian fear conditioning

Pavlovian fear conditioning, one of the most used memory-assessing behavioral paradigms in neuroscience (Maren, 2008), utilizes rodents' defensive behaviors elicited by unconditioned aversive stimulus (usually, mild foot shock). Fear conditioning comes in several types (*Fig. 4-1*): cued fear conditioning uses a particular sensory stimulus (blinking light or a sound) as a conditioned stimulus (CS), whereas during context fear conditioning animals are just placed in a certain box where foot shocks are delivered. After conditioning, one tests amount of freezing, natural defensive reaction of rodents to inescapable threats: to the CS in context different from the training one in case of cued fear conditioning, or to the same context where conditioning took place in case of context fear conditioning. Reliable freezing in context fear conditioning suggests that animals can abstract information into coherent context representation rather than integrate all stimuli composing the environment separately.

Dorsal hippocampus (a conventional site for place cell research) is involved differently in two types of Pavlovian fear conditioning. While lesions in dorsal hippocampus do not affect freezing amount in cued fear conditioning compared to unlesioned animals, in context fear conditioning paradigm subjects with hippocampal lesions showed drastic reduction of freezing levels compared to control animals (Kim and Fanselow, 1992). These results suggest that dorsal hippocampus is necessary for building representation of spatial context in context fear conditioning paradigm but it is not crucially important for processing information about

the CS. Interestingly, ventral part of hippocampus exhibits different set of functions in fear conditioning and other behavioral paradigms that involve emotional processing (*see Box 4-1*).

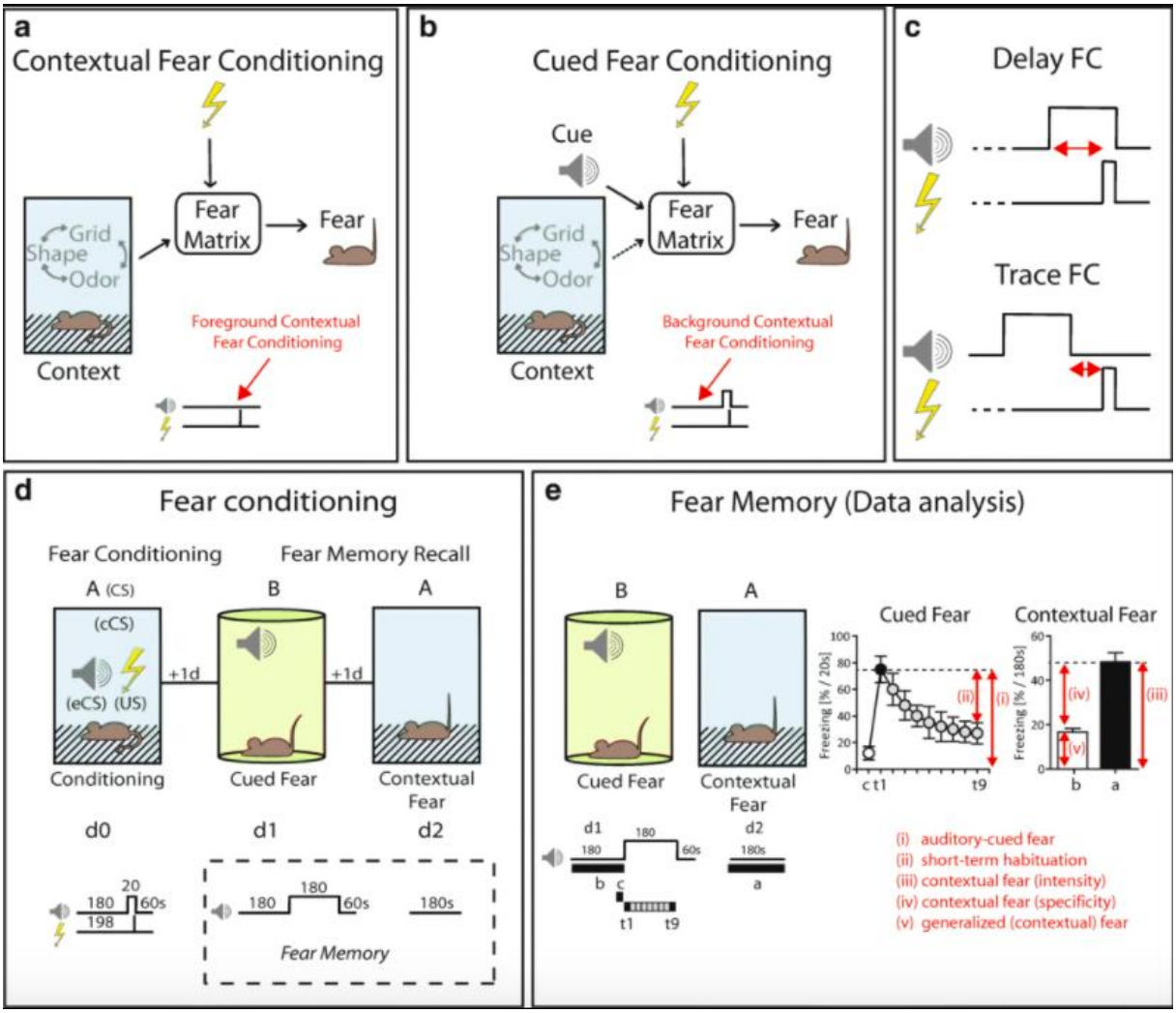

Figure 4-1. Types of fear conditioning. Adapted from Wotjak, 2019. *Fear conditioning (FC) can be based on protocols without (**a**) and with explicit pairing (**b**) of a discrete sensory stimulus with a foot shock, whereby stimulus (CS) and electric shock (US) may overlap or be separated by a temporal gap (**c**). In a prototypic fear conditioning (FC) experiment (**d**), mice receive a single tone–shock pairing in the conditioning context (A), followed by re-exposure to the tone in a different test context (B) followed by re-exposure to the original conditioning context (A), each separated by 24 h. During analysis of conditioned fear (**e**) one measures freezing to CS in case of cued FC or aversive context in case of context fear conditioning.*

The majority of cued fear conditioning use its delay version, when conditioned stimulus starts first and unconditioned stimulus (US) coincides with the last seconds of CS; minimal involvement of dorsal hippocampus was observed in such configuration. In contrast, trace fear conditioning seems to be largely supported by dorsal hippocampus networks. During trace fear conditioning, there is a stimulus-free interval between the end of CS and the beginning

Box 4-1 - ventral hippocampus

Idea that hippocampus is not unitary structure across its longitudinal axis was explicitly articulated in several influential reviews (Moser and Moser, 1998; Fanselow and Dong, 2010 – *also see Fig. 4-2*). Indeed, there are remarkable differences in connectivity and functional significance of dorsal and ventral hippocampi.

Ventral hippocampus is less involved in spatial navigation than its dorsal counterpart: lesions in ventral hippocampus had no effect for solving Morris water maze (Moser et al., 1995), or radial arm maze (Pothuizen et al., 2004), whereas lesions of dorsal hippocampus significantly impair performance in these tasks. Place coding in ventral hippocampus shows much less specificity than in dorsal one (Jung et al., 1994).

It is believed that ventral hippocampus is implicated in processing of stress and emotional behavior. Indeed, lesions of ventral hippocampus resulted in increased exploration of unprotected arms of elevated plus maze (Kjelstrup et al., 2002), as well as in reduced innate defensive reactions to predator odor in rats (Pentkowski et al., 2006). Moreover, neurons in ventral hippocampus that fire exclusively in anxiogenic situation of open elevated plus maze arms were identified (Ciocchi et al., 2015). Ventral hippocampus lesions have been shown to increase the number of stress-induced gastric ulcers (Henke, 1990). Selective impairments of ventral hippocampus in cued and context fear conditioning brought more mixed results. Inactivation of ventral hippocampus with muscimol before training disrupts cued fear conditioning but not the context fear conditioning (Maren and Holt, 2004; Sierra-Mercado et al., 2011). However, only lesions in ventral CA3 but not in ventral CA1 caused deficits in acquisition cued fear conditioning suggesting further fine-grained dissociation of function across hippocampal subregions (Hunsaker and Kesner, 2008). Importantly, both ventral CA3 and CA1 were necessary for retrieval of aversive memory.

It is tempting to conclude that dorsal and ventral hippocampus split their functions as spatial coding and emotional contents of space, respectively. However, situation seems to be more complex. Ventral hippocampus definitely plays bigger role in processing of motivation than dorsal hippocampus; however, degree of its involvement as well as its precise functions during spatial learning remain vague. Interestingly, no modulation of task-related ventral striatum neurons by ventral hippocampus SWRs was found (Sosa et al., 2019). Authors identified subpopulations of nucleus accumbens neurons that were either modulated by reward and reward-related information or not modulated. Opposite to what was expected, only dorsal hippocampus SWRs had effect on firing of task-related subpopulation.

Dorsal and ventral hippocampi also have different connectivity patterns (Fanselow and Dong, 2010). Dorsal hippocampus mostly projects to subiculum, restrosplenial and anterior cingulate cortices, structures involved in memory and spatial exploration in rodents. There are

also massive links with anterior thalamic complex, lateral mamillary nuclei, and indirect connections to ventral and dorsal striatum. All these circuits are thought to play crucial roles in locomotion, exploration and food foraging.

Ventral hippocampus, in turn, shares abundant bidirectional connections with amygdala, olfactory bulb and piriform cortex. Neurons of ventral hippocampus projecting to amygdala also project to infralimbic and prelimbic prefrontal cortices. Ventral hippocampus also sends descending axons to hypothalamic nuclei. This circuitry is implicated into processing of motivation and emotions, neuroendocrine and autonomic body functions.

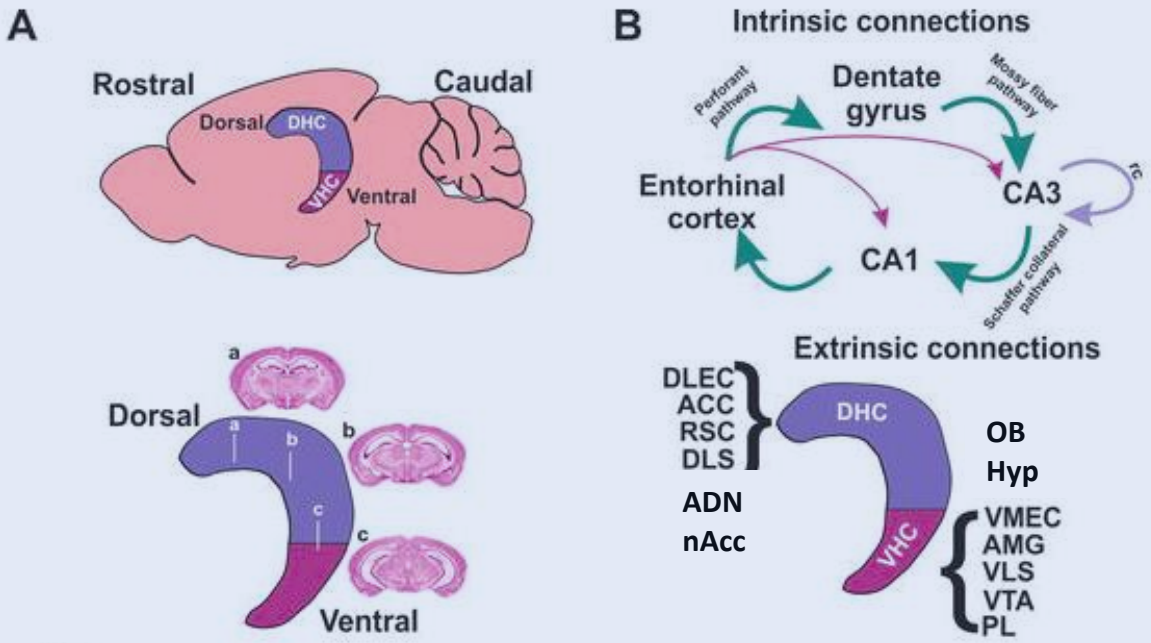

Figure 4-2. Organization of hippocampus along the longitudinal axis. Adapted from Harland et al., 2018 with modifications. ***A.*** *Location of hippocampus in the brain with distinction along the longitudinal axis. Microphotographs of cresyl violet-stained coronal sections are shown at corresponding locations of the scheme.* ***B.*** *Simplified representation of trisynaptic loop and projections of dorsal and ventral hippocampus. DLEC: dorsolateral entorhinal cortex; ACC: anterior cingulate cortex; RSC: retrosplenial cortex; DLS: dorsal lateral septum; ADN: anterior dorsal nucleus of thalamus; nACC: nucleus accumbens; OB: olfactory bulb; Hyp: hypothalamic nuclei; VMEC: ventromedial entorhinal cortex; AMG: amygdala; VTA: ventral tegmental area; PL: prelimbic portion of prefrontal cortex.*

of US. In modern literature, the principal difference between delay and trace fear conditioning protocol is the necessity of keeping the 'memory trace' to successfully learn the association in trace conditioning, while during delay conditioning temporal association is already present in the physical world (Raybuck and Lattal, 2014).

Interestingly, there is a number of studies that show that dorsal hippocampus is indispensable for both acquisition and retrieval of trace fear conditioning (Chowdhury et al., 2005; Raybuck and Lattal, 2011; Kitamura et al., 2014; Wilmot et al., 2019). It is generally thought in the community that hippocampus plays a large role in coding of time during that paradigm; however, direct evidence of this is lacking.

Spatial aspects of aversive learning

As we discussed above, dorsal hippocampus is necessary for acquisition and expression of context fear conditioning (Kim and Fanselow, 1992). It has been demonstrated that cognitive map undergoes partial remapping after context fear conditioning is performed on the animal; this effect was much less pronounced for cued fear conditioning protocol (Moita et al., 2004). Much stronger remapping was observed in the behavioral paradigm that exploited innate defensive behaviors triggered by predator odor (Wang et al., 2012). Despite moderate changes in behavior, large scale global remapping was observed, in which more than 65% of recorded cells shifted their place field either during exposure to the aversive odor or in the next hour. Similarly, predator odor applied on a portion of circular linear track induced massive remapping of place cells with place fields outside of the odor zone (Mamad et al., 2019). Interestingly, cells that shifted their place fields demonstrated elevated extra-field firing during conditioning sessions when animals were in the odor part of the maze. Different approach, which used a large robot looming towards animals and imitating predator, also induced remapping but mostly of place cells with place fields close to robot's location (Kim et al., 2015). In a longitudinal study, that recorded neuronal activity by means of Ca2+-imaging, authors assessed stability of cognitive maps in the inhibitory avoidance task (Schuette et al., 2020). They have found that several days of prominent defensive behavior in the setup coincides with global instability of place fields across days. Interestingly, new stable maps progressively emerged with gradual extinction of aversive associations. Thus, **strong remapping usually observed in the studies with escapable threats that induced visible biases in exploration patterns.**

In contrast, no global remapping was detected in the study, in which authors used combination of reward-based and aversive learning: animals had to go through the zone where a foot shock was delivered to obtain the reward (Oler et al., 2008). Authors stressed that in this task no modification of trajectories took place which means that changes in cognitive map could not be explained by variations in occupancy of certain trajectories. However, even in this report minor proportion of place cells with place fields inside the shock zone remapped.

Unfortunately, rare study addressed the question of how do place cells remap after aversive experience? Do they remap randomly, outwards or towards the aversive zone? The only report that has done such investigation describe that place cells shifts their place fields mostly towards aversive zone bearing resemblance to analogous overrepresentation effect in reward domain (Mamad et al., 2019).

Likewise, aversive location was found to be overrepresented in awake reactivations observed after aversive learning (Wu et al., 2017). Authors have used inhibitory avoidance linear track, in which half of the track is exposed to light, and half of the track is dark where rodents naturally prefer to be. Animals are shocked in the dark compartment which results in strong avoidance of the dark segment and partial remapping of place cells towards the shock zone. Despite absence of trajectories within the shock zone, replays representing trajectories towards the shock zone were detected significantly more often than other trajectories during post-learning trials. However, observed elevation in shock zone reactivation rate could hypothetically be explained by increased pre-learning behavioral occupation of the shock zone.

Different study was using air puffs on the linear track to induce aversive effect on behavior (Girardeau et al., 2017). In this study, NREM sleep reactivations of the whole environment was observed separately in hippocampus and basolateral amygdala, and the stronger effect was found for inter-regional cell populations, especially if neurons in amygdala were modulated by SWRs. In addition, it has been shown that inter-regional neuronal ensembles that represent trajectories including aversive locations are reactivated stronger compared to other trajectories.

Unfortunately, very few studies report changes in place cell coding after aversive experience, and even less we know about differences and similarities between reward-based and aversive spatial learning. In this thesis, **we aim to systematically compare hippocampal spatial coding after strong place preference and strong place avoidance were indu**ced. How stable is cognitive map after both types of affective learning and if not, towards which location is it preferentially biased? How similar are awake and sleep reactivations to behavioral correlates of emotional behavior? How similar are reactivations during learning and in following sleep? These are the questions we will address in this study.

Résumé

There are two types of affective learning that are opposite in some sense but share a lot of similarities in other sense: reward-based and aversive learning. Both of them drastically change animals' exploration patterns. Is this change reflected in cognitive map architecture and place cell coding?

During reward-based learning, cognitive map seems to follow behavioral occupation: place fields accumulate around reward locations. Moreover, place cells representing reward

zones are reactivated stronger and more often in quite wakefulness and NREM sleep following learning compared to neurons with place fields outside of the zone.

Dopaminergic activity plays important role in the establishment and processing of reward-related memories. Dopamine released from terminals that originated in ventral tegmental area and locus coeruleus supports learning of reward-place associations and selective attention to reward. Activity in nucleus accumbens, direct target of VTA projections, is tightly coordinated with reactivations of spatial information in dorsal hippocampus.

Normal functioning of dorsal hippocampus is necessary for acquisition and retrieval of context fear conditioning and trace cued fear conditioning. It has been shown that remapping of place cells occurs after context fear conditioning, as well as in other forms of aversive learning. It seems that mostly place fields shift towards the aversive zone bearing resemblance with similar effect in reward-based learning (superimposed on opposite behavioral patterns and motivational value).

There are still very few studies that investigate effect of aversive spatial learning on hippocampal reactivations. Existing ones report that aversive zones are overrepresented in reactivations detected during quiet wakefulness (however, without controlling for baseline occupation of the aversive zone), or coordinated reactivations of aversive trajectories between dorsal hippocampus and amygdala during NREM sleep.

To our knowledge, no systematic investigation of differences and similarities between reward-based and aversive spatial learning was performed. In this study, we will both try to fill the gaps in our understanding of hippocampal place coding during and after aversive spatial learning and to compare it in the situations of reward-based and aversive spatial learning.

Questions

- Are avoided locations reactivated during calm wakefulness and NREM sleep? – chapter 5

- How stable is the cognitive map after reward-based and aversive spatial learning and if not stable, towards which location is it preferentially biased?

- How similar are reactivations during affective learning and in following sleep? – chapter 5

- Is there a link between reactivations after affective learning and post-learning behavior? – chapter 5

Conclusion and questions that we attempted to answer in this thesis

We have seen in the introduction that spatial navigation and spatial memory rely on the activity of place cells and their reactivations during offline states. Hippocampus-dependent spatial memory is the clearest available model of episodic memories we can study in rodents. Most types of learning (i.e. memory formation) require explicit reinforcers: rewards to promote behaviors or aversive stimuli to punish behaviors that could be maladaptive. It is logical to think, given that there are numerous examples of successful spatial learning around us, that nervous system can incorporate information about affective stimuli into representation of space. However, up to this date we do not have full mechanistic understanding of how exactly this process happen.

In our opinion, it is interesting to look at this problem from several theoretical perspectives. First, if we look at the particular instance of memory, what does it constitute? **Do neutral declarative knowledge and information about reinforcement that helped to solidify this memory exist separately? Or are they intricately interwoven in the brain, to the point they become one?** In other words, mechanism of association between spatial and emotional information is wanted. Moreover, reinstatement of newly formed spatial memories should incorporate the motivational valence of reinforcement but we don't know to which degree. What does it mean to remember: to reinstate the shade of neutral information or to relive the moment again, including emotions that the learning process brought up?

Second, keeping in mind that cognitive map can be represented by the whole available set of place fields, we already know that positive reinforcements warp cognitive map towards reward sites. What governs this warping effect? **Is it general for all possible types of reinforcements, which would suggest that cognitive map is biased towards the most relevant locations of the environment?**

Third, given ever-changing conditions in real world, it is fairly probable that an object or a place associated with certain motivational valence will change this valence in the future. How does the brain deal with such conflicts? Are appetitive-to-aversive and aversive-to-appetitive modifications symmetrical?

Most of these questions will not be tackled in the present thesis, however they are motivated us to design and perform this study. There are several concreate problems we will address below.

How does aversive stimulus change representation of space?

Throughout the introduction, I tried to demonstrate that there are gaps in how we think about affective spatial learning. Indeed, reward shifts place fields of the cognitive map towards reward location, and those locations are overrepresented also in reactivations observed in offline states. It is still unclear, whether this effect is caused by motivational salience of rewarded locations, or simply by the fact that they are the most visited places on the map?

In this thesis, we investigated spatial maps as well as rate and contents of hippocampal reactivations during and after aversive spatial learning (which induces avoidance of motivationally salient location) and (partly) compared the results with reward-based learning.

Are there fear-related neurons in dorsal hippocampus?

Up to this date, there was no consensus about how hippocampus represents fear-related stimuli and locations. There are two main ideas: either processing of aversiveness occur outside of hippocampus (for example, in amygdala), or there are specialized neurons within hippocampus that respond to fear-related objects. In this thesis, we have opportunity to explore data for the evidence of the second possibility.

Can we reverse aversive spatial association using counterconditioning?

Given the expertise in affective spatial learning, we designed the experiment that aims at reversing aversive associations during sleep using pairing hippocampal reactivations to intracranial rewarding stimulation. This enormously complex task requires a reliable decoder of reactivations (see below). However, before we launch ourselves into this experiment, can we confirm that such counterconditioning works during wakefulness?

Can one build effective online decoder based on artificial neural network?

To be able to effectively manipulate hippocampal place cell activity during offline states, we need a position decoder that is accurate, fast and that would not require any manual curation of input data. Artificial neural networks are good candidates for such task. In this thesis, we attempt to build a decoder that satisfy all requirements for the closed loop experiment.

Chapter 5. Results I. Hippocampal reactivations in reward-based and aversive spatial learning

Introduction

Large part of hippocampus-oriented research in mammal models is concentrated around 'spatial memory' concept. Place cells recorded from areas CA1 and CA3 of hippocampus fire in the specific locations of a particular environment called 'place fields' (O'Keefe and Dostrovsky, 1971). Activity of place cells is highly organized in temporal domain. During active exploration periods, hippocampal local field potentials (LFP) are dominated by theta oscillations that organize place cell firing in sequences using the mechanism of theta phase precession (O'Keefe and Recce, 1993; Skaggs et al., 1996). These sequences, representing past, present and future position of an animal, can be considered trajectories constructed in the abstract space of a cognitive map. Interestingly, sequences that emerged during exploration are reactivated during calm states, such as sleep or immobile wakefulness (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Foster and Wilson, 2006; Diba and Buzsáki, 2007; Pfeiffer and Foster, 2013). Hippocampal reactivations are thought to support memory consolidation (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010; de Lavilléon et al., 2015; Grydchin et al., 2020), planning and decision-making (Pfeiffer and Foster, 2013; Ólafsdóttir et al., 2017; Pfeiffer, 2020).

Historically, hippocampal reactivations were studied in the behavioral paradigm of 'free foraging' that uses food scattered randomly in the environment as an incentive to explore. Therefore, a big share of reported results was obtained in the situations similar to appetitive learning. It has been shown that rewarded locations are reactivated more often compared to other explored locations (Singer and Frank, 2009; Dupret et al., 2010). What makes the rewarded location to be more reactivated than the others? It is entirely plausible to explain elevated rate of rewarded location reactivations by the fact that animals spend more time in these zones consuming reward. Indeed, more visited places during wakefulness have been shown to be reactivated more often during sleep (O'Neill et al., 2008). Alternative hypothesis suggests subcortical structures processing emotional stimuli (such as nucleus accumbens (NAc), ventral tegmental area (VTA) or amygdala) to guide selection process for contents of reactivations (Lansink et al., 2009; Atherton et al., 2015; Girardeau et al., 2017).

Fearful events that happen in certain locations also guarantee remarkable modifications in exploration patterns – much like during appetitive learning but of different sign. Animals increase their occupation of rewarded place, whereas behavioral occupation of aversive zones is drastically reduced. However, behavioral relevance (i.e. potential to induce learning) is similarly present in both situations. Would aversive locations be reactivated more compared to other zones, as if contents of reactivations depend more on importance of the location for an animal, or would aversive locations be underrepresented in reactivations, as if places visited less often than the others?

Spatial learning with aversive associations investigated far less than spatial learning with reward. One study has demonstrated that trajectories directed into aversive zone are reactivated despite the behavioral avoidance that follows aversive learning (Wu et al., 2017).

However, behavior of animals before learning was significantly biased towards the future aversive zone, which does not allow dissociation of potential baseline overrepresentation of shock location and consequences of aversive learning. In different study, coordinated reactivations of the aversive trajectory between hippocampus and amygdala have been shown to occur (Girardeau et al., 2017). In this study, authors have chosen air puff as an aversive stimulus; learning with air puff did not result in strong avoidance of the aversive zone of the environment.

In the present report, we aimed to compare hippocampal reactivations in aversive and reward-based learning. In our behavioral paradigm, baseline exploration was homogeneous across environment before learning and very similar across types of learning. Two groups of mice were trained in aversive place association learning sessions and in rewarded place association learning using intracranial stimulation of dorsolateral periaqueductal grey matter (dlPAG) and medial forebrain bundle (MFB), respectively. Learning procedure resulted in decreased time spent in the stimulation zone in case of aversive learning and increased time spent in the stimulation zone in case of reward-based learning. We investigated how representation of the full environment and the stimulation zone specifically are reactivated during immobile wake periods and NREM sleep following learning.

Results

Rewarding and aversive spatial learning protocol bias the behavior towards one zone

Mice implanted with recording electrodes in the area CA1 of hippocampus were trained in the U-shaped maze (UMaze, for full protocol see *Fig. 5-1A,* C). Inspired by both place preference set-up and linear mazes, the UMaze has two compartments of equal size. Experiment starts with the free exploration of the maze when animals are not exposed to any affective (i.e. potentially reinforcing stimuli). After free exploration phase, one arm of the maze (randomly assigned) serves either as a shock zone (in case of aversive protocol), or reward zone (in case of appetitive protocol). During these 'conditioning' sessions, animals were free to choose any trajectory within the environment – intracranial stimulation was triggered when a mouse crossed the border of the stimulation zone, and it was repeated each 6 s until the animal leaves the zone.

As a rewarding stimulus, we used intracranial stimulation of medial forebrain bundle, (MFB), a complex fiber tract that passes through lateral hypothalamus (Nieuwenhuys et al., 1982). It has been shown that place preference can be created by triggering MFB stimulation in a particular location (Kobayashi et al., 1997; Talwar et al., 2002; Kobayashi et al., 2003; Mamad et al., 2017). Intracranial stimulation of dorsolateral periaqueductal gray matter (dlPAG) served as an aversive stimulus. Activation of dlPAG proved successful in replacing unconditioned stimuli in fear conditioning and avoidance paradigms (Kim et al., 2013; Deng et al., 2016).

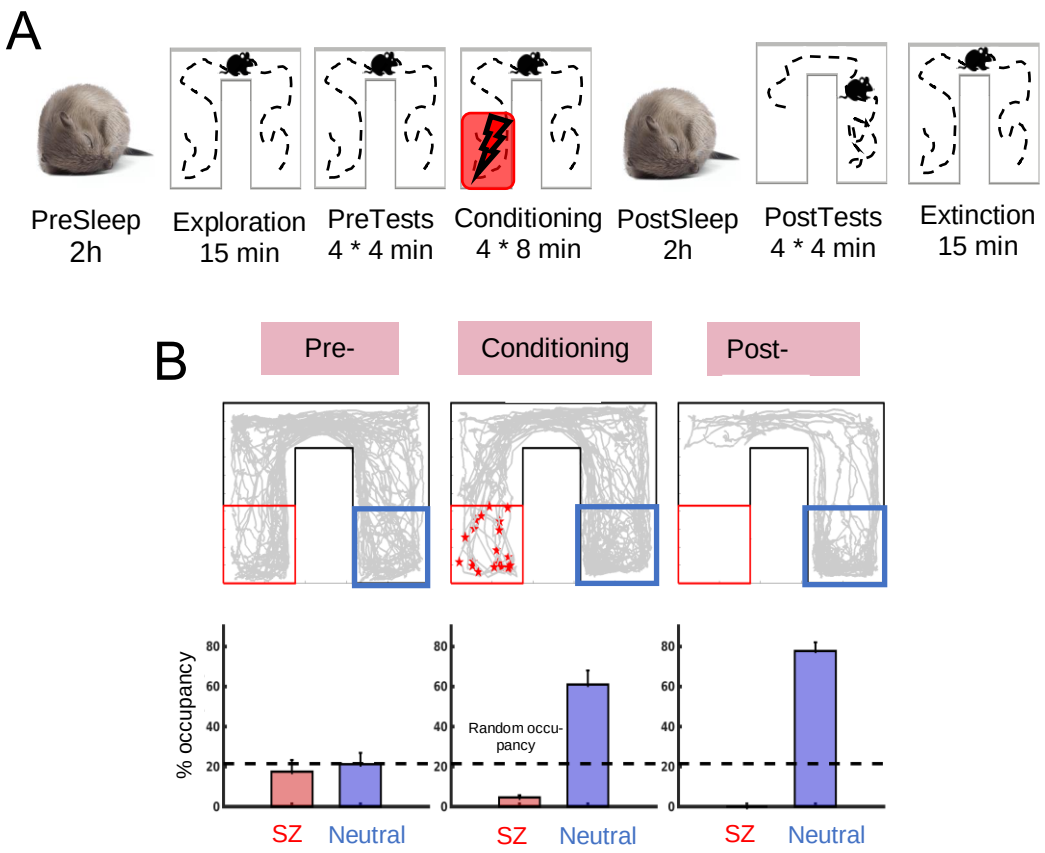
A
PreSleep 2h
Exploration 15 min
PreTests 4 * 4 min
Conditioning 4 * 8 min
PostSleep 2h
PostTests 4 * 4 min
Extinction 15 min
B
Pre-
Conditioning
Post-
% occupancy
Random occu-pancy
SZ
Neutral
C
PreSleep 2h
Exploration 15 min
PreTests 8 * 2 min
Conditioning 8 * 4 min
PostSleep 2h
PostTests 4 * 4 min
Extinction 15 min
D
Pre-
Conditioning
PostTests
% occupancy
Random occupancy
RZ
Neutral

Figure 5-1. Overview of behavioral tasks used in the study. *Two groups of mice were trained in two very similar protocols: aversive place association learning (**A**) and reward-place association learning (**C**). Both experiments start with 2 h long sleep session (PreSleep) that was followed by 15 min long free Exploration session. Then animals underwent PreTest sessions, 16 min in total. In aversive place association protocol, animals did 4 PreTests, 4 min long each; in reward-place association protocol, animals did 8 PreTests, 2 min long each. Afterwards, mice spent 32 min in Conditioning sessions: each time an animal crossed the border of stimulation zone, it received aversive or appetitive intracranial stimulation. Stimulations were performed (ISI = 6 s) until the mouse left the zone. In aversive place association protocol, animals did 4 Conditioning sessions, 8 min long each; in reward-place association protocol, animals did 8 Conditioning sessions, 4 min long each. Conditioning was followed by 2 hours of PostSleep and then PostTests, which repeated the structure of PreTest sessions of the current protocol. Both protocols were finished by 15 min of free exploration session called Extinction. **B**. Summary of the aversive place association experiment for an example mouse. **Top:** trajectories during Pre-Tests, Conditioning sessions and PostTests. Location of shocks are indicated by red stars. **Bottom**: Occupancy percentage of the shock zone and neutral counterpart in the opposite arm in PreTests, Conditioning sessions and PostTests. **D**. Summary of the reward-place association experiment for an example mouse. **Top:** trajectories during PreTests, Conditioning sessions and PostTests. Location of rewarding stimulations are indicated by green stars. **Bottom**: Occupancy percentage of the reward zone and neutral counterpart in the opposite arm in PreTests, Conditioning sessions and PostTests.*

To assess behavioral changes induced by affective spatial learning, we used two parameters: occupancy of the zone (neutral or stimulated) and the latency to enter the zone. Before learning procedure, animals explored future stimulation zone and its neutral counterpart in the opposite arm equally (*Fig. 5-2)*.

Conditioning procedure dramatically altered animals' behavioral patterns (*Fig. 5-1B, D for examples, Fig. 5-3 for overall results*). In appetitive protocol, animals increased time spent in the reward zone and latency to enter the shock zone decreased. In contrast, after aversive spatial learning, mice drastically reduced time spent in the shock zone and time to enter the shock zone increased.

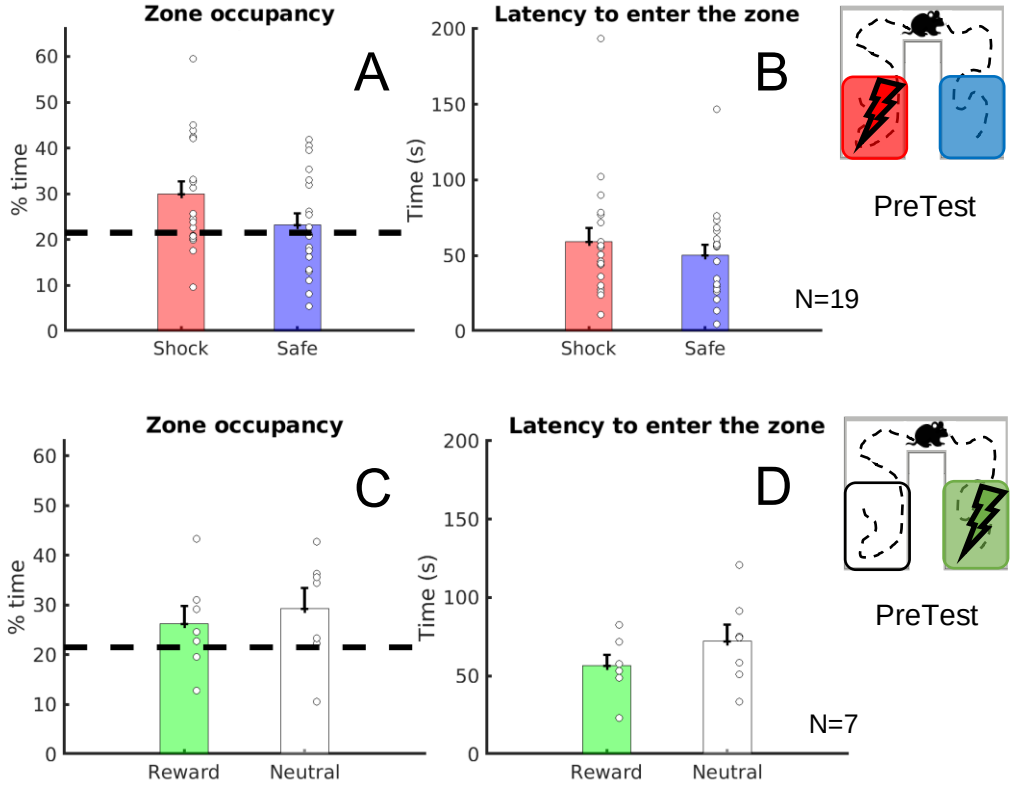

Figure 5-2. Stimulation and neutral zones were explored equally in both experiments during *PreTests* (before learning had started). ***A, C.*** *Occupancy of stimulation zone and its neutral counterpart during PreTests in aversive place association protocol (**A** -* 29.9±2.8% vs 23.2±2.6%, Wilcoxon ranksum p=0.1*) and in reward place association protocol (**C** -* 26.2±3.7% vs 29.3±3.7%, Wilcoxon ranksum p=0.62*). Dashed line indicates random occupancy rate for the zone. **B, D**. Latency to enter the stimulation zones and its neutral counterpart during PreTests in aversive place association protocol (**B** -* 59.1±9.3 s vs 50.2±7.2 s, Wilcoxon ranksum p=0.62*) and reward-place association protocol (**D** -* 56.5±7.1 vs 72.1±10.8 s, Wilcoxon ranksum p=0.26*).*

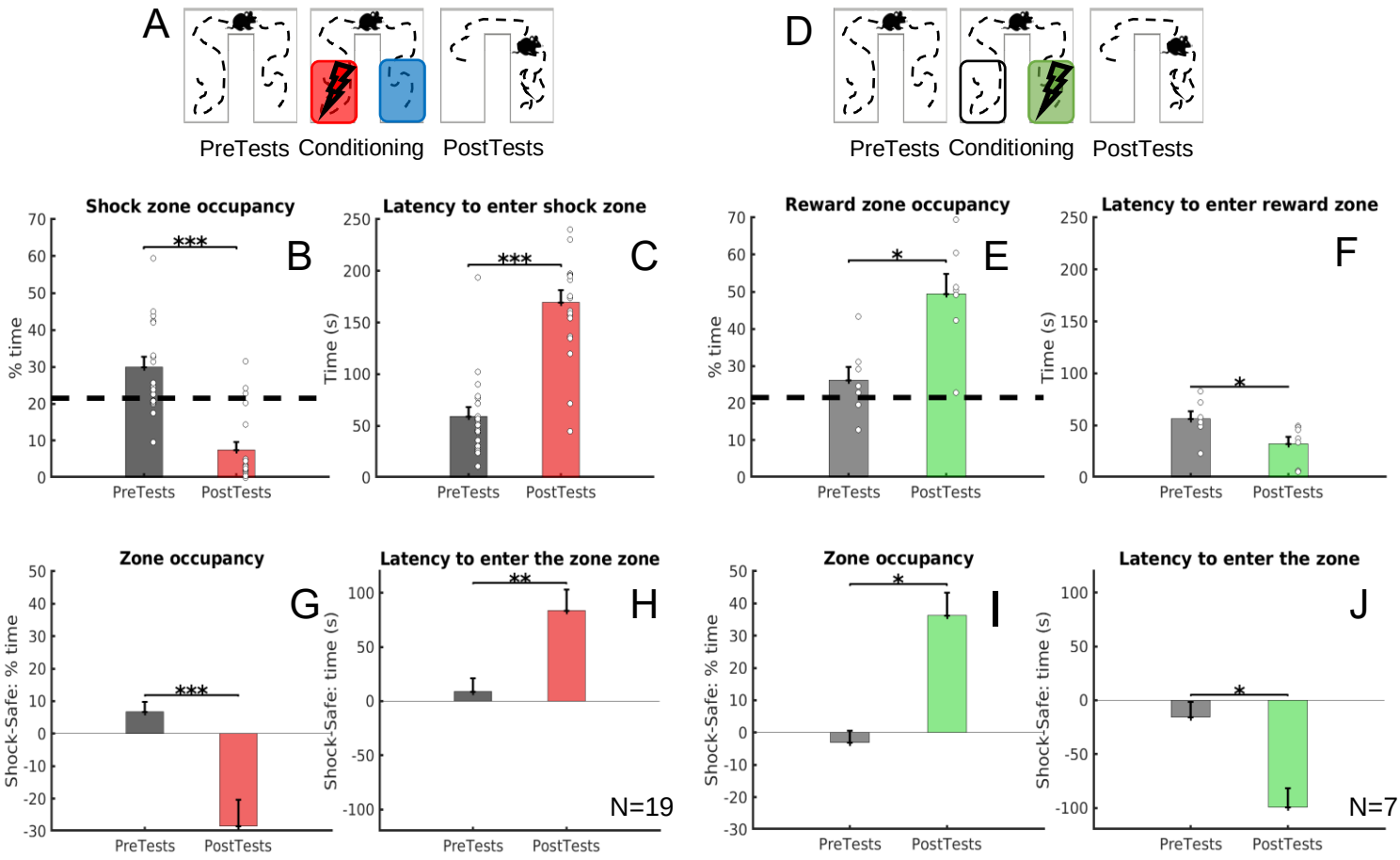

Figure 5-3. Aversive place association protocol and reward-place association protocol resulted in strong place avoidance and place preference, respectively. ***A, D****. Simplified schematics of aversive place association protocol (**A**) and reward-place association protocol (**D**). **B, E.** Occupancy of the shock zone before (PreTests) and after (PostTests) aversive place association learning (**B -** 29.9±2.8% vs 7.4±2.3%, Wilcoxon ranksum p=10^{-5}) and reward-place association protocol (**E -** 26.2±3.7% vs 49.4±5.4%, Wilcoxon ranksum p=0.01). **C, F.** Latency to enter the shock zone before (PreTest) and after (PostTests) aversive place association learning (**C -** 59.1±9.3 s vs 169.3±12.3 s, Wilcoxon ranksum p=8 x 10^{-6}) and reward-place association protocol (**F -** 56.5±7.1 s vs 32.2±7.1 s, Wilcoxon ranksum p=0.01). **G, I.** Difference between occupancies of the shock and safe zones during Pre- and PostTests in aversive place association learning (**G -** 6.7±3.2% vs -28.4±8.1%, Wilcoxon ranksum p=0.0006) and reward-place association protocol (**I -** (-3.1±3.8% vs 36.2±7.2%, Wilcoxon ranksum p=0.0006). **H, J.** Difference between latencies to enter the shock and safe zone during Pre- and PostTests in aversive place association learning (**H -** 9.0±12.3 s vs 83.5±19.6 s, Wilcoxon ranksum p=0.002) and reward-place association protocol (**J -** 15.6±14.5 s vs -99.5±17.9 s, Wilcoxon ranksum p=0.007).

It is worth to stress that modification of behavior within the stimulation zone in both protocols was specific to the stimulation zone (*Fig. 5-3G-J*). Indeed, in aversive place association protocol, difference between occupancies of the stimulation and neutral zones was slightly positive before start of the learning procedure, and it dropped drastically after the learning. Difference of latencies to enter the zone increased after aversive spatial learning. On the contrary, in reward-place association protocol, difference between occupancies of the stimulation and neutral zone increase after the learning procedure and difference between latencies to enter the zones decreased.

Thus, while animals showed very similar behavioral patterns before learning took place, exploring the whole UMaze in an unbiased manner, for both aversive place and reward-place association protocols, after learning behavioral patterns looked utterly opposite with respect to behavior in the stimulation zone. Indeed, dlPAG stimulation results in decreased occupancy of the stimulation zone, whereas MFB stimulation reduces occupancy of the arm opposite to stimulated zone. And vice versa, MFB stimulation boosts occupancy of the stimulation zone while mice become biased towards the zone opposite to aversive zone after dlPAG conditioning (*see also Fig. 5-15 in appendix*).

Sleep physiology and sleep SWRs after affective spatial learning

Sleep architecture was not modified by neither aversive place association learning nor reward-place association learning (*Fig. 5-4*). It is widely accepted that hippocampal reactivations mostly happen during sharp-wave ripples (SWRs) that could be observed during NREM sleep (Nadasdy et al., 1999; Buzsáki. 2015). Both aversive and appetitive learning resulted in increased SWR rate during PostSleep NREM stage compared to PreSleep NREM stage (*Fig. 5-4E, J*).

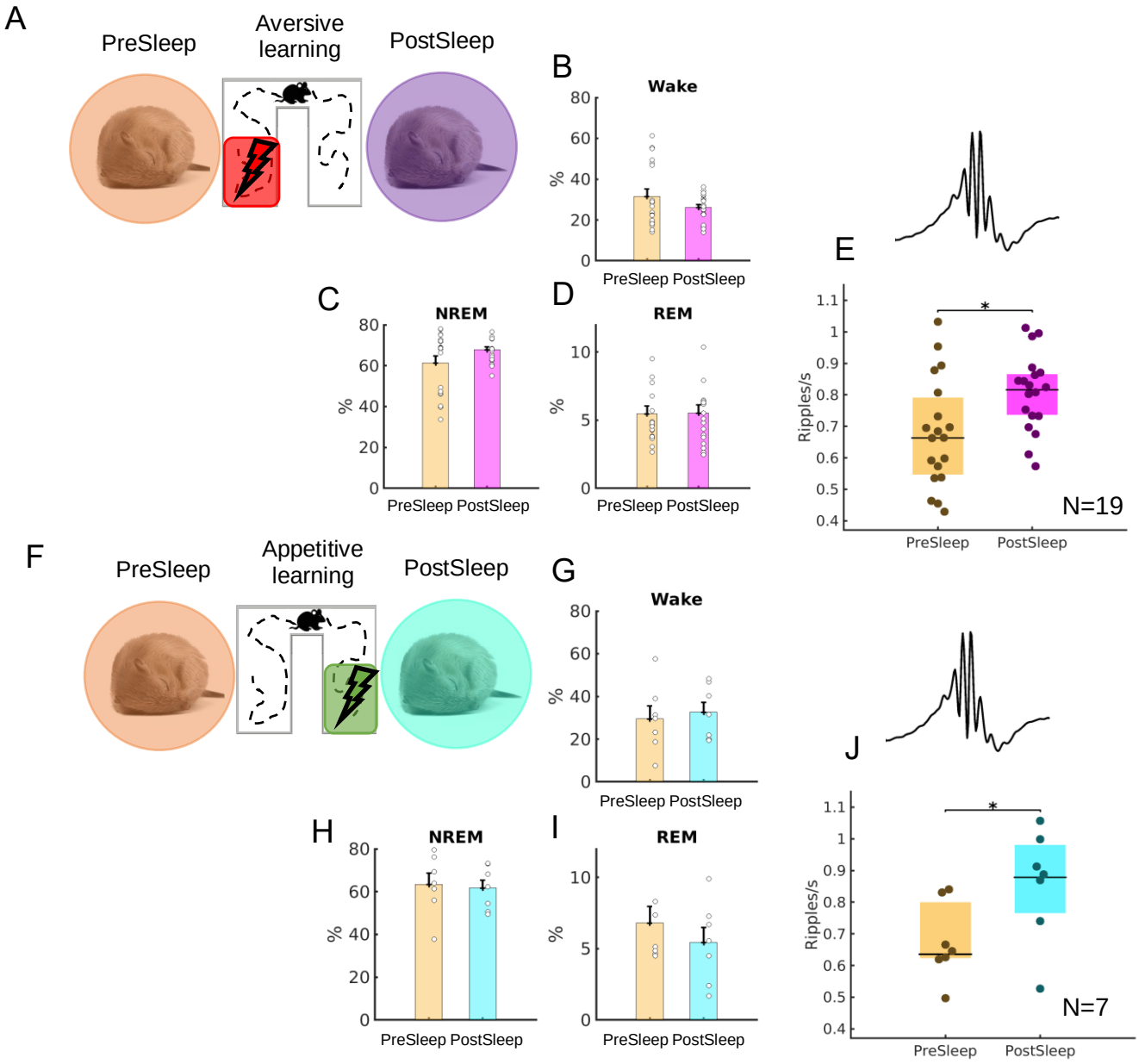

Figure 5-4. Sleep architecture and sharp wave ripples (SWR) rate in aversive place association protocol and reward-place association protocol. ***A****. Simplified schematics of aversive place association protocol.* ***B****. Percentage of wakefulness during Pre- and PostSleep sessions in aversive place association protocol (*31.4±3.4 vs 25.0±1.6 %, Wilcoxon ranksum p=0.45). ***C****. Percentage of NREM sleep during Pre- and PostSleep sessions in aversive place association protocol (*62.3±3.2 vs 68.02±1.5 %, Wilcoxon ranksum p=0.35). ***D****. Percentage of REM sleep during Pre- and PostSleep sessions in aversive place association protocol (*5.9±0.5 vs 6.1±0.6 %, Wilcoxon ranksum p=0.64). ***E****. SWR rate in the first 30 min of NREM state of Pre- and PostSleep sessions in aversive place association protocol (0.*68±0.04 Hz vs 0.81±0.03 Hz, Wilcoxon ranksum p=0.015*).* ***F****. Simplified schematics of reward-place association protocol.* ***G****. Percentage of wakefulness during Pre- and PostSleep sessions in reward-place association protocol protocol (*29.6±6.0 vs 32.6±4.8 %, Wilcoxon ranksum p=0.62). ***H****. Percentage of NREM*

*sleep during Pre- and PostSleep sessions in reward-place association protocol (*63.5±5.3 vs 61.6±3.9 %, Wilcoxon ranksum p=0.62). ***I.*** *Percentage of REM sleep during Pre- and PostSleep sessions (*6.8±1.2 vs 5.4±1.1 %, Wilcoxon ranksum p=0.46). ***J.*** *SWR rate in the first 30 min of NREM state of Pre- and PostSleep sessions in reward-place association protocol (*0.67±0.05 Hz vs 0.86±0.07 Hz, Wilcoxon ranksum p=0.04*).*

Reactivations of hippocampal neuronal ensembles after both appetitive and aversive spatial learning

We have recorded both local field potentials and ensembles of neurons from area CA1 of hippocampus in all animals. Among 19 recorded sessions in the aversive place association experiment, 7 sessions comprising 296 neurons in the pyramidal layer were stable and are included in the analysis. Likewise, among 7 recorded sessions in the reward-place association experiment, 3 sessions comprising 111 neurons were stable and are included in the analysis.

Reactivations of hippocampal neural ensembles were assessed by means of explained variance (EV), which represents how much of variance of populational pairwise correlations during PostSleep can be explained by pairwise correlations of activity recorded during the task after ruling out background correlations observed during sleep that precedes the task. A good control value, called reversed explained variance (REV), swaps Pre- and PostSleep correlation matrices. We looked at three different periods during the task in search of reactivations of the neuronal activity at these different moments: reactivations of neutral environment were assessed by calculating the EV for the free exploration periods before affective learning, reactivations of neuronal patterns observed during active behavior during learning and reinstatement of neuronal activity detected during awake SWRs that occurred during learning. EV and REV are presented for NREM sleep.

We have found that in both reward-based and aversive protocols neuronal activity observed before and during affective learning is reactivated in the NREM sleep following the task (*Fig. 5-5A, B, D, E*). Moreover, co-firing patterns observed during SWRs during learning are also reinstated in the following NREM sleep in both protocols (*Fig. 5-5C, F).*

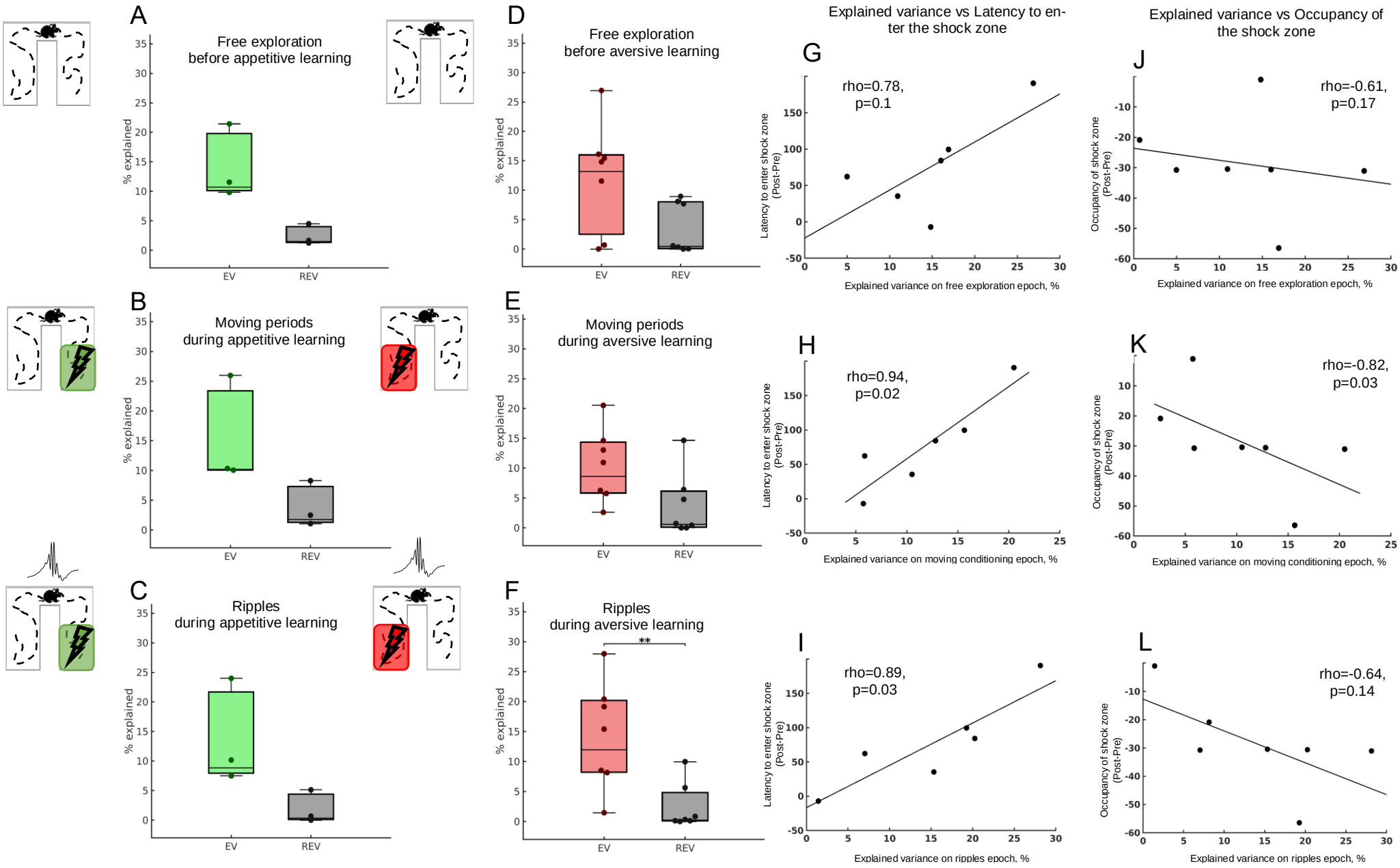
A
Free exploration
before appetitive learning
% explained
EV
REV
B
Moving periods
during appetitive learning
C
Ripples
during appetitive learning
D
Free exploration
before aversive learning
E
Moving periods
during aversive learning
F
Ripples
during aversive learning
**
Explained variance vs Latency to enter the shock zone
G
rho=0.78,
p=0.1
Latency to enter shock zone (Post-Pre)
Explained variance on free exploration epoch, %
H
rho=0.94,
p=0.02
Explained variance on moving conditioning epoch, %
I
rho=0.89,
p=0.03
Explained variance on ripples epoch, %
Explained variance vs Occupancy of the shock zone
J
rho=-0.61,
p=0.17
Occupancy of shock zone (Post-Pre)
K
rho=-0.82,
p=0.03
L
rho=-0.64,
p=0.14

Fig. 5-5. Hippocampal neurons reactivate co-firing patterns during NREM sleep. Strength of reactivations linearly correlate with avoidance behavior. ***A****. EV and REV during NREM sleep for active periods before reward-based learning (EV=14.3*±3.6 %, REV=*2.5*±1.0 %, N=3). ***B.*** *EV and REV during NREM sleep for active periods during reward-based learning (EV=15.5*±5.3 %, REV=*4.0*±2.2 %, N=3). ***C.*** *EV and REV during NREM sleep for neuronal activity registered during ripples in reward-based learning (EV=14.3*±5.5 %, REV=*2.1*±1.7 %, N=3). ***D****. EV and REV during NREM sleep for active periods before aversive learning (EV=13.0*±3.2 %, REV=*3.9*±1.6 %, N=7, Wilcoxon ranksum p=0.03). ***E.*** *EV and REV during NREM sleep for active periods during aversive learning (EV=10.6*±2.3 %, REV=*3.9*±2.0 %, N=7, Wilcoxon ranksum p=0.07). ***F.*** *EV and REV during NREM sleep for neuronal activity registered during ripples in aversive learning (EV=14.4*±3.4 %, REV=*2.4*±1.5 %, N=7, Wilcoxon ranksum p=0.007). ***G.*** *Linear correlation between explained variance calculated for active periods before aversive learning and difference between latencies to enter the shock zone before and after learning* (Spearman's rho=0.78, p=0.1). ***H.*** *Linear correlation between explained variance calculated for active periods during aversive learning and difference between latencies to enter the shock zone before and after learning* (Spearman's rho=0.94, p=0.02). ***I.*** *Linear correlation between explained variance calculated for neuronal activity registered during ripples in aversive learning and difference between latencies to enter the shock zone before and after learning* (Spearman's rho=0.89, p=0.03). ***J.*** *Linear correlation between explained variance calculated for active periods before aversive learning and difference between the shock zone occupancies before and after learning* (Spearman's rho=-0.61, p=0.17). ***K.*** *Linear correlation between explained variance calculated for active periods during aversive learning and difference between shock zone occupancies before and after learning* (Spearman's rho=-0.82, p=0.03). ***L.*** *Linear correlation between explained variance calculated for neuronal activity registered during ripples in aversive learning and difference between shock zone occupancies before and after learning* (Spearman's rho=-0.64, p=0.14).

In aversive spatial learning, strength of reactivations predicts avoidance behavior

We have demonstrated that, similar to what is observed after appetitive spatial learning, animals reactivate experience related to aversive spatial learning in the following NREM sleep. Can we predict the degree of the avoidance in the behavioral tests after aversive learning?

We have linearly correlated EV calculated for neuronal activity observed at different periods during the task. Latency to enter the shock zone correlated strongly with EV calculated for both active periods during learning phase and SWRs observed in the learning phase (*Fig. 5-5H,I*). Moreover, shock zone occupancy was anti-correlated with the EV calculated on active periods during learning. These results indirectly point to the fact that NREM sleep reactivations of neuronal activity observed during learning could be important to support avoidance behavior.

Neuronal ensembles reactivated during PostSleep after aversive spatial learning are also active during learning phase

We have confirmed existence of reactivations after aversive spatial learning. However, EV does not provide neither spatial, nor temporal resolution to deeper investigate contents and the time course of reactivated neuronal co-firing patterns. To address these issues, we used reactivation strength technique (Peyrache et al., 2010). Using this method, one can extract co-active neuronal ensembles and then match their activity (in the form of templates) to the activity in question. Such matching process yields a measure that we will call similarity score, which is high when similarity between the template and the matched neuronal activity is high.

Sharp-wave ripples (SWRs), fast oscillatory events that can be recorded in hippocampus are the good proxy for assessing rate of reactivation events (Diba and Buzsáki, 2007; Buzsáki. 2015). Templates were constructed using periods of SWRs detected in the NREM sleep following aversive learning phase. Thus, we tried to identify similarities between potential replay events that occur during sleep SWRs and awake activity in search of neuronal patterns that contribute the most to reactivations. Mean similarity score across two first most significant templates is reported.

We have found that average similarity score is indeed higher for NREM sleep following aversive learning than in NREM sleep before learning (*Fig. 5-6A*), suggesting different contents of SWRs before and after learning. Interestingly, templates that weakly match activity during learning occur with the same similarity score in NREM sleep before and after learning, while templates with strong similarity to activity observed during learning phase exhibit significant differences in similarity score measure between Pre- and PostSleep (*Fig. 5-6B, C*). **These observations suggest that activity during aversive learning rather than reinforcement-free free exploration mostly contributes to the reactivations during SWRs.**

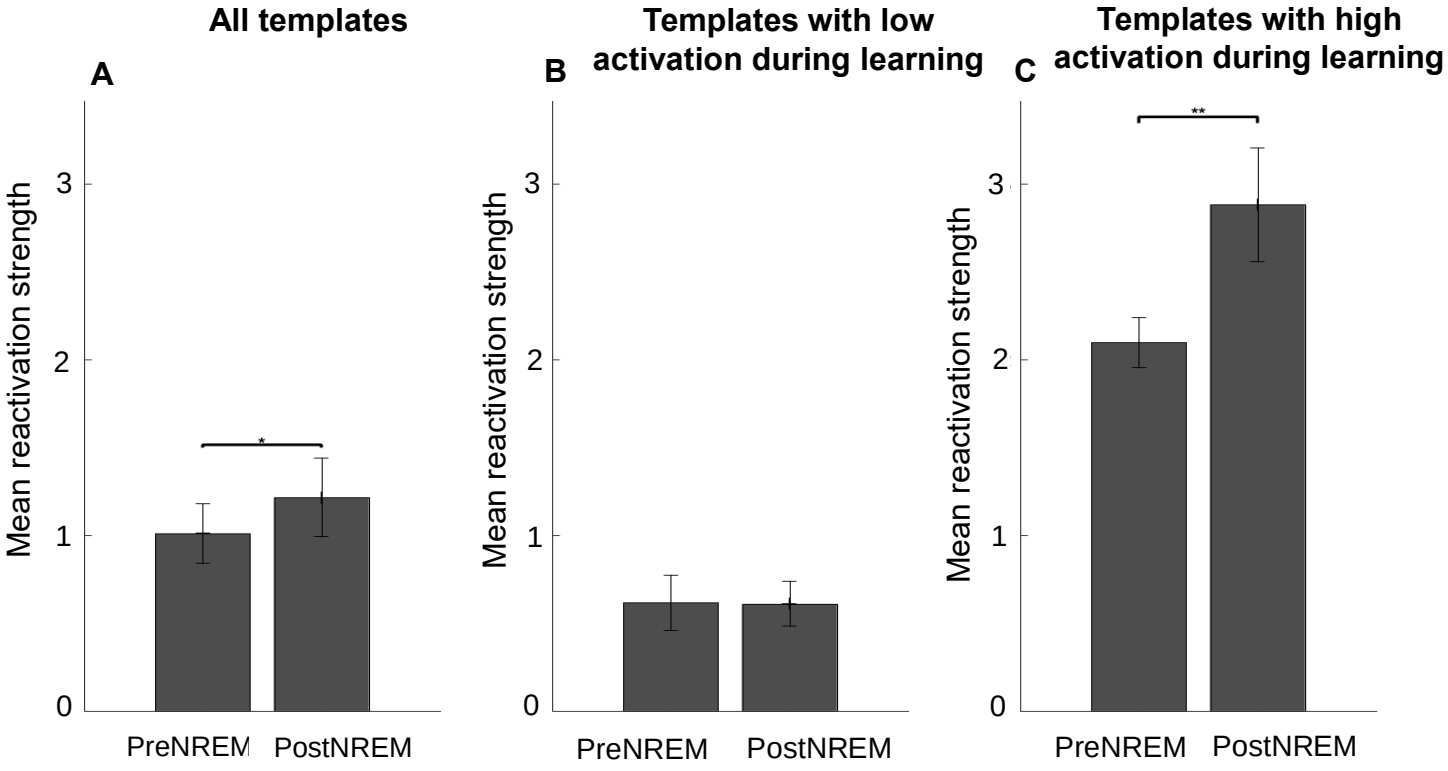

Figure 5-6. Ensembles that were active during aversive learning are reactivated during NREM sleep following the learning. ***A.*** *Mean similarity score across all templates in NREM sleep before and after aversive learning (1.22±0.22 vs 1.0±0.23, paired t-test p=0.03).* ***B.*** *Mean similarity score across templates with low similarity score during learning phase in NREM sleep before and after aversive learning (0.62±0.16 vs 0.61±0.13, paired ttest p=0.89).* ***C.*** *Mean similarity score across templates with high similarity score during learning phase in NREM sleep before and after aversive learning (2.1±0.14 vs 2.88±0.33, paired ttest p=0.009).*

Neuronal ensembles reinstated in NREM sleep are also reactivated at SWRs during aversive learning session

In contrast to free exploration sessions when minimal number of SWRs was detected, during both appetitive and aversive learning substantial number of SWRs was observed. Interestingly, in both protocols, maximum density of SWRs occurrence occurred at the most visited locations: thus, during reward-place association protocol most of SWRs occurred in the stimulation zone whereas during aversive place association protocol most of the SWRs were detected in the zone opposite to the shock zone (*Fig. 5-7*). There is a strong tendency of detecting more SWRs observed during aversive learning compared to reward- based one (0.45±0.1 (N=19) vs 0.04±0.01 (N=7) ripples/s).

We have noticed that similarity score of multiple templates significantly increases during aversive learning phase compared to free exploration before learning (*Fig. 5-*

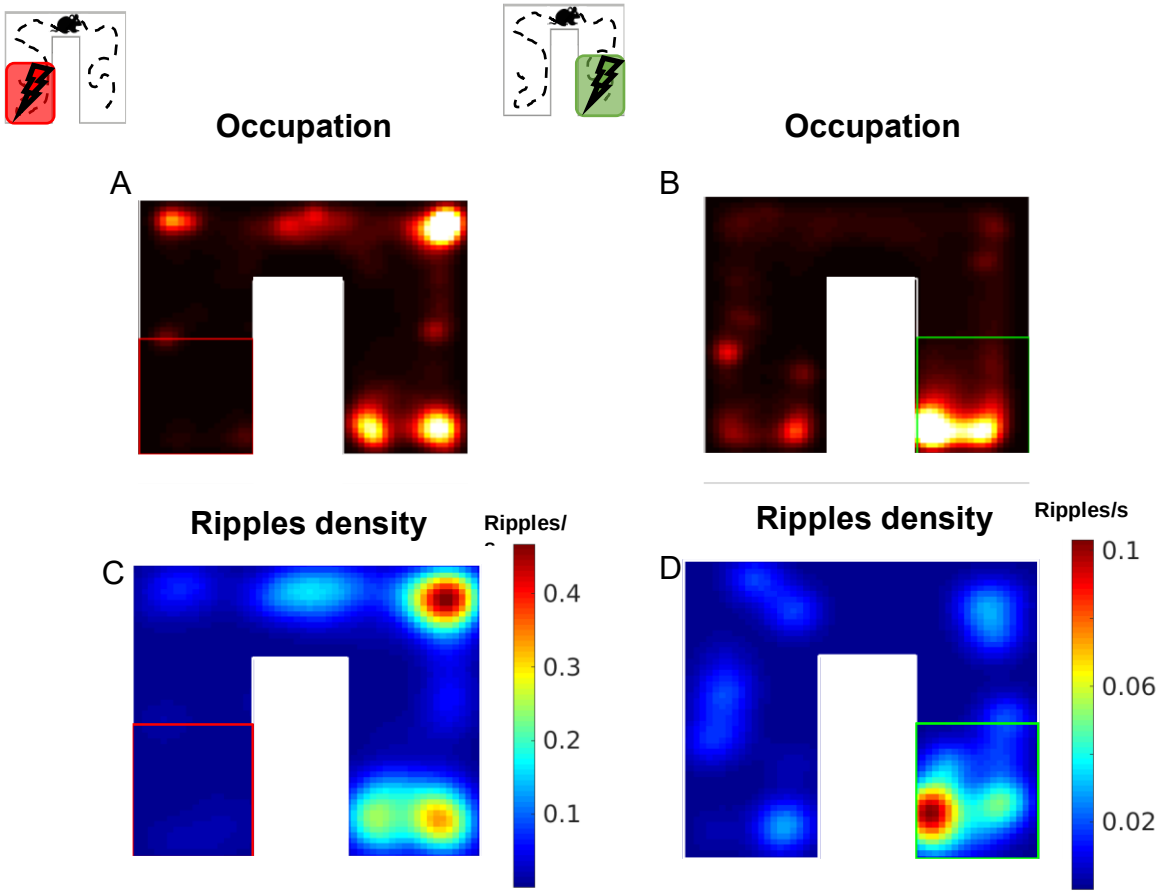

Figure 5-7. Sharp-wave ripples (SWRs) occur in the most visited locations in the environment both during reward-based and aversive spatial learning. ***A, B.*** *Occupation maps during learning phase in aversive place association protocol (**A**) and in reward-place association protocol (**B**).* ***Note that during aversive spatial learning freezing behavior often observed at the most visited locations. C, D.*** *Density of SWRs across the environment in aversive place association protocol (**C**) and in reward-place association protocol (**D**). Note that density of ripples during aversive learning is higher than density of ripples during reward-based learning.*

8A, B). This increase was often accompanied by the increase in SWR rate (Fig. 5-8C), and similarity score peaked at the time of awake SWRs occurrence (Fig. 5-8D). When we separated moments of SWR occurrence and the rest of the learning phase, it turned out that similarity score at the periods of awake SWR is approximately 5 times higher than in periods outside SWR, and even more so in free exploration session (Fig. 5-9). These results indicate that reactivations that we see during NREM sleep following aversive spatial learning are dominated by the activity of neurons during awake SWRs inside the learning experience.

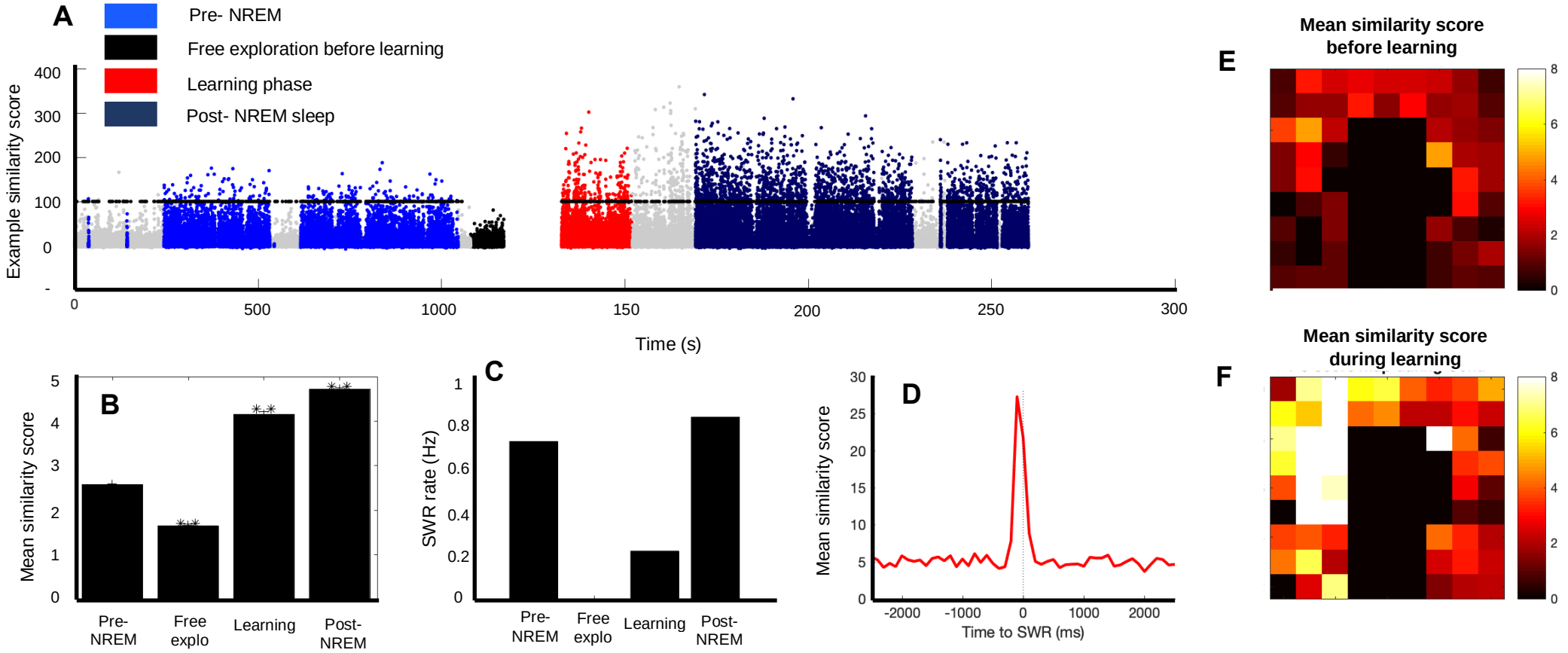

Figure 5-8. Similarity score during different stages of the aversive learning experiment for an example mouse. Activity during Post-NREM SWRs was taken as templates. ***A***. *Similarity score across the experiment of an example template. Black dots ranged across horizontal line are SWRs.* ***B***. *Similarity score of all significant template during different experimental phases.* ***C***. *SWR rate across different experimental phases.* ***D***. *Mean similarity score triggered at the time of SWR.* ***E***. *Spatial map of mean similarity score before aversive spatial learning.* ***F***. *Spatial map of mean similarity score during aversive spatial learning.*

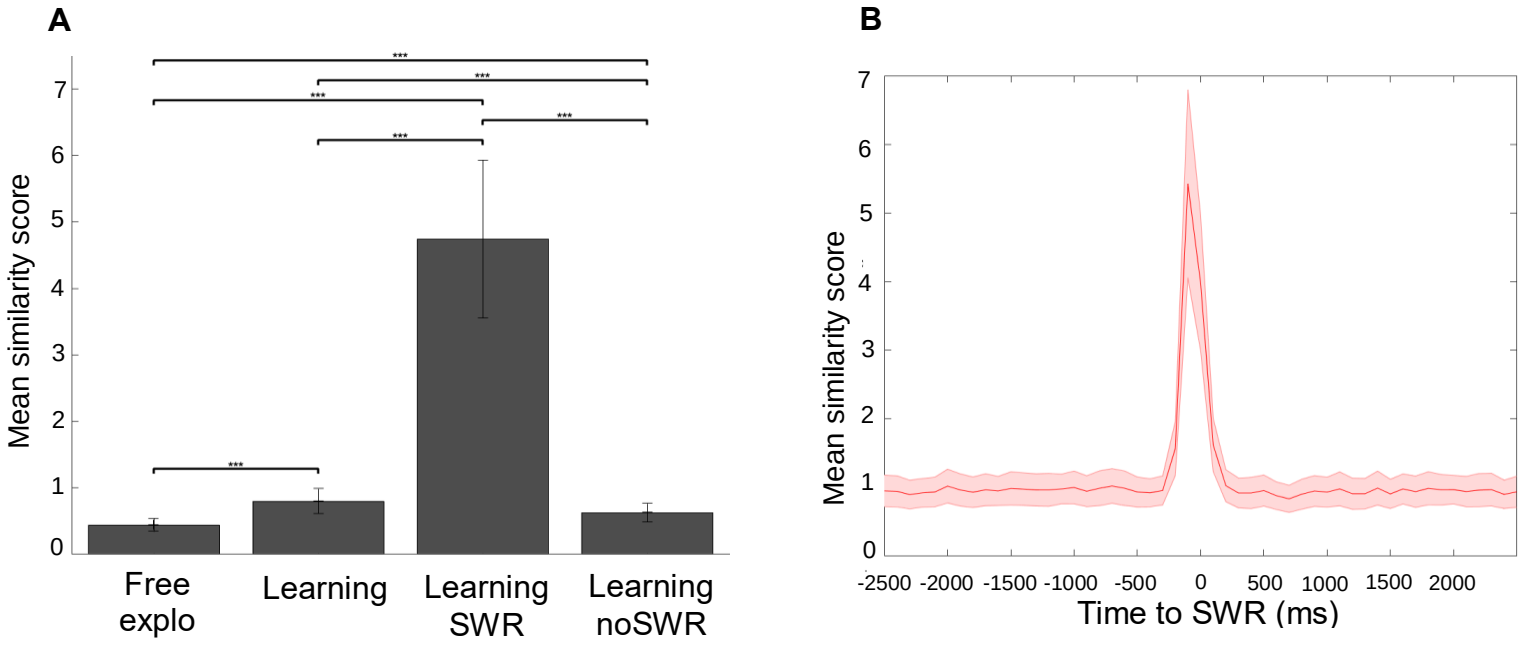

Figure 5-9. Neuronal activity during learning awake SWR during makes strongest contribution to NREM sleep reactivations. ***A****. Mean similarity score during different periods of the task (Free exploration: 0.43±0.1, Learning: 0.79±0.15, SWRs during learning: 4.74±1.19, Learning except SWRs: 0.62±0.14; paired t-test – free exploration vs learning: p=0.002; Free exploration vs SWRs during learning: p<0.001; free exploration vs learning except SWRs: p=0.007; learning vs SWRs during learning: p<0.001; learning vs learning except SWRs: p=0.001; SWRs during learning vs learning except SWRs: p<0.001).* ***B.*** *Mean similarity score triggered on awake SWRs.*

During learning phase of aversive place association experiment animals reactivate locations adjacent to the shock zone

When analyzing aversive place association protocol data, we have discovered hippocampal reactivations that occur mostly during SWRs in the learning phase and keep getting reactivated in subsequent NREM sleep. However, contents of these reactivation still remain an open question. To answer it, we investigated spatial distribution of average similarity score measure during free exploration period before learning and during aversive spatial learning.

While no clear location was more similar to the post-learning sleep SWRs than other location before learning began, average similarity score during learning phase had highest values in the shock zone (*Fig. 5-10*). Interestingly, aversive zone and close locations were the least visited across the whole environment. Our results suggest that zones of high motivational salience rather than zones of the highest behavioral occupancy are replayed.

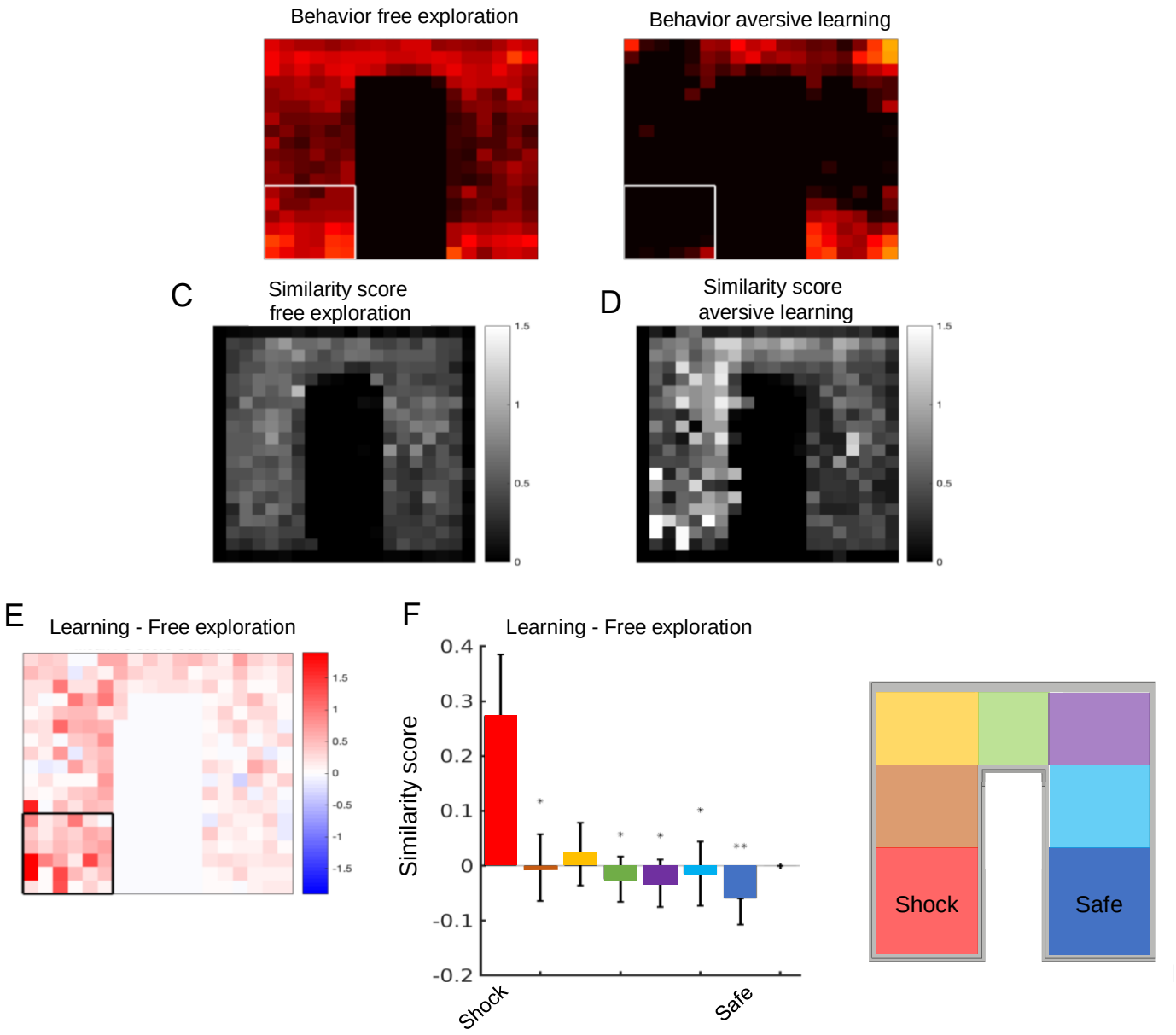

Figure 5-10. Average similarity score within the UMaze before and during learning. ***A, B****. Spatial distribution of behavioral occupancy across the UMaze location during free exploration before aversive learning (**A**) and during aversive learning (**B**).**C, D**. Average similarity score across the UMaze location during free exploration before aversive learning (**C**) and during aversive learning (**D**). **E.** Difference between average similarity score before and during aversive learning. **F.** (left) Average similarity score within 7 zones within the maze: from the shock zone to the safe zone. (right) Zones within the UMaze.*

Majority of place fields does not remap after aversive learning

It has been shown that affective learning can warp cognitive map by partial remapping of place fields (Hollup et al., 2001; Dupret et al., 2010; Moita et al., 2004; Wang et al., 2012; Mammad et al., 2017). However, there is still high controversy surrounding these results due to the fact that affective learning massively changes behavioral patterns of the animal – therefore, it is very difficult to evaluate place fields in an unbiased manner. Usually, stability of place

fields across sessions is assessed by rate maps correlation, which is very sensitive to slight shift in peak firing rates and even more sensitive to changes in exploration.

We have correlated rate maps from free exploration before learning and learning itself recorded during aversive place association protocol and compared them with intra-session stability assessed during free exploration only (*Fig. 5-11*).

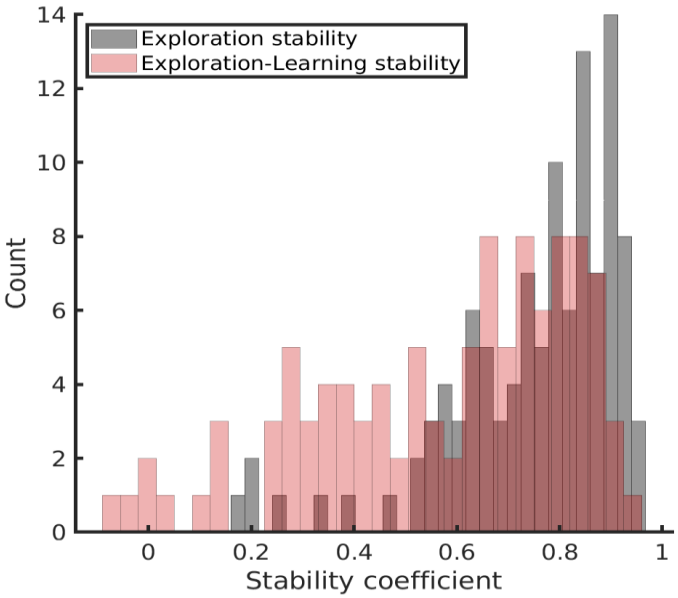

Figure 5-11. Distribution of place fields stabilities coefficients that assess stability between free exploration and learning periods.

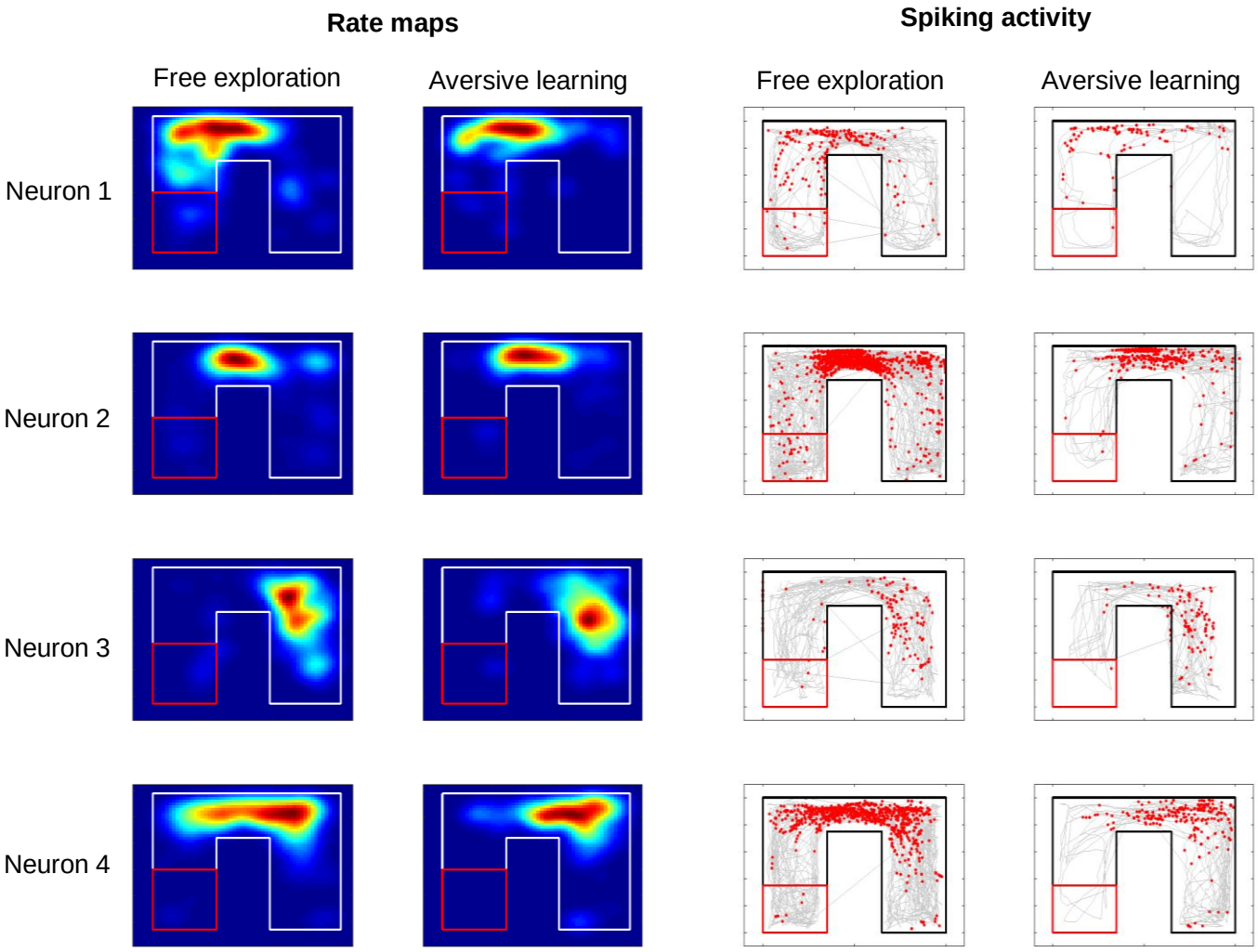

Figure 5-12. Examples of place cells with stable place fields before and during learning.

Within-session stability of free exploration period unsurprisingly gravitates towards 1 (identical rate maps), however we have observed to peaks in the distribution of exploration-to-learning stability: one closer to 1 and another around 0.5. Overall, while 90% within-session stability coefficients lie above 0.5 (*for such examples, see Fig. 5-12*), lesser proportion – 70% of exploration-to-learning stability coefficients were higher than 0.5. Nonetheless, 70% is still high enough to conclude that the majority of place cells remain stable.

To confirm our interpretation, we visually investigated place cells with low exploration-to-learning stability for convincing signs of remapping (*Fig. 5-13*). We have found that most cases of low stability can be attributed to drastically modified behavioral patterns during learning. The least stable neurons usually had their place fields in the least visited locations of the environment during learning, but closer visual inspection confirm remainders of location-specific firing. These observations confirm our interpretations that cognitive map remains stable during aversive spatial learning.

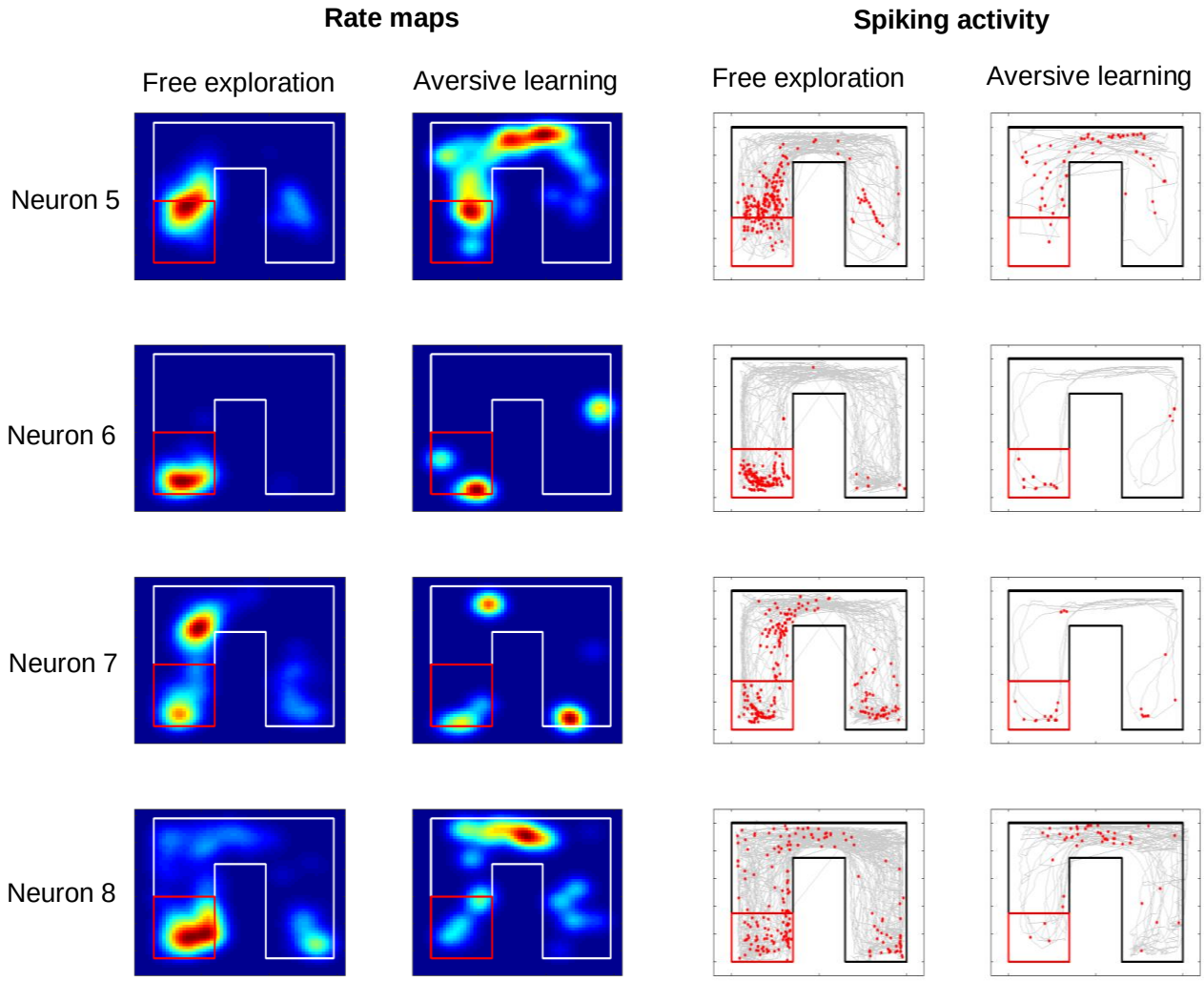

Figure 5-13. Examples of place cells with unstable place fields before and during learning.

Discussion

We have demonstrated that neuronal co-firing observed during the task, which includes spatial learning, was reinstated in the following NREM sleep for both valences of reinforcement used in the study. Reactivations of co-firing patterns recorded during sleep SWRs in the aversive learning phase linearly correlated with latency to enter the shock zone, one of the most important metrics of approach/avoidance behavior. Moreover, we have found that neuronal activity during awake SWRs contributed the most to reactivations observed during NREM sleep SWRs. Importantly, contents of these reactivations include mostly aversive zone representation.

Reactivations in aversive spatial learning

Classically, aversive spatial learning is much less studied than appetitive spatial learning. Such bias is explained by drastic behavioral modifications that is induced by moderate aversive stimuli. Indeed, aversive protocols usually result in elevated amounts of freezing and significant reduction of exploration, which impairs researcher's ability to assess location-specific firing. Therefore, existing studies use either very 'light' aversive stimuli, such as predator's odor or the air puff (Wang et al., 2012; Girardeau et al., 2017), and/or include strongly motivating appetitive reinforcement in the aversive learning – in other words, utilizing approach/avoidance conflict (Oler et al., 2008).

To our knowledge, there are two studies that assessed hippocampal reactivations after aversive spatial learning on the fine time scale. In one of them, authors have used inhibitory avoidance behavioral paradigm and have found that trajectories towards the shock zone are replayed significantly more often than other trajectories at choice locations in the environment (Wu et al., 2017). Important feature of this study is that aversive stimuli were presented in the dark compartment of the linear track, a place where animals naturally spent the most time before learning. Potentially, such bias can be reflected in the reactivations. Another study has demonstrated coordinated NREM sleep reactivations both in the hippocampal network and in the ensembles of neurons that comprise both dorsal hippocampus and basolateral amygdala (Girardeau et al., 2017). In this report, very mild aversive stimulus (an air puff) was used. Air puff induced minor modifications of foraging behavior – a hesitation in front of the stimulus zone, and the degree of its aversiveness remains an open question.

Unlike in the studies reviewed above, our behavioral paradigm does not favor behavioral biases before the start of learning, uses strong aversive stimulus (intracranial dlPAG stimulation), and it allows evaluating of both awake and sleep reactivations. In such conditions, vast majority of place cells did not change their place fields during aversive learning. According to previous reports, strong remapping is observed in the paradigms that induce massive behavioral modifications (Wang et al., 2012; Mamad et al., 2019), however only partial remapping was detected in the studies with weak or partial changes in exploration patterns (Moita et al., 2004; Kim et al., 2015). In our report, aversive learning induced major modification in exploration patterns, which was often characterized by the absence of trajectories in

some locations. We have observed that place fields that were supported by trajectories in both before learning and learning sessions remained stable.

Our results generally confirm that fear-related experience is reactivated during NREM sleep. We have observed NREM sleep reactivations of neural activity recorded during pre-learning free exploration phase as well as NREM reactivations of neuronal co-firing patterns during SWRs. Importantly, activity recorded during awake SWRs made the strongest contribution to NREM sleep reactivations in PostSleep.

Similar to already published data (Wu et al., 2017; Girardeau et al., 2017), neuronal activity that represents the aversive zone contributed the most to subsequent reactivations. Our results complement existing reports that have found awake reactivations of the aversive location (Wu et al., 2017), and NREM sleep reactivations of the aversive location after moderately aversive stimulation (Girardeau et al., 2017). Behavior in the latter paradigm implied travelling through the aversive zone, whereas our task was designed to evoke strong avoidance of the aversive zone. Therefore, behavioral adaptations in two studies are different, however the results are similar, which suggests that motivational salience as the main factor that drives behavioral changes.

Magnitude of SWRs-related reactivations after aversive learning could predict strength of avoidance behavior, as reflected by the linear correlation analysis. Potentially, this observation opens the possibility to construct mechanistic model of how reactivations of affective experience support subsequent behavioral adaptations induced by them. Despite several brilliant reports demonstrating necessity of hippocampal reactivations (Girardeau et al., 2009; Ego-Stengel and Wilson, 2010; de Lavilléon et al., 2015; Grydchin et al., 2020), few researchers tried to follow specific place representations throughout experiments and in their relation to behavior.

Reward-based and aversive spatial learning are similar

Despite the lack of data within the reward place association group, we have been able to show that reward-based and aversive spatial learning are opposite with respect to the behavioral modifications but similar with respect to hippocampal reactivations. While MFB and dlPAG stimulations lead to mirrored behavioral patterns in the UMaze, they both increased SWRs occurrence rate and both resulted in reactivations of co-firing patterns observed during the task.

It was suggested that stronger awake and sleep reactivations of rewarded locations after appetitive learning is a by-product of increased occupancy of rewarded zones: more explored trajectories and locations are replayed the most. We have demonstrated that the locations of the strongest reactivations are located neither in the aversive zone (the most motivationally salient but the least visited), nor in the opposite safe zone (neutral but the most visited). Locations adjacent to the shock zone are mostly represented in reactivation events, however aversive zone reactivations also made significant contribution to replays. Speculatively, these zones could represent not spatial locations but avoidance behavior, similar to the

one observed by Wu et al., 2017. Given the fact that neuronal activity representing locations adjacent to the aversive zone made the strongest contribution to NREM sleep reactivations, we suggest that spatial correlates of behavioral adaptations to the new conditions are reactivated during and after affective learning.

Such interpretation goes against the hypothesis that hippocampus codes for and reactivates purely spatial information. There is ever-growing evidence that hippocampal coding includes other external and internal parameters in addition to space (Aronov et al., 2017; Eichenbaum, 2016; Barron et al., 2020). Moreover, one group of authors has shown that separate subpopulations exist in the CA1 of hippocampus: one represents space as a set of locations and demonstrates strong location-specific firing, and another one represents context as a whole without keeping sharp place-tuning of each individual neuron (Tanaka et al., 2018). Their results suggest that a smaller proportion of hippocampal neurons in CA1 represents broad context-specific experience. Activity of such neurons could become very useful when motivationally salient events happen: upon introduction of new conditions, experience-coding neurons would be able to represent altered circumstances, while keeping general representation of space (reflected in the activity of location-tuned neurons) intact. Theoretically, neurons that are preferentially reactivated during SWRs in affective spatial learning are experience-coding rather than space-coding.

A model of affective spatial learning

To further illustrate this line of thought, we suggest a very simple model, the validity of which can be tested in an experiment (*Fig. 5-14*). Reinforcing stimuli trigger behavioral modifications during learning phase as well as occurrence of SWRs with awake reactivations necessary for early consolidation processes. Contents of these awake reactivations would define future behavioral patterns induced by affective stimuli: we hypothesize that animals would reactivate rewarded zone in case of reward-based learning and zones that are important in the context of fear-related behavior in case of aversive learning. Awake reactivations strengthen functional connections between neurons that participate in them, and partly for that reason the same neuronal patterns are reinstated in NREM sleep following the learning, further continuing memory consolidation processes. After sleep, upon exposure to the same environment, behavior is constrained within the locations that were mostly reactivated during wakefulness and sleep. This model is falsifiable and allows for causal intervention in multiple points to test its validity.

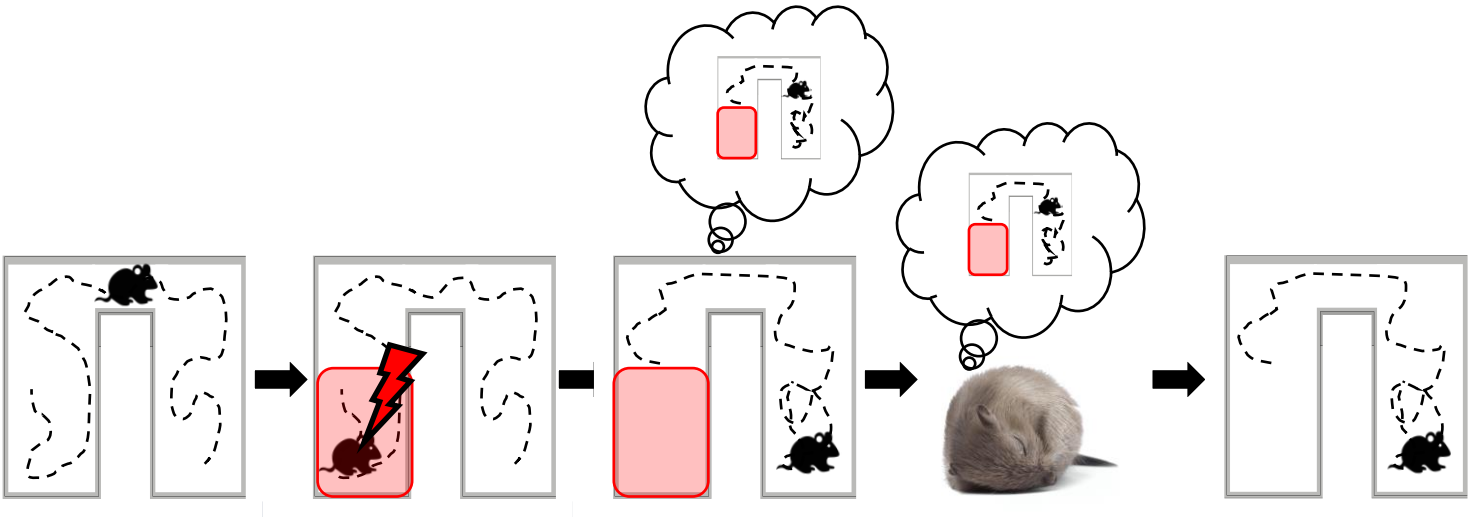

Figure 5-14. A model of affective spatial learning (explained on the example of aversive spatial learning). *Upon receiving motivationally relevant stimulus (fear-inducing stimulation or reward), animals modify their behavior (go to safe zones or to reward zones). This modification represents adaptation to the new conditions in the environment and is accompanied by elevated SWR rate. During awake learning SWRs, reactivations of the newly adapted behaviors are observed (no-go in the shock zone or goal-directed behavior towards reward zone). In the following NREM sleep, animals keep reactivating the same behaviors, further consolidating behavioral adaptations. After sleep, behavior is constrained by the consolidated behavioral patterns which are adaptive if the conditions stay the same.*

In summary, we have shown that, equivalent to the reward-based spatial learning, aversive spatial learning result in NREM sleep reactivations of neuronal activity observed during the task. These sleep reactivations are mostly explained by the neuronal activity during awake SWRs and represents the aversive zone. Strength of reactivations of activity recorded during awake SWRs correlates with the amplitude of avoidance behavior. We hypothesized that, during affective learning, the locations corresponding to fresh behavioral adaptation to reinforcing stimuli are reactivated both during wakefulness and sleep. Trajectories consolidated by means of these reactivations would constrain the following behavior, which leads to behavioral patterns we usually observe after learning. Direct test of this hypothesis will require more experiments, and they would include recording more neurons per animals as well as causal perturbation of hippocampal activity.

Appendix

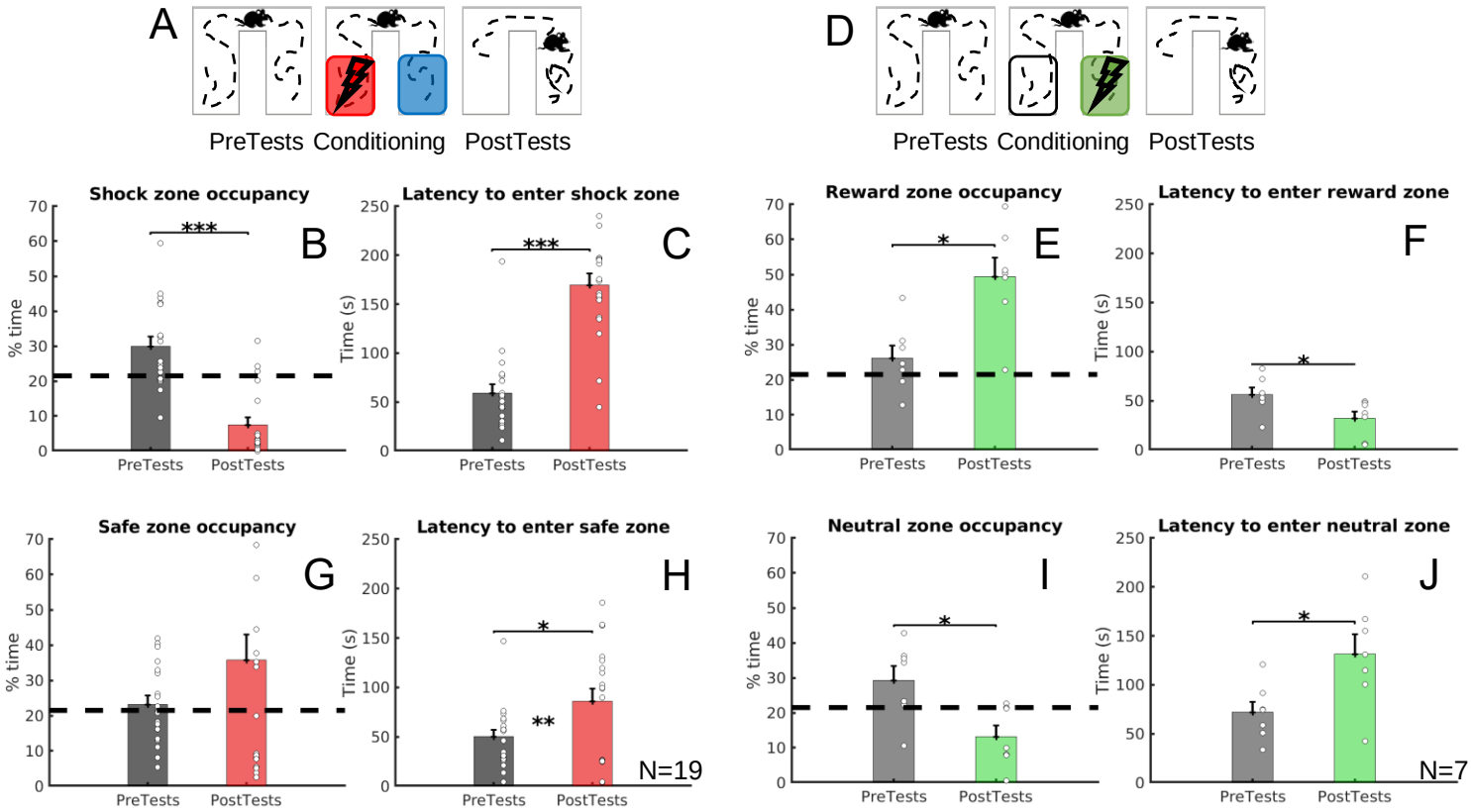

Figure 5-15. Aversive place association protocol and reward-place association result in the opposite behavioral patterns for the stimulation and neutral zone. ***A, D****. Simplified schematics of aversive place association protocol (**A**) and reward-place association protocol (**D**). **B, E.** Occupancy of the shock zone before (PreTests) and after (PostTests) aversive place association learning (**B**) and reward-place association protocol (**E**). **C, F.** Latency to enter the shock zone before (PreTest) and after (PostTests) aversive place association learning (**B**) and reward-place association protocol (**F**). **G, I.** Occupancy of the neutral zone before (PreTests) and after (PostTests) aversive place association learning (**G**) and reward-place association protocol (**I**). **H, J.** Latency to enter the neutral zone before (PreTest) and after (PostTests) aversive place association learning (**H**) and reward-place association protocol (**J**).*

Chapter 6. Results II. Plug-and-play position decoding from high-pass filtered hippocampal activity

Introduction

Firing of place cells recorded from areas CA1 and CA3 of hippocampus is tuned to the current location of an animal (O'Keefe and Dostrovsky, 1971). In a particular environment, each place cell has one or several receptive fields ('place fields') that are stable over long periods of time (Thompson and Best, 1990). However, if an animal is exposed to different environment place fields remap globally (Leutgeb et al., 2005). Activity of place cells is highly organized in temporal domain, both during active exploration when neurons with adjacent receptive fields form theta sequences and during calm states when explored trajectories are 'replayed' (Hartley et al., 2014). Due to the fact that place cell coding unambiguously represents the map of occupied space, it is possible to decode current location of the animal from the activity of place cells with high accuracy. The standard approach to such decoding problem is Bayesian framework that effectively calculates probability of an animal being in certain place given the population vector of neuronal firing (Zhang et al., 1998).

Bayesian approach comes with two heavy assumptions: spikes of an individual neuron is considered to be distributed according to Poisson distribution, and firing of any given neuron is thought to be independent from firing of others. In reality, place cells are much more variable than is expected from Poisson process (Fenton and Muller, 1998; Buzsáki., 2015), and they are not statistically independent from each other as described in vast literature on reactivations and replays (Wilson and McNaughton, 1994; Kudrimoti et al., 1999; Buzsáki, 2015). Thus, despite the fact that Bayesian position decoding demonstrates great accuracy, it is hard to say to which extent its assumptions constrain its performance.

Artificial neural networks (ANNs) are known for their success in learning dependencies within high dimensional data. Moreover, ANNs place no assumptions on data distribution (Yang and Yang, 2014). Recent advances in deep learning and increased accessibility of cheap computing power suggest the use of ANNs as alternative algorithms to decode animal's position in space from electrophysiological signal recorded from hippocampus (Richards et al., 2019). Indeed, a small two-layered recurrent neural network significantly outperformed Bayesian decoder in decoding animal's position from 2D environment (Tampuu et al., 2019). Authors used long short-term memory (LSTM) neural network, which is designed to detect patterns in the data unrolling in time, on large (0.2 – 4 s) chunks of pre-sorted single units' activity. Important insight of this study is that LSTM layers can be successful in parsing temporally organized neural data from hippocampus. However, use of sorted spikes as input data remarkably inflates the time needed to do an iteration of the experiment, and also introduces human-related biases in the pipeline. This drawback was overcome in different report where convolutional neural network (CNN) applied to morlet wavelets computed from raw electrophysiological data was utilized for successful decoding of animal's position (Frey et al., 2019).

Decoding algorithms are often applied to closed-loop systems, in which speed of computation is of crucial importance (Brumberg et al., 2010; Ciliberi et al., 2018). Bayesian decoder was successfully adapted for online use (Ciliberti et al., 2018), but published ANN-based position decoders demands large amount of time to prepare input data. While decoding of current

position of animal during active behavior places relaxed demands on speed of the algorithm, to decode contents of replays, online ANN-based position decoder should be able to make inference using short (20-80 ms) time windows of data. On such a short timescale, human treatment of the input data becomes impossible, and time needed for decoding should be reduced to minimum.

In this study, we have tested the ANN-based position decoder that comprises CNN layers to extract features from raw electrophysiological signal and LSTM layers to learn a link between temporally organized hippocampal spikes and position of an animal. In addition, our algorithm utilizes Bayesian neural network approach to construct probability distribution across possible position that can be used as a confidence handle during decoding. We have been able to show that algorithm shows lower error on time bins decoded with higher confidence compared to time windows with lower confidence. Moreover, we demonstrated in this report that suggested architecture decodes with high accuracy both on large (> 200 ms) and short (36 ms) time windows. In a closed-loop decoding system, our algorithm decoded position with the roundtrip latency of less than 72 ms.

On a more general scale, our approach to decoding of animal's position can provide a framework to decode any variable of interest encoded in spiking neuronal data without placing any assumptions on the input data. Importantly, it does not require human treatment of the neural signal and can be used in closed-loop systems that demand low roundtrip latencies.

Results

To test our architecture (see *Methods* section), we used the data obtained from a mouse implanted with one 4-shank silicon probe in the area CA1 of hippocampus. Raw electrophysiological signals (64 channels, 20 kHz sampling rate) were recorded while the animal was freely exploring the U-shaped maze. These raw data were fed into the ANN-based position decoder that comprises convolutional and LSTM layers (*Fig. 6-1*). We compared performance of the ANN-based decoder with a classical memoryless Bayesian decoder that takes sorted spikes as inputs and uses historical exploration distribution as a prior. Real and inferred coordinates were linearized for the representation of results unless the contrary is explicitly claimed.

ANN-based decoder decoded position with higher accuracy than Bayesian decoder on all tested time windows (see *Tables 6-2–6-5 in appendix and Fig. 6-2–6-4*). Predictions from two decoders overlapped (*Fig. 6-5*); however, they overlapped more at longer time windows used for decoding (probably, due to poor performance of Bayesian decoder at short time scale), and they overlapped less at points decoded when the animal was immobile or moved at low speed compared to high speed (speculatively, due to the fact that two algorithms predict reactivations differently).

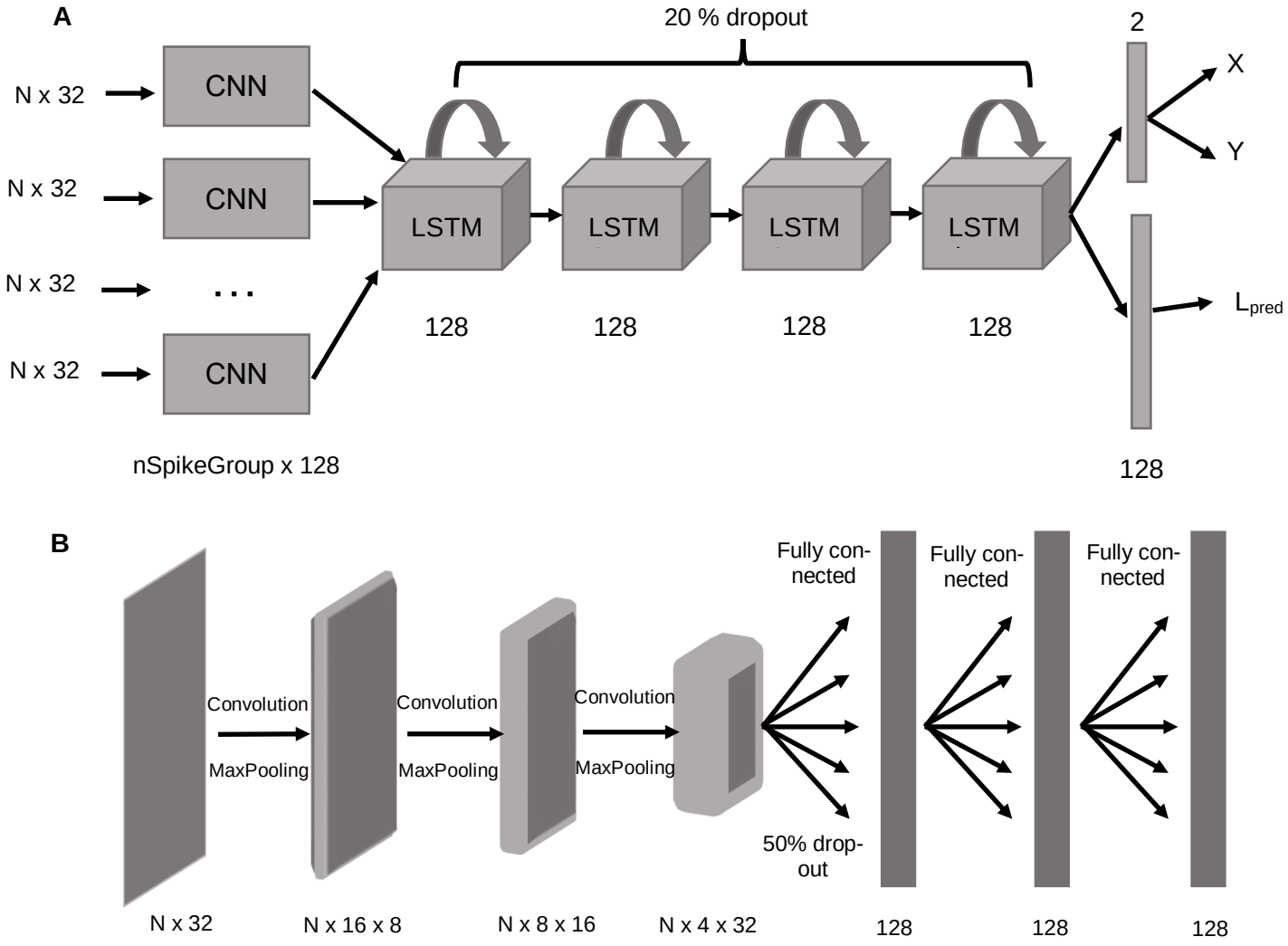

Figure 6-1. Architecture of position decoder artificial neural network used in the study.

A. *Waveforms from N channels that compose one spike group are sent into separate convolutional network, each of which outputs 128 units. These units are pooled to four consecutive LSTM layer are applied to them. The last layer is connected to a dense layer which outputs 2 values: X and Y coordinate of an animal.* ***B.*** *Architecture of the CNN. Waveforms from N channels that compose one spike group are sent into 3 consecutive convolutional layers with kernel size of (2,3), and then into three dense layers of 128 units each.*

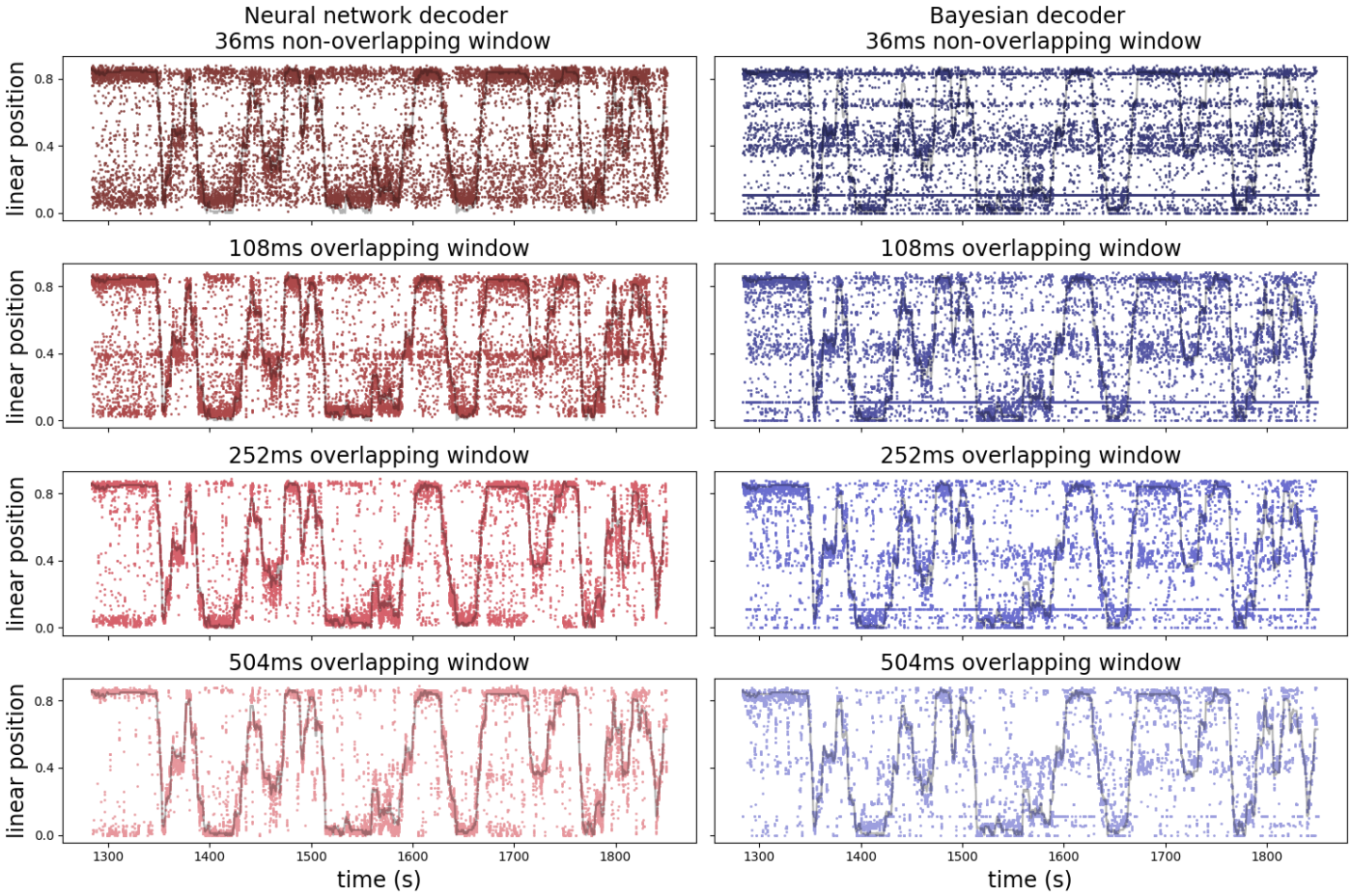

Figure 6-2. Example of inference using both the ANN-based decoder and Bayesian decoder on the linearized Euclidian coordinates. ***Left.*** *Performance of the ANN-based decoder.* ***Right.*** *Performance of Bayesian decoder. Each line represents a particular time window that was used to perform decoding: from top to bottom – 36, 108, 252 and 504 ms.*

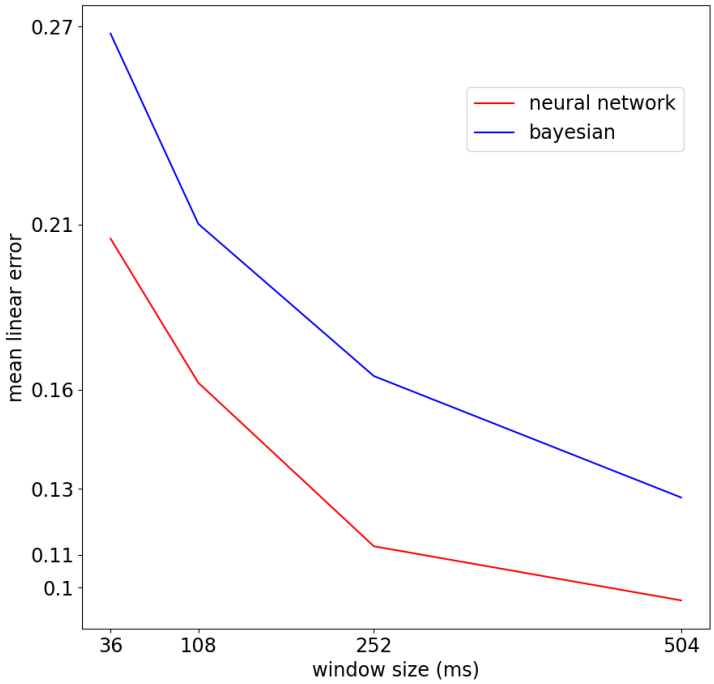

Figure 6-3. Mean linear error for different decoding window sizes both the ANN-based decoder and Bayesian decoder. *ANN-based decoder outperforms Bayesian decoder on all tested window sizes.*

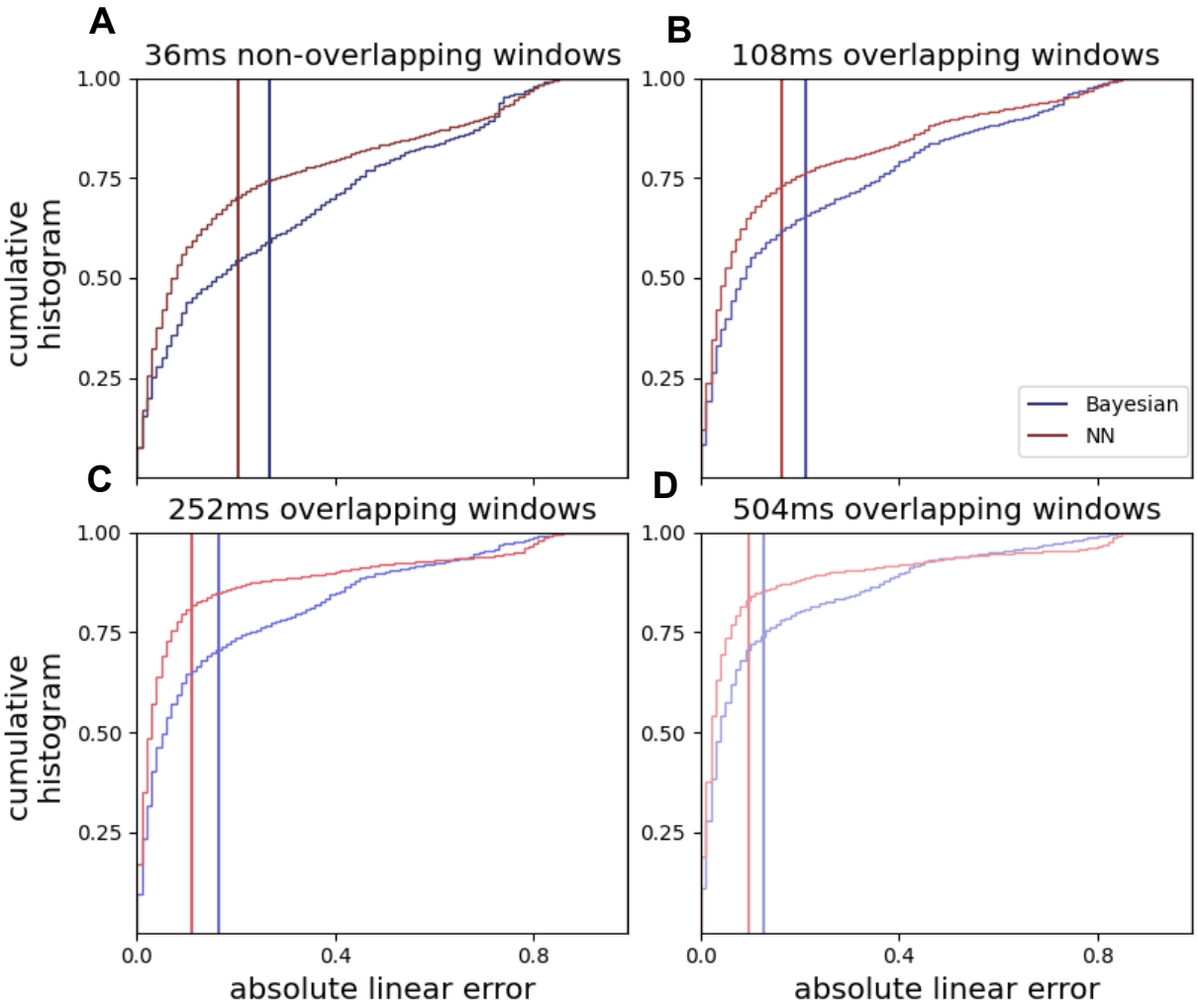

Figure 6-4. Cumulative histograms of linear error for both ANN-based and Bayesian decoder at different time windows. *Vertical lines indicate mean linear error for respective decoder and window size.* ***A.*** *Distribution of the linear error for 36 ms-long windows.* ***B.*** *Distribution of the linear error for 108 ms-long windows.* ***C.*** *Distribution of the linear error for 256 ms-long windows.* ***D.*** *Distribution of the linear error for 504 ms-long windows.* ***E.*** *Mean linear error on the full test dataset.*

In addition to predicting the position, we constructed the ANN-based decoder to minimize distance between real loss function and Euclidean error. Output value, which was obtained on every time step using such loss function, was called '**Predicted loss'**. Predicted loss is low when position loss and real Euclidean error are similar, and high otherwise. Therefore, the value of predicted loss could be used as a 'confidence' measure of prediction on every time step.

Predicted loss was inhomogeneously distributed across positions of the maze (*Fig. 6A*). Most likely, this is due to the fact that recorded place cells did not cover the whole maze evenly (*Fig. 6B*). Thus, behavior of the 'confidence' measure follows the expected pattern: with low amount of evidence, we expect lower confidence (higher predicted loss, in our case).

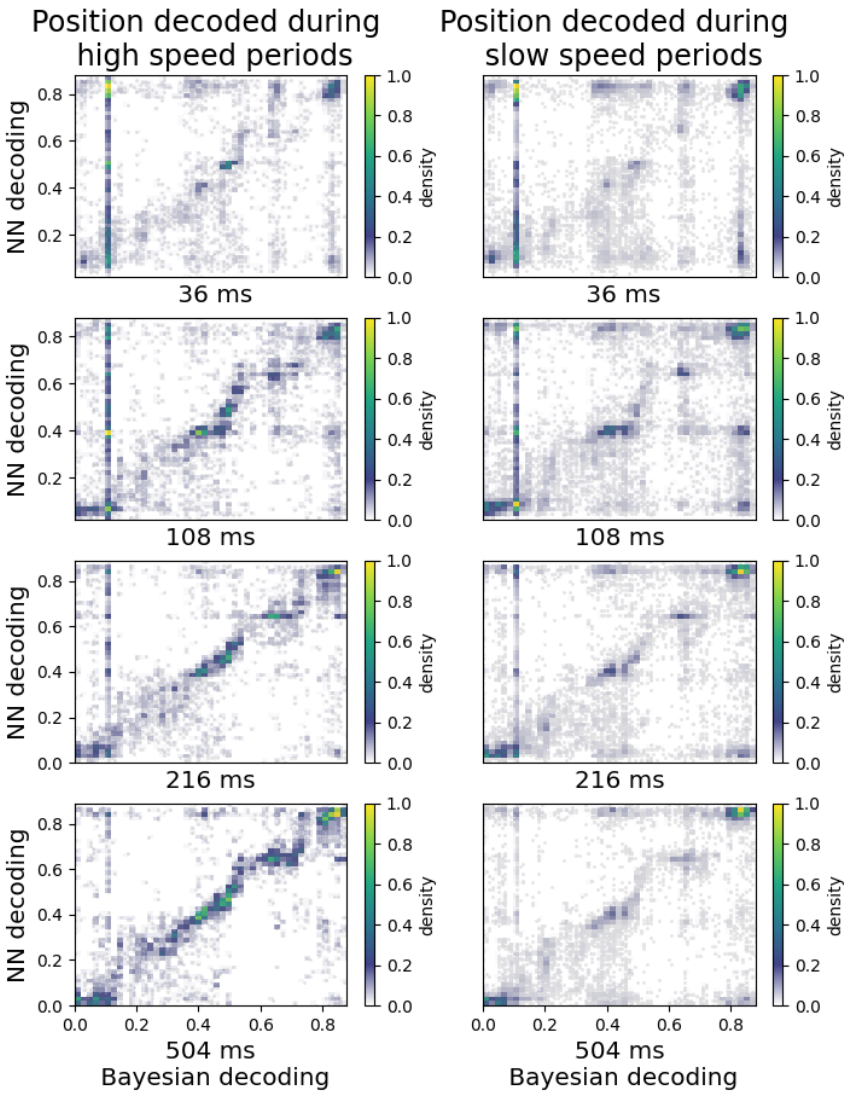

Figure 6-5. Overlap between predictions of the ANN-based and Bayesian decoders. *Window-to-window correspondence of predictions for ANN-based decoder and Bayesian decoder.* ***Left****: points decoded during periods of high speed.* ***Right****: points decoded during period of low speed. Every row represents decoding with a particular window size.*

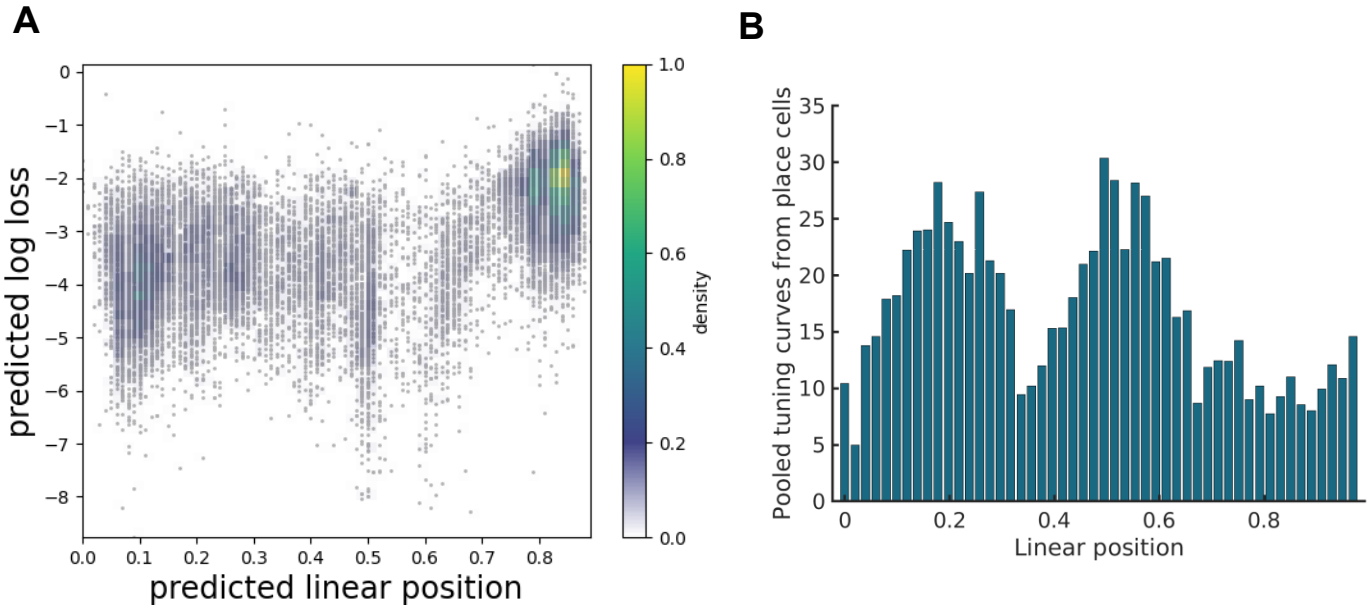

Figure 6-6. Predicted loss depends on linear position in the same manner as the tuning curves of place cells. Decoder was trained on 36 ms-long windows. ***A.*** *Predicted loss distributed unevenly across position.* ***B.*** *Tuning curve of all place cells pooled together.* ***Note that positions with less accurate spatial representation exhibit higher predicted loss.***

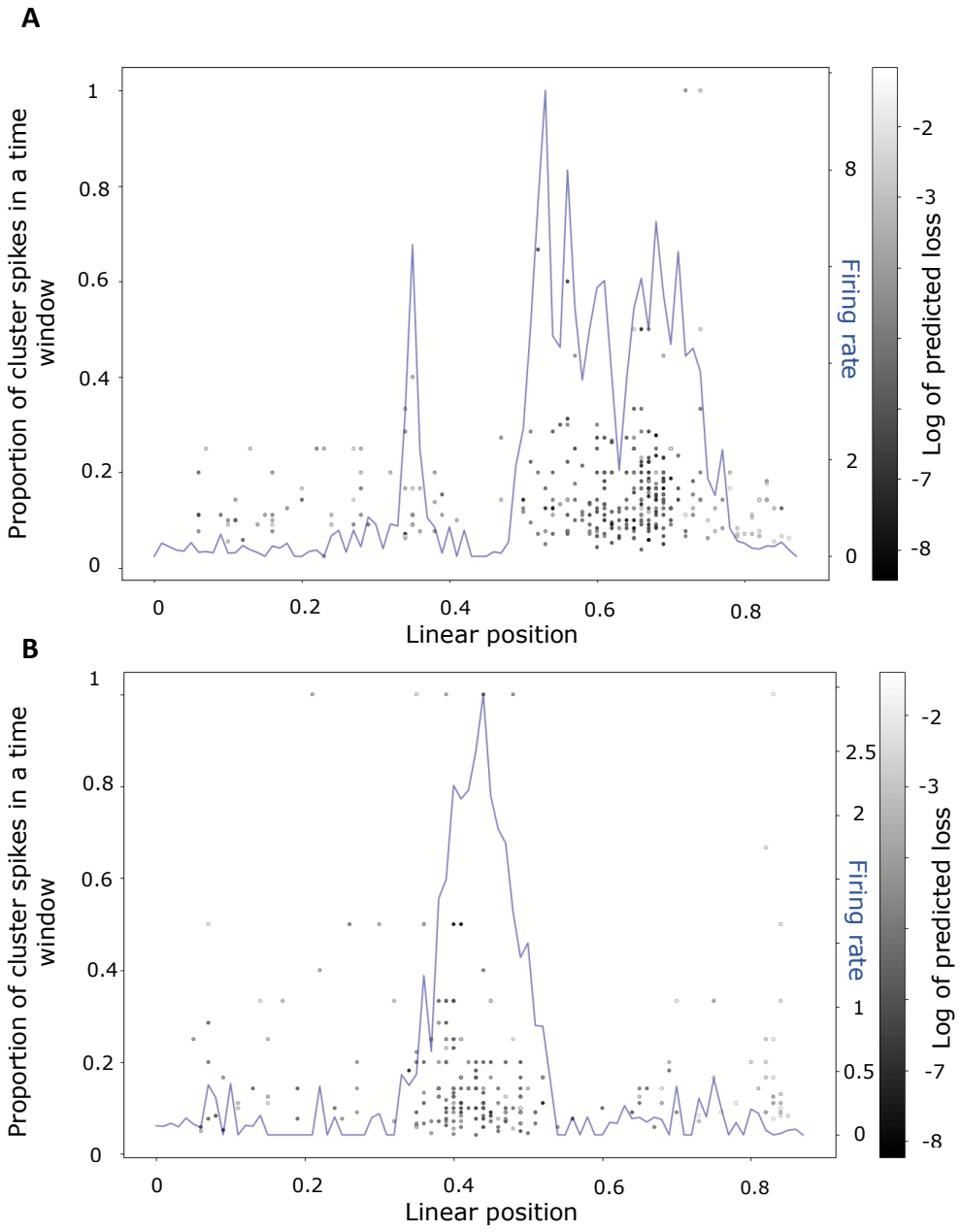

Figure 6-7. Predictions that coincide with firing of place cells are concentrated in the place field. ***A and B***. *Two example place cells.*

Does that mean that the ANN-based decoder used information contained in the firing of place cells? Indeed, positions inferred within the time windows when sharply tuned place cells fired spikes concentrated strongly in the place fields (*Fig. 6-7*).

Predicted loss value can be used to filter out inaccurate predictions. Indeed, accuracy calculated on the points that correspond to the lowest 30% of predicted loss distribution is around 2 times lower the accuracy calculated on the full dataset (*Table 6-1; Fig. 6-8*). Decoder that used 36 ms-long time windows demonstrated increased linear error at the lowest predicted loss (*Fig. 6-9*) – however, the error still remained at very low levels. It can be explained by the fact that there are less than 50 points that are decoded with the predicted loss less than 7 – and outliers start to have large influence on the results.

	Full dataset		**High speed filtered dataset**	
Mean liner error: window size	All points	Points of lowest 30% predicted loss	All points	Points of lowest 30% predicted loss
36 ms	0.21	0.1	0.16	0.07
108 ms	0.16	0.08	0.11	0.06
252 ms	0.11	0.08	0.07	0.05
504 ms	0.1	0.06	0.06	0.04

Table 6-1. Accuracy of the ANN-based decoder filtered by low predicted loss values.

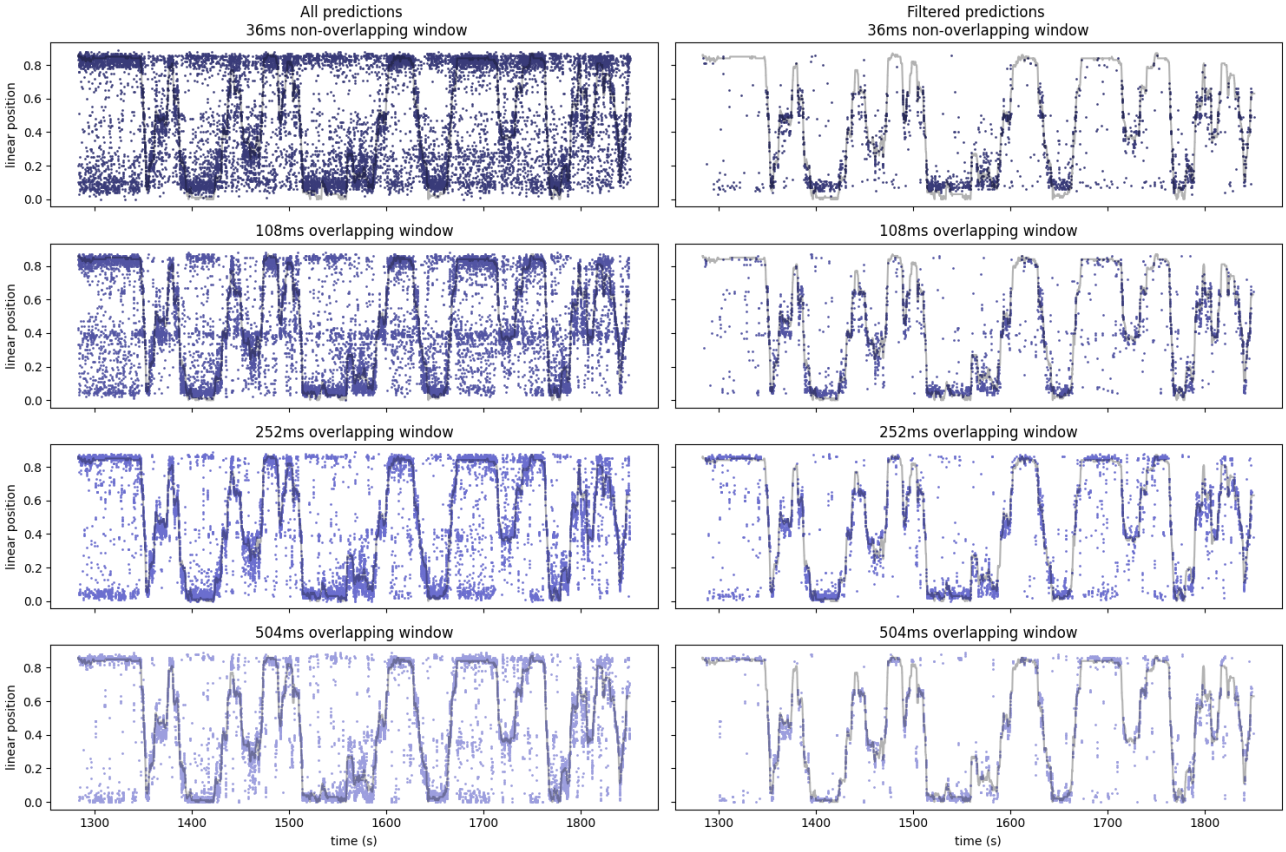

Figure 6-8. Example of points decoded with the ANN-based decoder: either all decoded points or only points decoded with high confidence. ***Left.*** *Performance of the ANN-based decoder.* ***Right.*** *Performance of the ANN-based decoder filtered by the 30% lowest predicted loss*

values. Each line represents a particular time window that was used to perform decoding: from top to bottom – 36, 108, 252 and 504 ms.

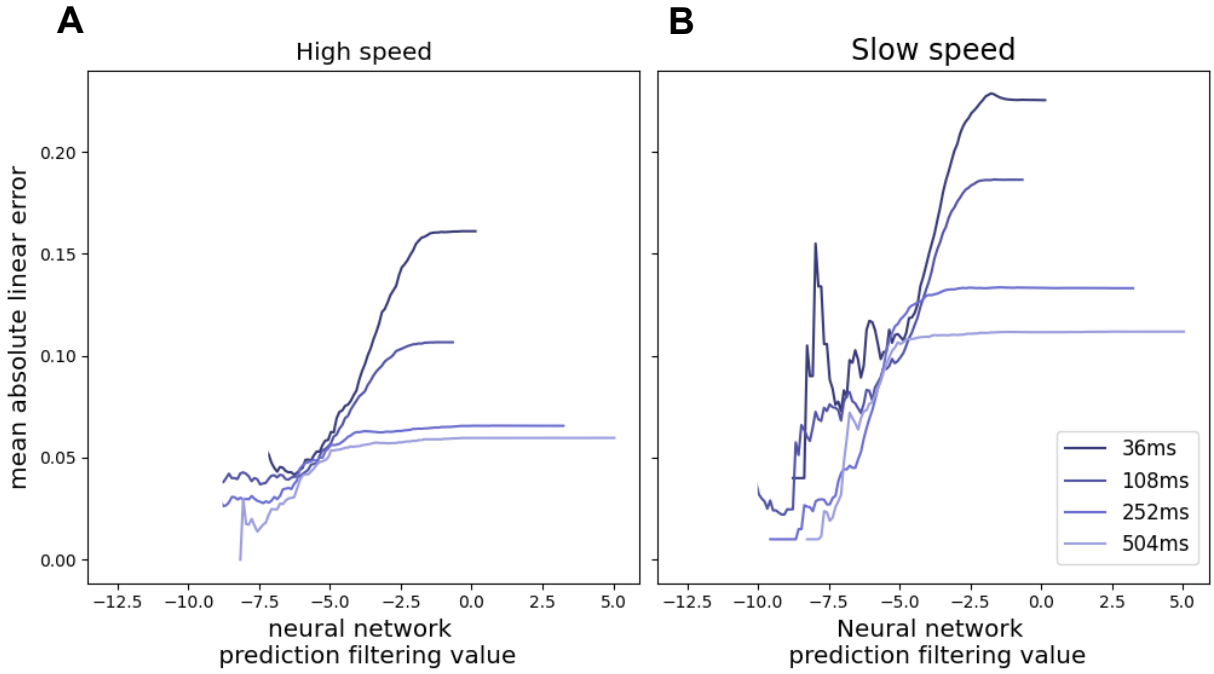

Figure 6-9. Mean linear error drops dramatically if we take only low entropy predictions. ***A.*** *Mean linear error on the points with high speed if we filter out all values with predicted loss higher than filtering value.* ***B.*** *Mean linear error on the points with slow speed if we filter out all values with predicted loss higher than filtering value.*

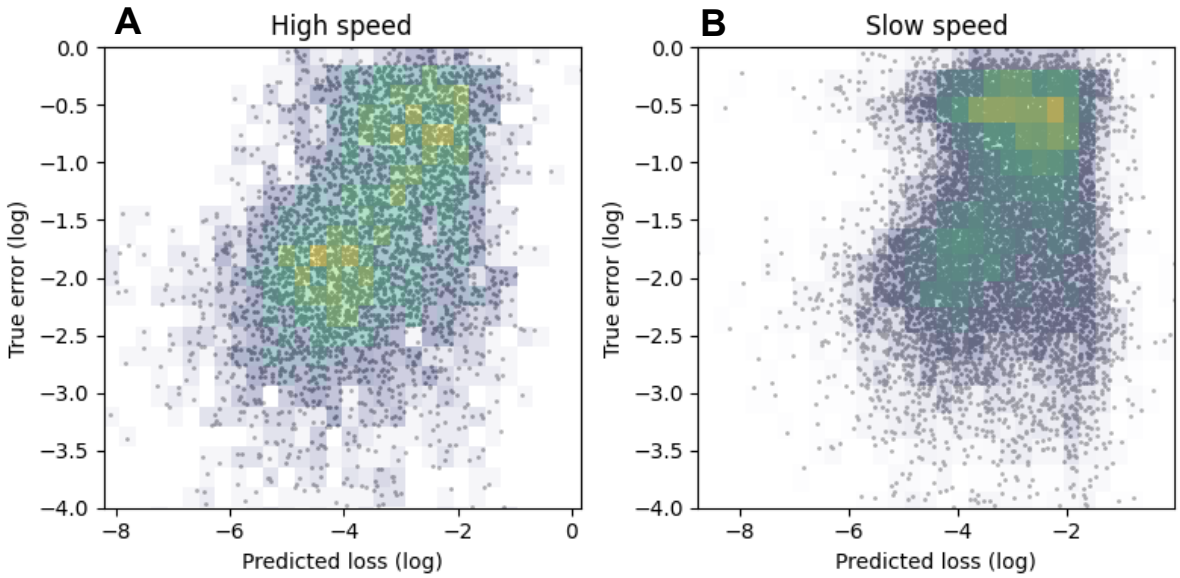

Figure 6-10. Predicted loss and true linear error on the testing set. *During periods of high speed (**A**), points decoded with low predicted loss tend to have low linear error. In contrast to them, points with high predicted loss tend to have high error. For low speed periods such dependence is much less clear.*

During periods of high speed (when neuronal firing strongly predicts the position of the animal) predicted loss and linear error tend to form two clouds: one with low predicted loss and low predicted error and one with high predicted loss and high predicted error (*Fig. 6-10*). Thus, predicted loss measure, in our opinion, can be used as a confidence handle to filter out spurious predictions during online decoding.

The ANN-based decoder presented here was designed as a tool for online decoding of hippocampal reactivations during quiet wakefulness and sleep in closed-loop experiments. Unfortunately, no ground truth (except cases with explicit data simulations) exists for determining time and contents of hippocampal reactivations as all detection techniques have their flaws (Tingley and Peyrache, 2020). To confirm validity of the ANN-based decoding for the inference of reactivations contents we performed an indirect check.

It is widely accepted that majority of reactivations occur during sharp-wave ripples (SWRs) – fast oscillatory event that can be recorded from the hippocampal CA1 and that coincides with massive excitation of pyramidal cells (Buzsáki, 2015). If neuronal activity during SWRs represents locations in space better than surrounding signal, positions inferred during SWRs should correspond to lower predicted loss. We have found exactly the effect we were looking for (*Fig. 6-11*). This result points in the direction that our ANN-based decoder can be used to decode reactivations.

To confirm that the proposed algorithm can be used in the online experiment, we have recorded the time between the end of the decoded time window and the stimulation event that was triggered by the decoder (or a round-trip latency – *Fig. 6-12*). Among 1219 stimulations made, 48% happened in the next 36 ms after decoding took place, 82% were found within 50 ms and 99% fell into 72 ms period (two time windows after decoding). Arguably, this is acceptable for online experiment involving reactivations.

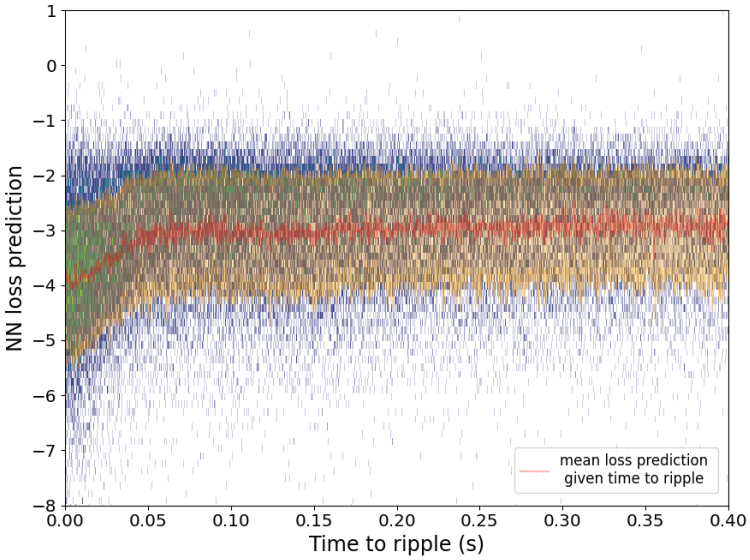

Figure 6-11. Time windows containing sharp-wave ripples are predicted with lower predicted loss than other time windows.

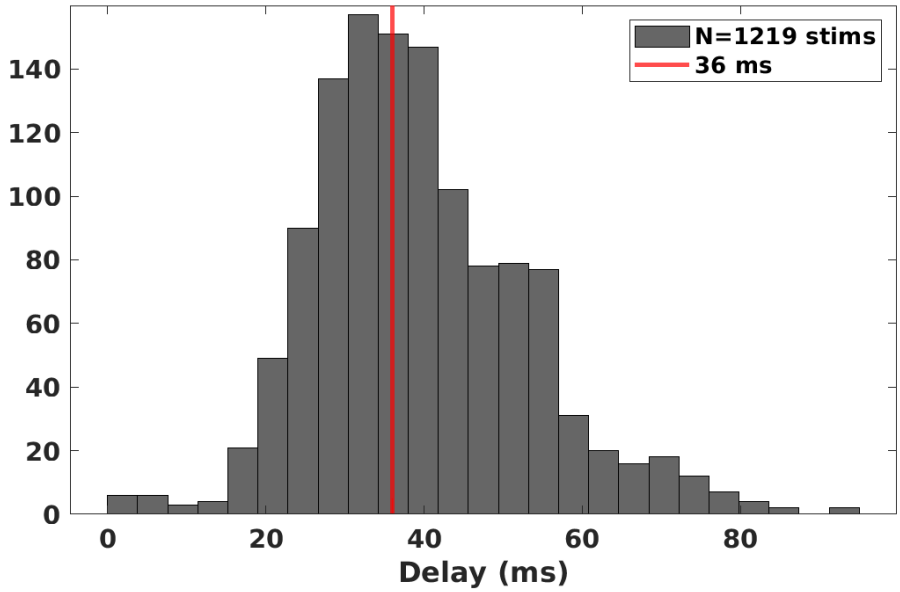

Figure 6-12. Distribution of round-trip latencies (time between position decoding and intracranial stimulation) in the closed-loop set-up. *Window size is 36 ms. 99% of stimulations happened within the first 2 time windows after detection (72 ms).*

Discussion

We have successfully validated our approach to position decoding that requires no manual treatment of input data and that can be used in closed-loop experiments to decode fast reactivation events. We based our decoder on artificial neural networks: more specifically, the stack of convolutional layers to extract features from raw electrophysiological signal and LSTM layers, a subclass of recurrent neural networks, to decode position of the animal. It has been shown that the ANN-based decoder largely outperforms Bayesian decoder on both ultra-short and large time windows.

Similar to the first published ANN-based decoder (Tampuu et al., 2019), we used LSTM layers as the core learning algorithm. During exploratory periods, activity of hippocampal neurons is organized into sequences by theta oscillations (Wilson and McNaughton, 1993), and these sequences are replayed later in the quiet period (Lee and Wilson, 2002; Pfeiffer and Foster, 2013). LSTM was explicitly designed to detect temporal dependencies in the data (Hochreiter and Schmidhuber, 1997) – therefore, the choice of LSTM was natural for the inference of position from hippocampal neural recordings. In our study, we confirmed that LSTMs can be successfully used to decode position of animals from the hippocampal activity. Moreover, we have also discovered that LSTMs can accurately infer position if trained on ultra-short time windows (36 ms).

Tampuu and colleagues have used sorted spikes as an input to their decoder. Spike sorting is known as a very time-consuming procedure as well as the procedure that can po-

tentially confound dataset due to human restrictive intervention. We used convolutional layers to extract (most likely) meaningful patterns from raw spike-like data, treating each of them as an image. However, we did not train our CNNs to decode animal's position, as another study did (Frey et al., 2019). In the future, we would like to examine the output of the CNN network for better understanding of the data that serve as an input to LSTM layers.

In addition, we have established a single-value metrics that can be used as a confidence handle to filter out spuriously decoded positions. Indeed, time windows decoded with higher confidence demonstrated lower error. Using confidence as a filter, the user can significantly (2-fold on the tested dataset) increase accuracy of decoding. Another advantage of this metrics is that it comes 'for free', i.e. it is fully integrated in the decoder and requires no external calculations to be performed. Confidence measure can be used, for example, to decide whether to apply stimulation in a closed-loop experiment or not.

We have demonstrated in online experiment that decoder can be used to guide stimulations with the round-trip latencies less than 72 ms. Importantly, only 18% of stimulations triggered by decoding algorithm occurred later than 50 ms. Arguably, such set-up is fast enough to influence hippocampal networks at the time of reactivations.

There are several directions we have not undertaken yet. First, we report in detail results obtained only on one dataset. To fully validate the algorithm, we will apply the decoder to several different datasets as well as perform several-fold cross validations on each of them. Second, we do not report here the results of sleep reactivations decoding. This is mainly due to the fact that electrodes have moved during sleep in the dataset in question, and we need additional time to filter out potentially spurious results. Third, we have not investigated how robust the predictions are to the decreasing number of place cells. How many place cells does the ANN-based decoder need to accurately decode the position?

The presented ANN-based decoding algorithm was created to perform intracranial stimulations triggered by reactivation of the specific place cells ensembles in online closed-loop experiment. The results reported above ignite confidence that such highly complex and ambitious project can be successfully performed.

Appendix

36 ms

	ANN-based decoder			Bayesian decoder		
	Full dataset	High speed	Low speed	Full dataset	High speed	Low speed
Mean linear error	0.21	0.16	0.23	0.27	0.25	0.28
Median linear error	0.08	0.07	0.08	0.17	0.15	0.18
Mean Euclidean error	0.31	0.28	0.33	0.42	0.4	0.42
Median Euclidean error	0.24	0.2	0.26	0.37	0.34	0.38

Table 6-2. Mean and median accuracy for the ANN-based and Bayesian position decoder decoded on 36 ms bins.

108 ms

	ANN-based decoder			Bayesian decoder		
	Full dataset	High speed	Low speed	Full dataset	High speed	Low speed
Mean linear error	0.16	0.11	0.19	0.21	0.19	0.22
Median linear error	0.05	0.05	0.06	0.09	0.08	0.09
Mean Euclidean error	0.28	0.22	0.31	0.35	0.32	0.37
Median Euclidean error	0.17	0.14	0.2	0.27	0.23	0.29

Table 6-3. Mean and median accuracy for the ANN-based and Bayesian position decoder decoded on 108 ms bins.

252 ms

	ANN-based decoder			Bayesian decoder		
	Full dataset	High speed	Low speed	Full dataset	High speed	Low speed
Mean linear error	0.11	0.07	0.13	0.16	0.14	0.18
Median linear error	0.03	0.03	0.03	0.06	0.05	0.06
Mean Euclidean error	0.2	0.15	0.22	0.31	0.26	0.33
Median Euclidean error	0.12	0.1	0.13	0.2	0.18	0.22

Table 6-4. Mean and median accuracy for the ANN-based and Bayesian position decoder decoded on 252 ms bins.

504 ms

	ANN-based decoder			Bayesian decoder		
	Full dataset	High speed	Low speed	Full dataset	High speed	Low speed
Mean linear error	0.1	0.06	0.11	0.12	0.1	0.14
Median linear error	0.03	0.02	0.03	0.04	0.04	0.04
Mean Euclidean error	0.18	0.13	0.19	0.26	0.21	0.28
Median Euclidean error	0.1	0.08	0.11	0.16	0.13	0.18

Table 6-5. Mean and median accuracy for the ANN-based and Bayesian position decoder decoded on 504 ms bins.

Chapter 7. Results III. Freezing neurons in hippocampus and counter-conditioning

Freezing cells in hippocampus

Unlike neurons of ventral hippocampus, neurons of dorsal hippocampus are rarely observed to play roles outside spatial navigation and memory. In one such paper, very small subpopulation of dorsal hippocampus neurons that respond selectively to primary reward was identified (Gauthier and Tank, 2018). To our knowledge, there are no studies showing consistent response of hippocampal neurons to aversive stimuli but hippocampal neurons were found to have link with one of the most studied fear-related behavior, freezing (Schuette et al., 2020). Authors have shown using Ca2+-imaging that a group of neurons in dorsal hippocampus consistently change their firing during periods of time when mice froze. Interestingly, a group of freezing cells did not overlap a lot with a group of place cells.

In this short preliminary report, we would like to demonstrate using electrophysiological methods that, indeed, there are neurons in dorsal hippocampus, the firing of which is modulated by freezing behavior.

Results

We have recorded 737 neurons in the CA1 of dorsal hippocampus of mice during freezing behavior. Freezing occurred after intracranial stimulation of dorsolateral periaqueductal gray matter (dlPAG) in the U-shaped maze (see methods). In this task, animals received stimulation once they entered specific 'shock' zone in the maze. Stimulation of dlPAG leads to strong escape reaction and terminates with freezing periods – due to stereotypical behavioral pattern and the shape of the UMaze, mice rarely froze in the location where they have been stimulated.

Hippocampal neurons were differently modulated by freezing behavior (*Fig. S1A*). We have split neurons in three groups according to their response during freezing behavior: OFF-cells (N=90), cells not affected by freezing (N=547) and ON-cells (N=100) (*Fig. S1A, C*). Principal component analysis revealed that 11.9% is explained by the component that show strong modulation of firing during freezing (*Fig. S1D*). Interestingly, neurons that decrease their firing rate during freezing have larger firing rate and larger proportion of interneurons (*Fig. S1E, F*). Indeed, the majority of recorded interneurons (60%) are found in the OFF neurons group. Speculatively, these fast-spiking interneurons could disinhibit principal cells that we see in the ON group during freezing.

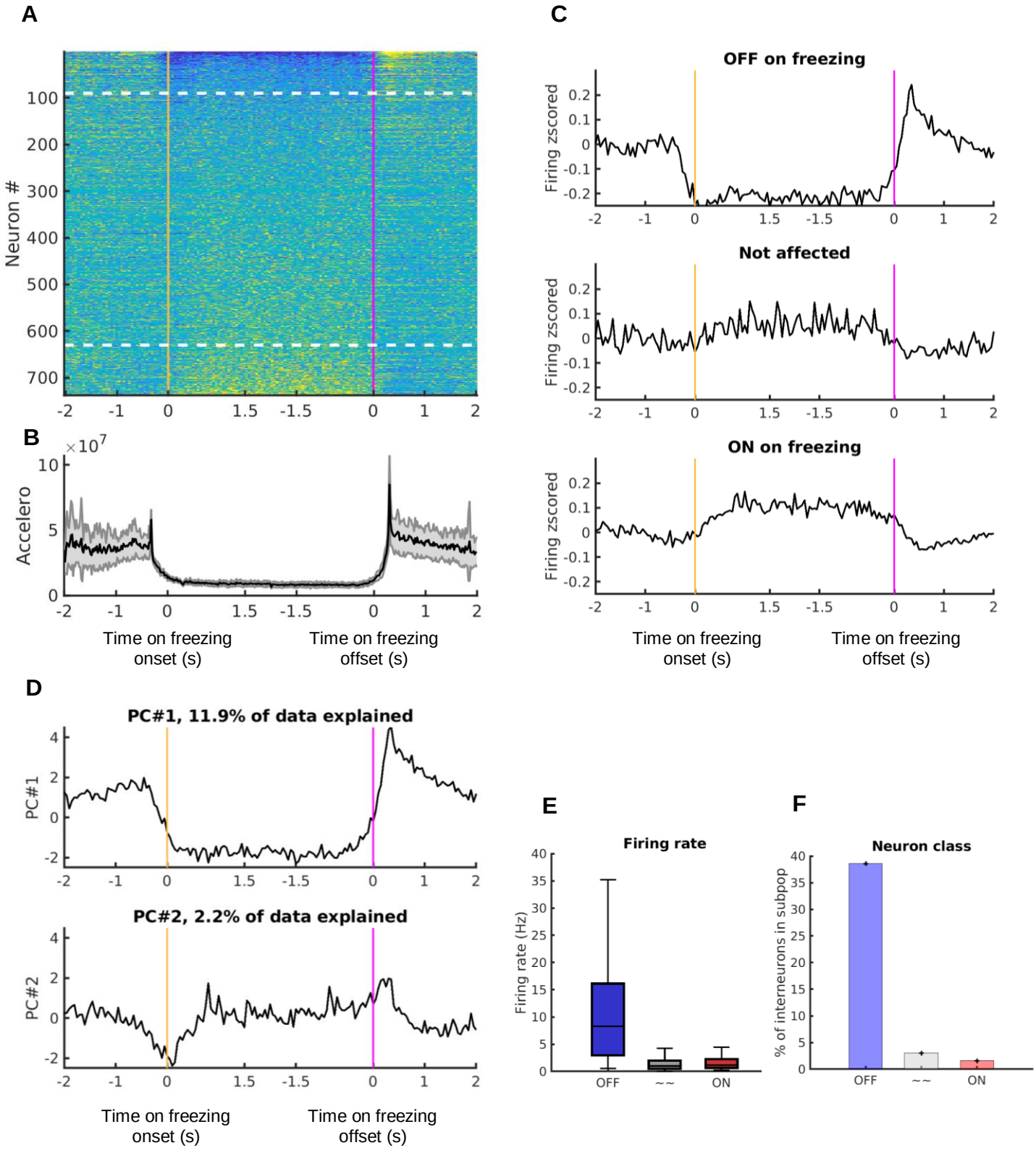

Figure S1. Neurons in the CA1 of dorsal hippocampus are differently modulated by freezing behavior. ***A.*** *Peri-event time histogram triggered on the onset (orange line) and the offset (purple line) of freezing periods. White dashed lines delineate three groups of neurons according to their firing rate during freezing.* ***B.*** *Average accelerometer trace during freezing periods.* ***C.*** *Average firing rate (z-scored) of three groups of neurons: neurons that decrease*

their firing during freezing (OFF), neurons that are modulated by freezing behavior (Not affected) and neurons that increase their firing during freezing (ON). ***D.*** *First two principal components calculated on the z-scored firing rates of hippocampal neurons during freezing.* ***E.*** *Firing rate of neurons in three groups of neurons.* ***F.*** *Percent of interneurons in each group of neurons*

Discussion

We have discovered that small subpopulation of neurons strongly decreases its firing rate during freezing behavior. Another neuronal group, which slightly, increased its firing was also identified, however net modulation effect seems to be weaker than for down-modulated group. This result confirms initial finding that was made independently in different research group using Ca^{2+}-imaging (Schuette et al., 2020). In their study, animals had visual contact with the place where shock was delivered. In the present study, mice mostly in the locations not only outside of the shock zone but without any visual contact with the aversive place (*see Fig. 5-1 in chapter 5*).

Moreover, we have found that neurons that decrease their firing during freezing have remarkably larger proportion of interneurons (39%) than the group average (6-7%). the majority of recorded interneurons (60%) are included in the down-modulated subpopulation. To our knowledge, this is a completely novel result: **hippocampal interneurons decrease their firing rate during fear-related behavior of freezing.**

It is very unlikely that firing rate reduction of dorsal hippocampus interneurons is necessary or required for expression of freezing behavior. Fast spiking interneurons in hippocampus are known to play an important role in regulating oscillatory dynamics in hippocampus (which in turn very much related to spatial navigation and memory function. – see *chapter 1*) as well as restraining firing rate of principal cells (Buzsáki, 2015; Pelkey et al., 2017). Speculatively, decreased firing rate of interneurons during freezing leads to disinhibition of principal cells (which we see in the ON group of neurons).

In chapter 5 (Fig. 5-7), we have demonstrated that freezing behavior is characterized by elevated rate of sharp-wave ripple occurrence. Decreased tonus of fast spiking interneurons could, in theory, modulate strength and rate of reactivation activity - however, the exact link between reactivations during freezing, sharp-wave ripples and interneurons remains to be unraveled.

Counterconditioning using intracranial stimulation during wakefulness

Recently, appetitive association with a previously neutral location was created during sleep by pairing hippocampal place cell reactivations with intracranial rewarding stimulations suggesting that sleep reinstatement of neural patterns that were active during wakefulness can be used for learning (de Lavilléon et al., 2015**).** Could one use the same technique but applied on an aversive association to perform counterconditioning during sleep? Treatment of persistent maladaptive aversive associations is the main focus in such psychiatric conditions as post-traumatic stress disorder (PTSD) or anxiety disorder. Sleep counterconditioning is one of the promising methods that was already tested in humans (Arzi et al., 2014). We aimed to refine the idea by identifying reactivations of a particular aversive memory and trying to neutralize it with intracranial rewarding stimulation.

In a preparatory phase to this extremely ambitious experiment, we developed a position decoding tool based on deep learning to effectively identify hippocampal reactivations (*see chapter 6*). In addition, we performed a counterconditioning experiment during wakefulness to confirm a fundamental possibility of counterconditioning using intracranial stimulation. We deployed place aversion and place preference caused by intracranial rewarding medial forebrain bundle (MFB) and aversive dorsolateral periaqueductal gray matter (dlPAG) stimulation to demonstrate counterconditioning effect.

Results

Ten mice were included in the protocol: five of them were assigned to experimental group and five of them were assigned in the sham group. In the sham group, rewarding stimulation was not given. Essentially, sham group has undergone extinction protocol after aversive learning – exposure to aversive location without negative reinforcement. Would counterconditioning procedure be more effective in reversing aversive association than extinction?

Experiments were performed in the U-shaped maze (UMaze – see methods). Full protocol of the experiment is shown on *Fig. S2*. We used a variation of place preference and place aversion protocols described in details in *chapter 5:* intracranial stimulations of medial forebrain bundle (MFB) and periaqueductal gray matter (dlPAG) were used as rewarding and aversive stimulation, respectively. To ensure that animals get minimal number of stimulations of each type, we blocked animals in the stimulation zone for short periods of time. During blocking periods, animals received a constant number of stimulations.

Aversive place learning resulted in strong avoidance of the stimulation zone (*Fig. S3*), similar to what we have observed in a stand-alone place aversion protocol (*chapter 5*). Sham group demonstrated slightly weaker avoidance in behavior in the tests after aversive learning (Fig. S4), probably due to the quality of implantation in sham group. However, occupancy of the stimulation zone after mice has undergone conditioning drastically increased whereas mice from the sham group almost did not elevated their time in the shock zone.

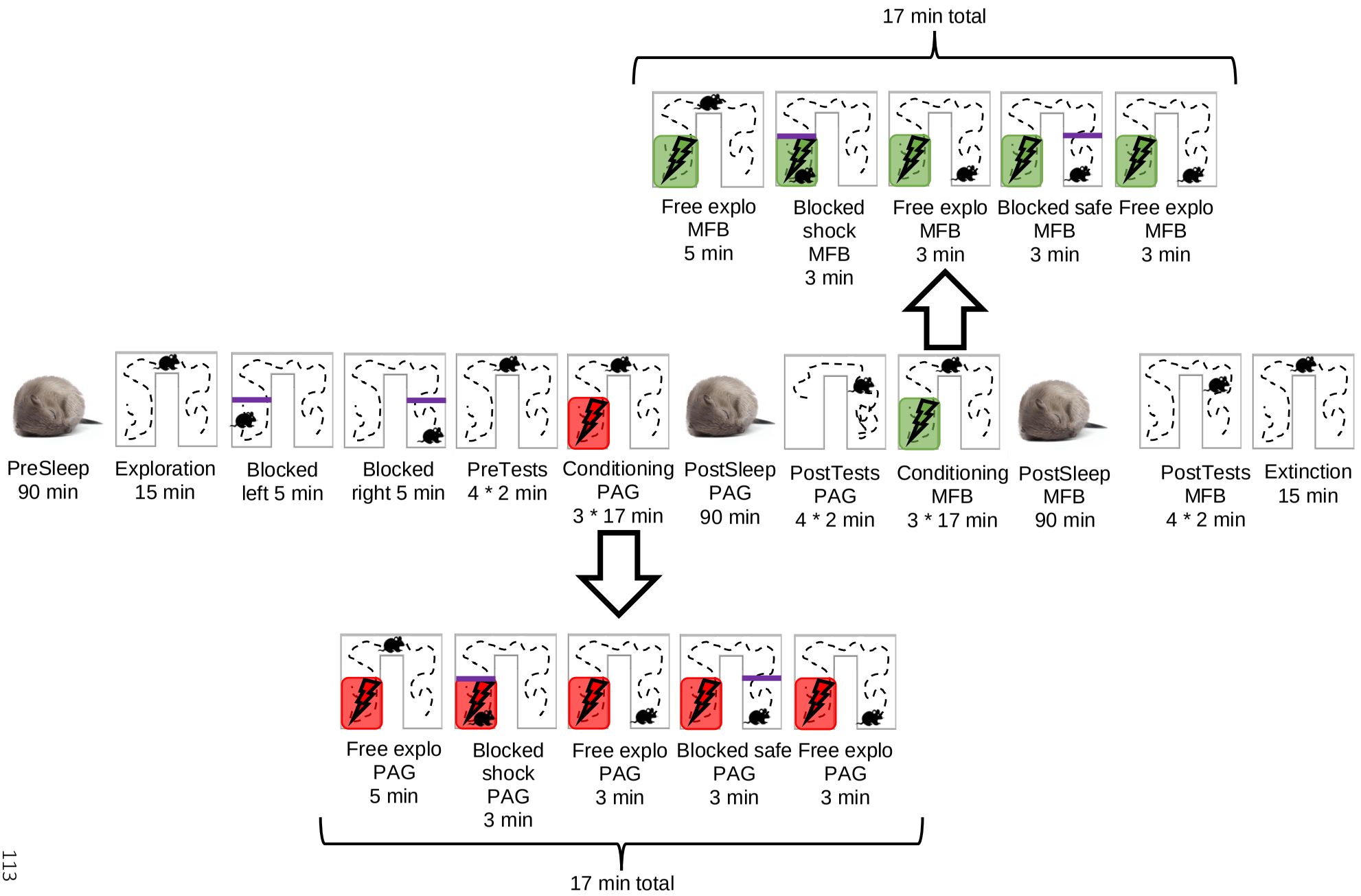
17 min total
Free explo MFB 5 min
Blocked shock MFB 3 min
Free explo MFB 3 min
Blocked safe MFB 3 min
Free explo MFB 3 min
PreSleep 90 min
Exploration 15 min
Blocked left 5 min
Blocked right 5 min
PreTests 4 * 2 min
Conditioning PAG 3 * 17 min
PostSleep PAG 90 min
PostTests PAG 4 * 2 min
Conditioning MFB 3 * 17 min
PostSleep MFB 90 min
PostTests MFB 4 * 2 min
Extinction 15 min
Free explo PAG 5 min
Blocked shock PAG 3 min
Free explo PAG 3 min
Blocked safe PAG 3 min
Free explo PAG 3 min
17 min total

Figure S2. Protocol of counterconditioning experiment during wakefulness. *Experiment started with sleep in the home cage for 90 min. After initial exploration of the UMaze (15 min), animals were habituated to blocking procedure on both sides of the maze (each 5 min). Then, 4 trials of 2 min-long free explorations were recorded as a baseline behavior. Each learning session ('Conditioning PAG') started with free exploration, in which PAG stimulation was delivered in a mouse was detected in the specific 'shock' zone (5 min). Then animals were blocked in the shock zone and received 4 shocks during 3 min, after which the wall blocking mice was raised and 3 min more of free exploration with 'shock' zone stimulations followed. Blocking procedure was repeated on the safe side. This conditioning sequence lasted 17 min in total and was repeated 3 times, after which animal rested in its home cage for 90 min ('PostSleep PAG'). Sleep was followed by 4 trials of 2 min-long free explorations where we assessed behavior after aversive learning ('PostTests PAG'). After them, another conditioning sequence was conducted, this time we used rewarding MFB stimulations ('Conditioning MFB'). Afterwards, mice slept in their home cage ('PostSleep MFB') and tested in 4 2 min-long trials again ('PostTests MFB'). Experiment concluded with 15 min of free exploration ('Extinction').*

Interestingly, average speed, number of entries to the stimulation zone and the latency to enter the stimulation zone, parameters that changed remarkably after aversive learning, became very similar after both counterconditioning and extinction. Indeed, both counterconditioning and extinction restore to normal levels behavior of mice outside of the shock zone. However, animals in the sham group spent much less in the stimulation zone than counterconditioned mice, suggesting that location of stimulation still evokes anxious behavior. This confirmed by the fact that occupancy of the shock zone after extinction is lower than baseline occupancy before any learning – and we see opposite tendency for the counterconditioning group.

Taken together, this chuck of results validates that counterconditioning using intracranial stimulations is more effective in neutralizing aversive associations – at least, at the length of one day.

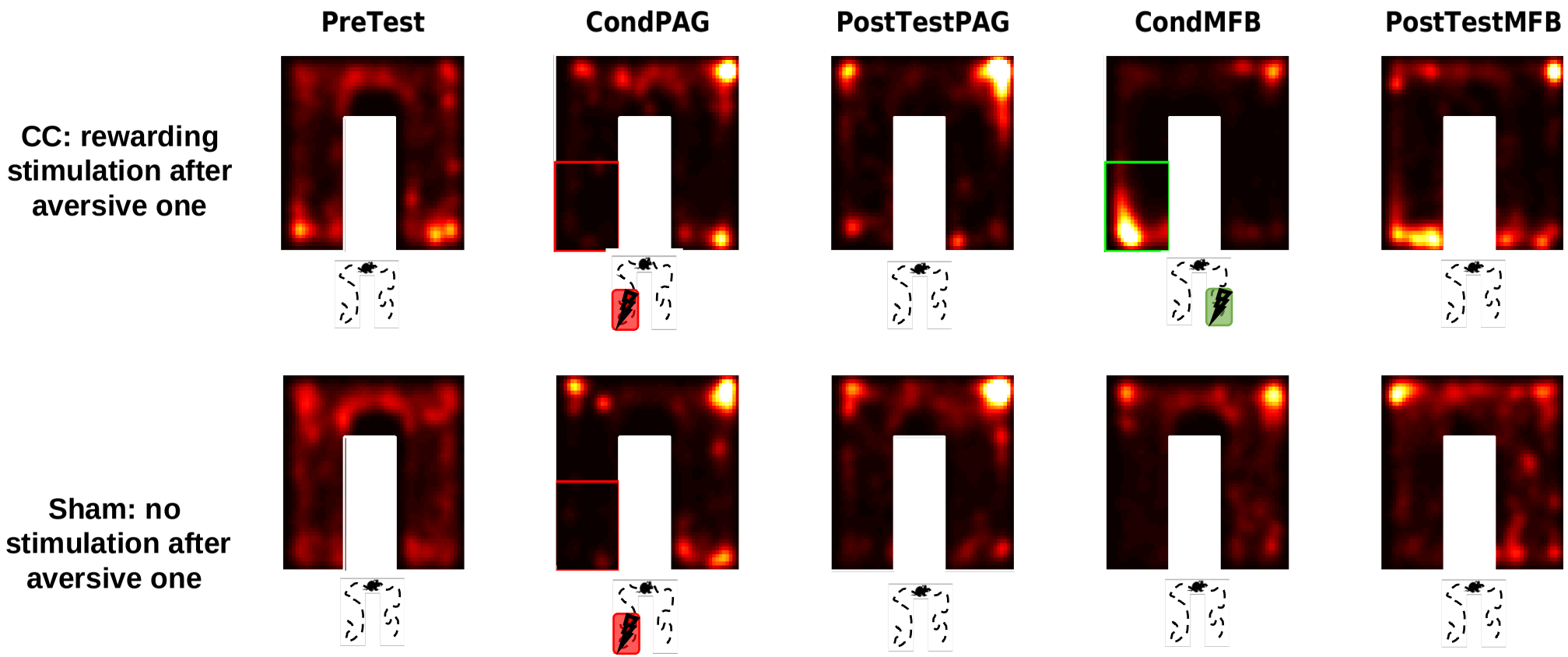

Figure S3. Occupancy maps in counterconditioning for the experimental (N=5) and the sham (N=5) groups.

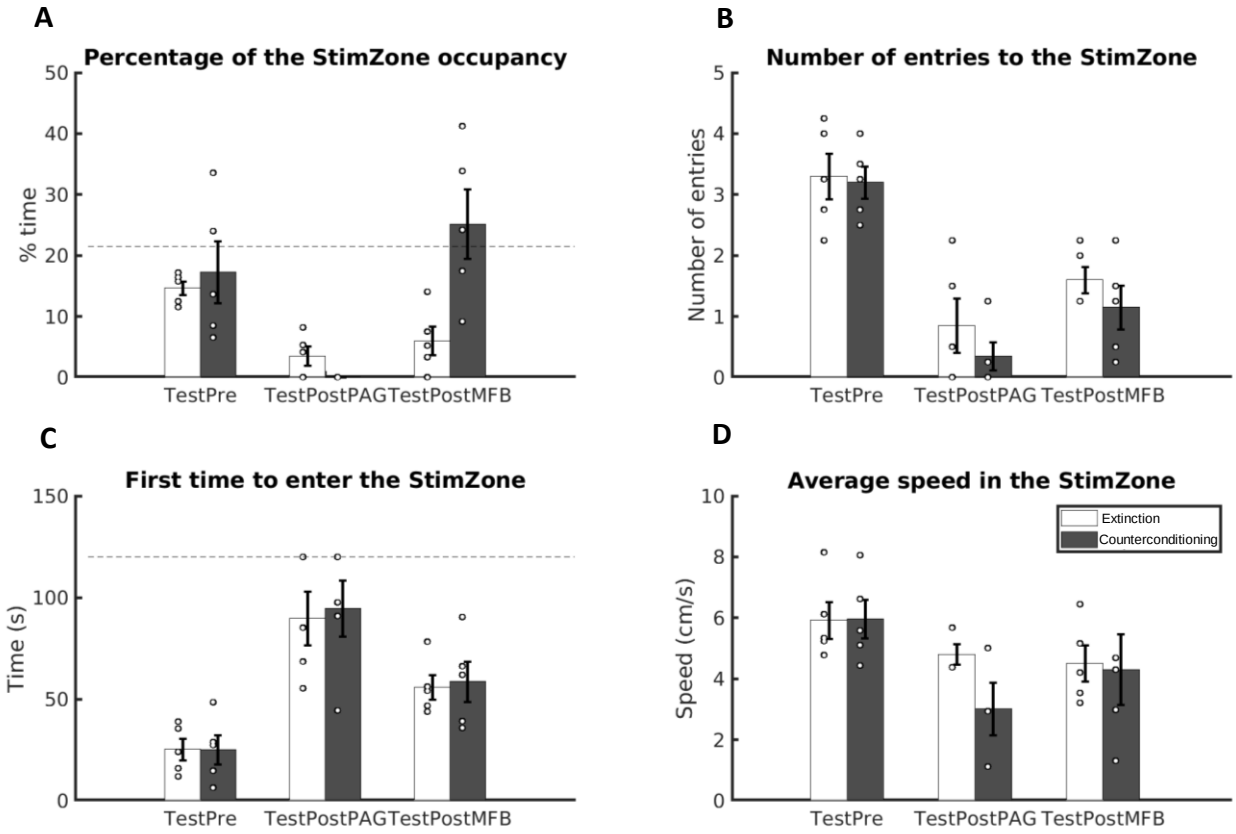

Figure S4. Behavioral results of the counterconditioning experiment. White bars represent results of the sham group, and black bars represent the results of counterconditioning group. ***A.*** *Percentage of the stimulation zone occupancy. Note that it drops after aversive learning (TestPostPAG) for both groups but it returns to pre-aversive values only for counter-motioned group.* ***B.*** *Number of entries in the stimulation zone. It decreases after aversive learning and slightly rebounds after either counterconditioning or extinction but never reaches pre-learning levels.* ***C****. Latency to enter the stimulation zone. It increases almost to maximum values after aversive learning and returns back after either counterconditioning or extinction but never reaches pre-learning levels.* ***D.*** *Average speed in the stimulation zone. It decreases after aversive learning starts but lot more for counterconditioning group, and stays similar even after both counterconditioning and extinction.*

Discussion

We have performed aversive-to-appetitive counterconditioning experiment during wakefulness using intracranial stimulation. This experiment was preparatory for counterconditioning during sleep: confirmation was needed that intracranial stimulation could successfully serve as unconditioned stimuli in affective spatial learning.

Counterconditioning was more effective in recovering pre-learning occupancy levels in stimulation zone than extinction protocol. Interestingly, other behavioral measures taken in the stimulation zone very similar in both groups of mice. Our interpretation is that despite moving far away from fear of the stimulation zone due to extinction, in this groups of mice stimulation zone is still anxiogenic, and animals leave the zone soon after they enter it. In

contrast, animals in counterconditioning group are staying for long periods of time, probably looking for reward, which is one of the signs of successful change in valence of place association.

Counterconditioning during sleep is an ultimate experiment that would follow. Fundamental possibility to alter valence associated with particular location during sleep was demonstrated earlier in our team (de Lavilléon et al., 2015). Now we would like to pair intracranial rewarding stimulation with reactivation of locations association with aversive experience in sleep after aversive learning. This procedure could possibly change valence of aversive association bringing up the proof of concepts that persistent aversive memories could be treated during sleep. We hope that sleep counterconditioning would become more 'user-friendly' and, possibly, provide more stable results than counterconditioning during wake, which is used for treatment of post-traumatic stress disorder and phobias nowadays (Keller et al., 2020).

We did not discuss stability of such counterconditioning as it was not our aim in wakefulness experiment. We hope that counterconditioning during sleep would be more stable than during wakefulness for several reasons. First, most likely it also utilizes reconsolidation mechanisms as it is thought that hippocampal reactivations could be the moments of labile memory reinstatement. Second, it provides completely different definition of learning context: it could be that learning during sleep is similar to learning in many different contexts as psychological distance between locations and environments may be warped.

General conclusion

Aversive experience is reactivated during NREM sleep

During the time of my thesis, I studied hippocampal reactivations after affective spatial learning. I, together with my team, have demonstrated that neuronal co-firing observed during the task, which includes spatial learning, is reinstated in the following NREM sleep for both reward-based and aversive learning. To our knowledge, we are the first team who showed that hippocampus reactivates fear-related experience in the behavioral paradigm with unbiased baseline behavior and that uses strong aversive stimulus to reinforce learning. Interestingly, strength of sleep reactivations after aversive learning correlated with magnitude of avoidance behavior.

Importantly, we have also shown that activity during awake SWRs observed during aversive learning contributes the most to NREM sleep reactivations, and this activity represents the aversive zone – the least visited location in the environment.

Effects detected in this thesis confirm observations made in similar studies as well as it adds new interesting observations of hippocampal reactivations after affective learning. **Our results suggest that instead of simple overrepresentation of motivationally salient or the most visited zones in the replays, hippocampus could reactivate the most relevant experience that comes as an adaptation to the reinforcing stimuli.** This perspective would predict that reward-based learning result in elevated rate of reward zone reactivations dur to the fact that animals after learning are attracted towards reward zone. To fully confirm this hypothesis, we would need to gather more data in reward place association protocol and perform causally motivated experiments.

There is large amount of evidence indicating that experience of novelty also triggers increased SWRs rate accompanied by reactivations. One of the further directions could be comparison of affective learning with novelty effect.

Interneurons in hippocampus are modulated by freezing

In addition, **we identified a small subpopulation of hippocampal neurons, the firing of which are down-modulated by freezing behavior**. The majority of these neurons are fast-spiking interneurons. Neurons modulated by freezing were found in the hippocampus in the recent study that used Ca^{2+}-imaging to record neuronal activity (Schuette et al., 2020). We extend their finding by providing specific neuronal type that alters its activity during freezing behavior.

These results are interesting as they suggest that dorsal hippocampal neurons participate in the representation of such defensive, motivationally relevant behavior as freezing. The following studies could try to unravel significance of this effect for disinhibiting principal cells and for modifications of reactivations during freezing episodes.

Can we reverse aversive association during sleep?

In the previous report published by our team, it was demonstrated that it is possible to use hippocampal reactivations to create novel appetitive association with a particular location during sleep (de Lavilléon et al., 2015). After this initial success, we suggested to use this technique to neutralize aversive association learned during wakefulness.

Such experiment was motivated by the problem of mental conditions, which are characterized by persistent aversive associations such as post-traumatic stress disorder (PTSD), anxiety disorder, etc. In those cases, clearly maladaptive aversive memories are very hard to extinguish. Repetitive nature of reactivations allows to draw a parallel with repetitive thoughts that are experienced by the patients. Therefore, **we designed the experiment to provide a proof of concept that strong aversive association can be neutralized by pairing hippocampal reactivations that represent the aversive zone with the intracranial rewarding stimulation.**

To verify that aversive spatial learning induces reactivations of the aversive zone, whose presence is indispensable for the success of the experiment, we performed aversive association place protocol. In this thesis, we confirmed that aversive experience is reactivated during sleep. However, not the aversive zone per se was the most reactivated in our study but the zone adjacent to it. Therefore, direct association of the aversive zone with positive reinforcement appear to be hard to perform. According to our speculative interpretations, instead of purely spatial representations, representations of experience are reactivated (in this case, reactivation of avoidance behavior). Yet, if it is true it would still yet be possible to perform proposed sleep counterconditioning experience in hope that if one pairs fear-related behavior to appetitive stimulation, aversiveness would be neutralized.

Another potential danger that has arisen is that, in this study, activity during free exploration phase before learning contributes the least to the NREM reactivations. On the contrary, the most contributing period was awake SWRs that occur during learning, which suggest that if one is to conduct such procedure in humans, one would need to strongly revive the aversive memory to be treated.

Thus, I believe that aversive-to-appetitive counterconditioning is still interesting to perform, however, several important adjustments should be made in our thinking about it:

- Stimulations should be tuned to locations that are most crucial in fear-related behavior rather than the aversive zone per se.
- We should keep in mind that experiment without reviving of experience before sleep (i.e. in the situation close to new learning to activate reactivations of fear-related behavior) could fail to produce positive results.

Aversive-to-appetitive counterconditioning using intracranial stimulations is possible during wakefulness

Before we attempt to perform sleep counterconditioning, we tested whether intracranial stimulations of opposite valence used in this study can be utilized to perform counterconditioning during wakefulness. Our results confirmed that such awake counterconditioning was more effective in returning behavior to pre-aversive levels than extinction procedure.

New position decoding tool is created to facilitate closed-loop reversal experiment

For successful sleep counterconditioning experiment, one needs an algorithm that would decode the position replayed during each reactivation event. In the seminal study, it was achieved by simple thresholding of a particular place cells, which is very costly in terms of time as it is very difficult to find sharply tuned place cell with amplitude large enough to threshold. To facilitate the process, we have designed new accurate position decoder algorithm which is based on artificial neural networks and can be used in online closed-loop experiment as it requires minimal curation of input data. Importantly, with each position inferred it also outputs a 'confidence' value that can be used to filter out spuriously inferred positions.

Methods

Animals

C57BL6jRj mice were used in this study. All behavioral experiments were performed in accordance with the official European guidelines for the care and use of laboratory animals (86/609/EEC), in accordance with the Policies of the French Committee of Ethics (Decrees n° 87–848 and n° 2001–464) and after approval by ethical committee (reference: 2016-09). Animal housing facility of the laboratory where experiments were made is fully accredited by the French Direction of Veterinary Services (B-75-05- 24, 18 May 2010). Animal surgeries and experimentations were authorized by the French Direction of Veterinary Services. All animals were housed individually after surgery (08:00–20:00 light) without any restrictions on access to food or water. Ambient temperature was maintained at 21±1°C and 50±10% humidity.

Surgery

Implantation took place when the mice were between 7 and 12 weeks of age. Animals were anaesthetized in an induction chamber with 5% isophlurane (Isotec3, Ohmeda, UK; 0_2 flow rate was 2.5 L/min) and kept at 1-1.5% isophlurane concentration throughout whole surgery. Buprenorphine (0.1mg/kg) was injected subcutaneously one hour before the induction.

9 animals were implanted with 6 formvar-insulated nichrome tetrodes (3 in each hemisphere) above hippocampus, and every triplet of tetrodes was placed on its own Microdrive (*AP = -2 mm; ML = ±2 mm from Bregma*). 17 animals were implanted with one 64-channel silicon probe (type E1, Cambridge Neurotech, UK) above the right hippocampus *AP = -2 mm; ML = ±1.5 mm from Bregma*). All mice (a total of 23 mice) were also implanted with 2-3 tungsten wires unilaterally in the olfactory bulb *(AP = 4.5 mm; ML = ±0.8 mm; DV = -1.2 mm from Bregma*), 2-3 wires in the right prefrontal cortex (*AP = 1.9 mm; ML = 0.6 mm; DV = -1.6 mm from Bregma*). Reference wires were placed above cerebellum.

All animals were implanted with at least two bipolar tungsten stimulation electrodes: one was implanted in dorsolateral periaqueductal grey matter (dlPAG) (*AP = -4.72 mm; ML = 1.2 mm; DV = -1.6 mm from Bregma; angle = 16°*), and one was implanted in medial forebrain bundle (MFB) (*AP = -1.4 mm; ML = 1.2 mm; DV = -4.8 mm from Bregma).*

Animals were treated with buprenorphine injections at least two days after surgery, twice a day. Experiments were performed at least two weeks after surgeries. Silicon probe was progressively lowered into the pyramidal layer of the hippocampus until ripple-containing channels were reached.

Data acquisition and stimulation

Electrophysiological data were acquired using a RHD2164 board and headstage preamplifiers (Intan technologies, California, USA). Amplified signals were sent to recording computers at 20 kHz sampling rate. Tracking of the animal was performed using overhead thermal camera (FLIR A325sc, Teledyne FLIR LLC, Oregon, USA) and custom MATLAB-based software (The Mathworks, Inc, Massachusetts, USA), which identified the center of mass of the hottest spot in the camera field of view as a mouse (*Fig. M1*). Acquisition rate of the camera was 15

Hz. Electrophysiology and tracking data were synchronized using Arduino-based code, which managed TTL pulses to log start and end of each recording session.

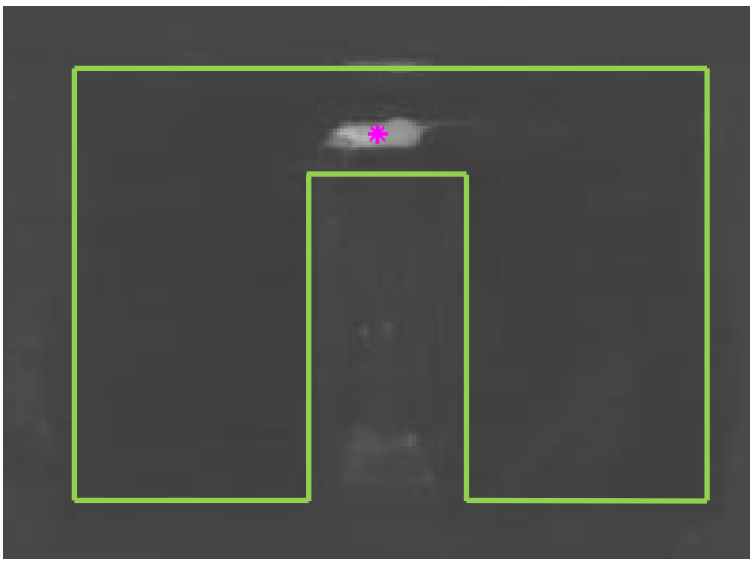

Figure M1. Field of view of thermal camera used for behavioral tracking. Environment is delineated in green. Center of the mass of a mouse was tracked (shown by magenta star).

PulsePal stimulator was used to perform intracranial electrical stimulations (Sanworks, NY, USA). Each stimulation was a train of 13 biphasic 1 ms short pulses with an interstimulus interval of 8 ms (125 Hz).

Calibration of medial forebrain bundle electrode in the nosepoke apparatus

Calibration of medial forebrain bundle (MFB) electrode took place in 20*20*30 cm open field made of white plexiglass (nosepoke chamber). A hole, 1 cm in diameter, was made 3.5 cm above the floor in one of the walls. Infrared-emitting diode (detector), located in this hole, was configured to send TTLs when any object was thrusted into the hole.

An animal was placed in the nosepoke chamber and allowed free exploration of the environment. Upon detection of animal poking nose into the hole, MFB stimulation was triggered. If animal performed continuous poke, stimulations were sent with 1 s interstimulus interval. Animal was allowed to explore for 100 s after the first poke. Number of pokes was counted.

Experiment started with baseline intensity of 0V. Intensity was increased 0.5 or 1V after each session of poking. Experiment stopped when animals reached plateau or when number of pokes started to decrease (speculatively, due to involvement of defensive circuits). Intensity with maximal number of self-stimulated nosepokes was chosen for the experiment.

Calibration of periaqueductal grey matter (dlPAG) electrode in the open field

Calibration of periaqueductal grey matter (dlPAG) electrode took place in 32*20*30 cm open field made of white cardboard.

An animal was placed in the open field and allowed free exploration of the environment for 180 s. Six PAG stimulations were performed in every 180 s long session: at 50, 70,

90, 110, 150, 170 s timestamps. Stimulation-induced immediate jumps and freezing following them were assessed in each session.

Experiment started with baseline intensity of 0V. Intensity was increased 0.5 or 1V after each session of 180 s. Experiment stopped when animals reached plateau in freezing amount or stimulation-induced jumps reached their maximum intensity. Intensity with maximal amount of freezing and stimulation-induced jump amplitude was chosen for the experiment.

UMaze

Most of the experiments took place in U-shaped maze (UMaze) environment. Dimensions could be seen in the *Fig. M2*. We used four different UMazes in the study: one was made from cardboard and fabric, and three UMazes were made from plexiglass. Every UMaze has unique set of proximal cues, different in different arms. Between each recording session UMaze was cleaned either with 30% ethanol or laboratory-purpose detergent (Surfa'Safe Premium, Laboratoire Anios, France).

We divided UMaze into 7 zones: Stim zone, Far Stim zone, Center Stim zone, Center zone, Center No-Stim zone, Far No-Stim zone and No-Stim zone (see fig. 2A).

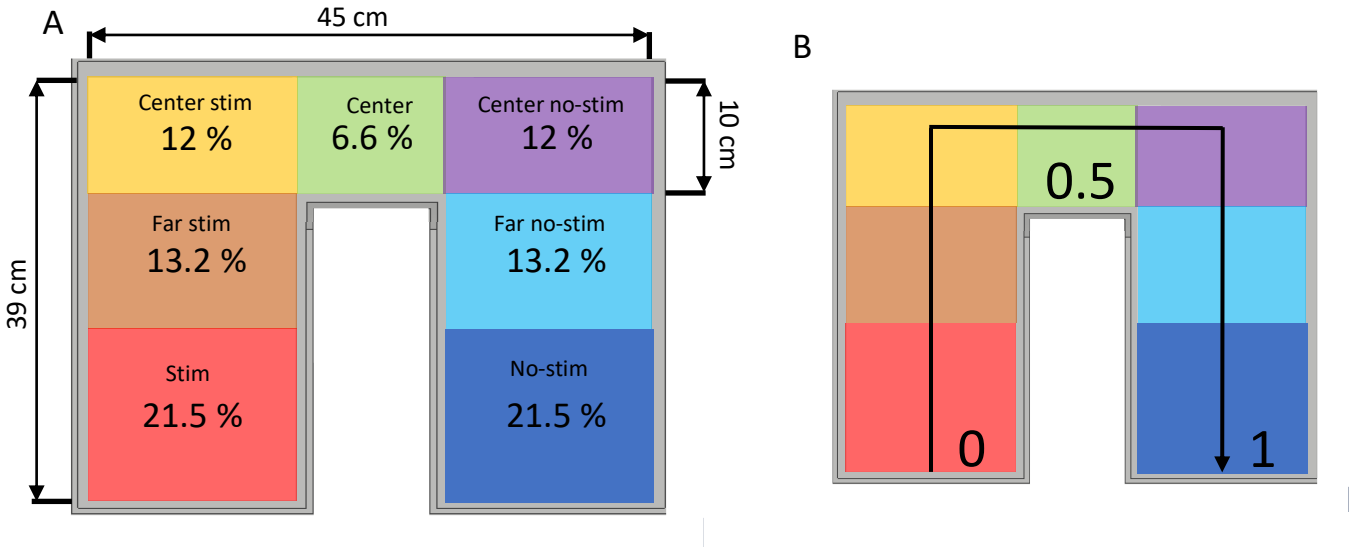

Figure 2. UMaze. ***A.*** *Dimensions of the UMaze. Colors indicate seven zones used in the analysis. Percentage indicates surface occupied by the zone.* ***B.*** *Linearized coordinates of the UMaze (linearization described below).*

UMaze behavioral protocols

UMaze protocol 1 – 'aversive place association learning'

For graphical depiction of the protocol, please see *Fig. M3a.*

Mice started the experiment in the home cage for a baseline sleep session ('*PreSleep*'), which lasted at least two hours. After animals ran through the following series of recording sessions (each session mouse started in the center zone of the UMaze):

1. Free exploration, 15 min;

2. 4 PreTests, each 4 min – these are free exploration sessions as well, they are used to assess baseline behavior;
3. 4 Conditioning sessions, each 8 min – if an animal crosses the border of shock zone, it gets PAG stimulation; and it kept getting them each 6 s until the mouse left the zone. If it returned to the zone, stimulation was triggered again;
4. PostSleep, 2 hours – mouse was placed back into its home cage for sleep;
5. 4 PostTests, each 4 min – free exploration sessions, used to assess aversive learning.
6. Extinction, 15 min – free exploration session.

UMaze protocol 2 – 'positive place association learning'

For graphical depiction of the protocol, please see *Fig. M3b.*

For rewarding stimulation experiment, we have changed duration of behavioral tests from 4 to 2 min because positive association is a subject to very fast extinction once an animal reaches previously rewarding locations and stayed there. Number of tests were increased to match the time in aversive experiment. We have also re-balanced duration and number of conditioning sessions to try to achieve more robust learning.

1. PreSleep, 2hours
2. Free exploration, 15 min;
3. 8 PreTests, each 2 min.
4. 8 Conditioning sessions, each 4 min – if an animal crosses the border of shock zone, it gets PAG stimulation; and it kept getting them each 6 s until the mouse left the zone. If it returned to the zone, stimulation was triggered again;
5. PostSleep, 2 hours – mouse was placed back into its home cage for sleep;
6. 8 PostTests, each 2 min – free exploration sessions, used to assess aversive learning.
7. Extinction, 15 min – free exploration session.

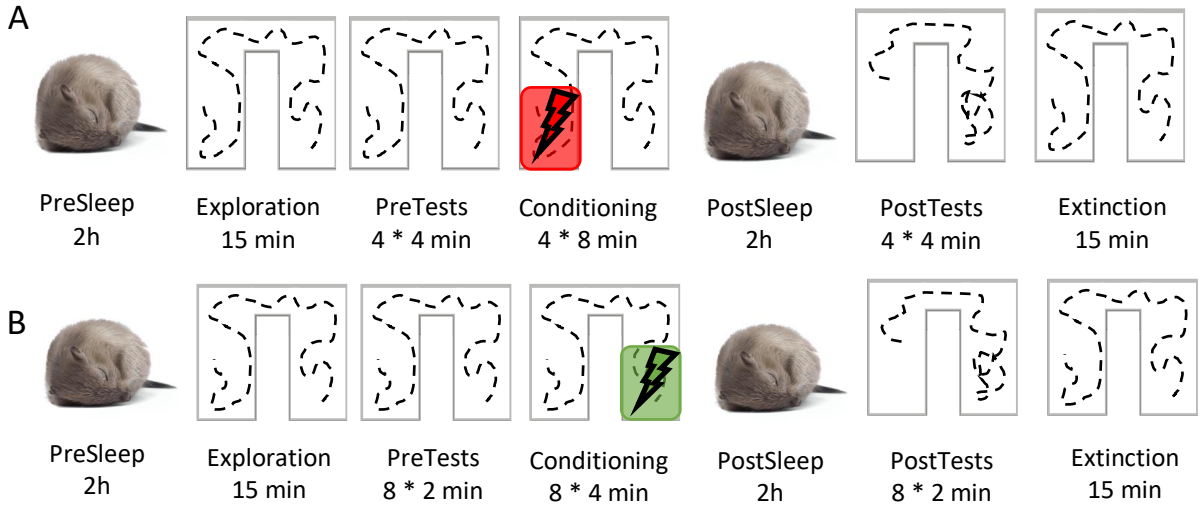

Figure M3. Protocol used in the experiment. ***A.*** *Aversive place association protocol.* ***B.*** *Positive place association protocol.*

Data analysis – behavior

Alignment and linearization of trajectories

Due to the fact that recordings were made in different rooms in slightly different visual settings, all raw trajectories were aligned to common coordinates.

Trajectories were also linearized by projecting instantaneous coordinate of an animal to the line that run from the bottom of the stimulation zone to the bottom of the opposite arm (*Fig. M2B*).

Data analysis – electrophysiology

Preprocessing and spike sorting

Raw data were recorded at 20 KHz sampling rate. Local field potentials were sampled at 1250 Hz. Analysis was performed in custom-written Matlab software, with the use of ts library (https://github.com/PeyracheLab/TStoolbox) and FMAT toolbox (https://source-forge.net/projects/fmatoolbox/). Bayesian decoding and deep learning applications were coded in Python using Tensorflow as backend (https://www.tensorflow.org/).

Spike sorting was performed on pre-extracted waveforms using KlustaKwik software for automatic clustering and Klusters software for manual sorting (neurosuite.source-forge.net). Recordings were visualized and processed using Neuroscope and Neurosuite plugins (https://sourceforge.net/projects/fmatoolbox/).

Sleep scoring

In this study, we used two sleep-scoring algorithms. In the majority of recordings, we used sleep scoring based on the power of gamma oscillations in olfactory bulb, or OB-based sleep scoring (Bagur et al., 2018 - for more details, please refer to original publication and see Fig. M4). LFP recorded in olfactory bulb were filtered in gamma (50-70 Hz) band, instantaneous amplitude was obtained by applying the Hilbert transform, and the resulting time series were smoothed using 3 s wide sliding window. **Sleep-wake threshold** was identified as the intersection of two gaussian fitted in the distribution of smoothed gamma recorded from olfactory bulb. To calculate NREM-REM threshold, LFP recorded in hippocampus was filtered in delta band (2-5 Hz) and theta band (5-10 Hz), and Hilbert transform was applied to each filtered signal. Ratio of theta and delta amplitudes was calculated and then smoothed using 3 s wide sliding window. Due to the fact that in our study sleep was limited for the animals, we have never observed bimodal distribution of theta/delta ratio (two modes corresponding to NREM and REM). For that reason, we identified **NREM-REM threshold** by finding value of ratio where gaussian fit to ratio distribution explains less than 50% of the data.

Recordings from animals, in which olfactory bulb signal was of bad quality, were sleep-scored using **accelerometer-based sleep scoring** algorithm. Amount of movement at each timestamp was calculated as follows:

$$Mov = x^2 + y^2 + z^2 ,$$

Where *x* is acceleration on X-axis, *y* is acceleration on Y-axis, and *z* is acceleration on Z-axis. Temporal profile of *Mov* was used to manually decide **sleep-wake threshold**. To calculate **NREM-REM threshold**, the same procedure as in OB-based sleep scoring was used.

In both algorithms, all detected states with durations less than 3 s were merged with neighboring states.

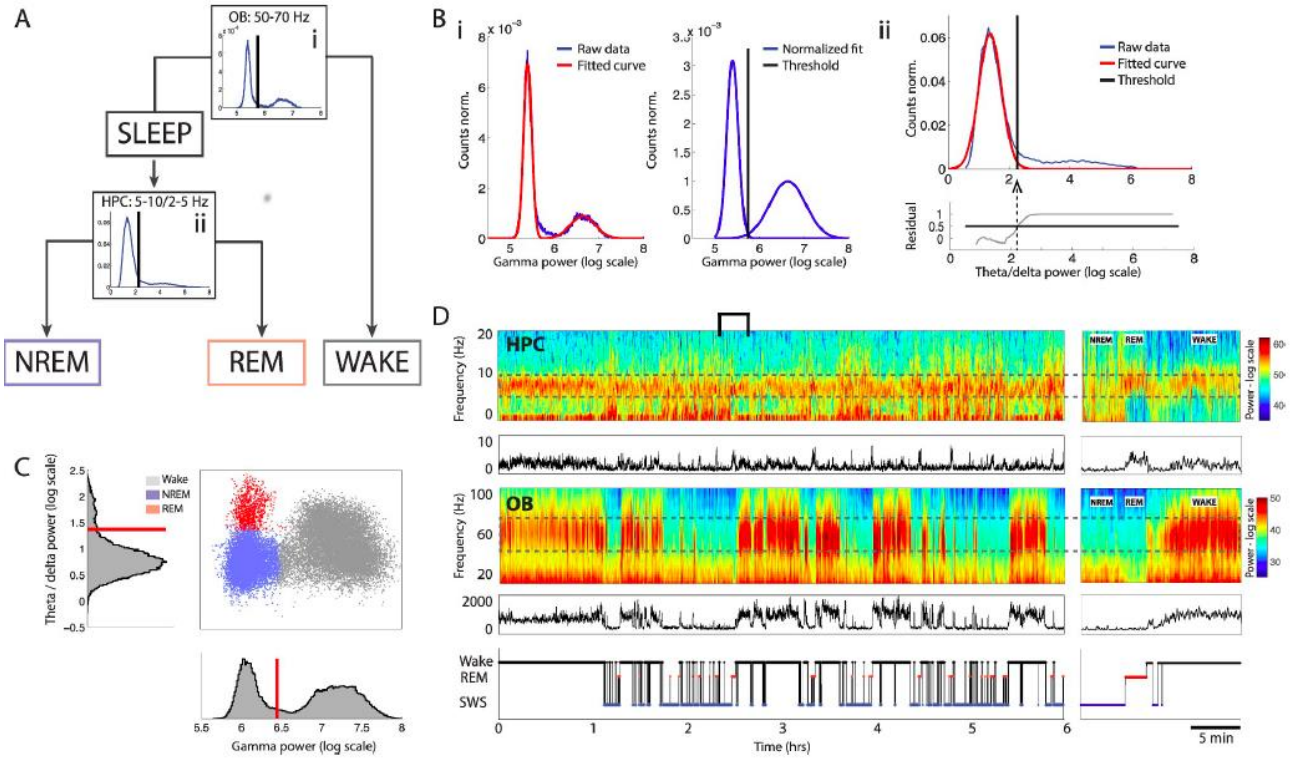

Figure M4. Schematic of sleep scoring method based on gamma power in olfactory bulb. Adapted from Bagur et al., 2018. ***A.*** *Flowchart of data through the scoring algorithm. Sleep and wake states are first classified based on OB gamma (i). Sleep data are then further classified into REM and NREM sleep based on HPC theta/delta power ratio (ii).* ***B.*** *Example of automatic thresholding of distributions. (i) Two Gaussian distributions are fit to the distribution of OB gamma power (left), and their areas are equalized (right). The threshold is placed at the intersection of the two distributions. (ii) A Gaussian distribution is fit to the distribution of HPC theta/delta power ratio during sleep. The residuals are shown in the bottom plot. The threshold is placed at the point where the fit explains less than 50% of the data.* ***C.*** *Phase space of brain states showing the distribution of NREM (blue), REM (red), and wake (grey) data for an example mouse. Corresponding histograms are shown along the relevant axis, with automatically determined thresholds in red.* ***D.*** *HPC low-frequency spectrogram with theta/delta power*

ratio below, OB high-frequency spectrogram with gamma power below, and hypnogram for the same mouse as in C. The relevant frequency bands are outlined by a dotted grey line. Right: bracketed area on an expanded timescale. HPC, hippocampus; NREM, non-REM; OB, olfactory bulb; REM, rapid eye movement.

Detection of sharp-wave ripples

LFP recorded from pyramidal layer of hippocampus was filtered in 120-220 Hz band. Two methods were used to detect SWRs. The first one identified SWRs as periods of time where filtered signal exceeded 4 standard deviations (STDs) with the peak value exceeding 6 STDs. The second one labeled periods of time where squared filtered signal exceeded 2 STDs with the peak value exceeding 5 STDs.

For both methods, STD was calculated on the whole day recording. For both methods, minimal duration of SWRs was set to 20 ms, maximal duration was set to 200 ms, and minimal inter-ripple interval was 15 ms. Results of both methods were merged to obtain the final set of SWRs.

Neuron classification

To classify waveforms into putative pyramidal cells and interneurons, we used the approach inspired by Csivari et al., 1999. K-means clustering with k=2 was performed on the following values characterizing each single unit: firing rate in Hz, time passed between two half-amplitudes of mean waveform and asymmetry index. Asymetry index is calculated using the formula as follows (Royer et al., 2012):

$$Asymetry = \frac{MaxAft - MaxBef}{MaxAft + MAxBef}$$

where MaxAft is the maximum before the negative peak and MaxBef the maximum before the negative peak calculated on the mean waveform.

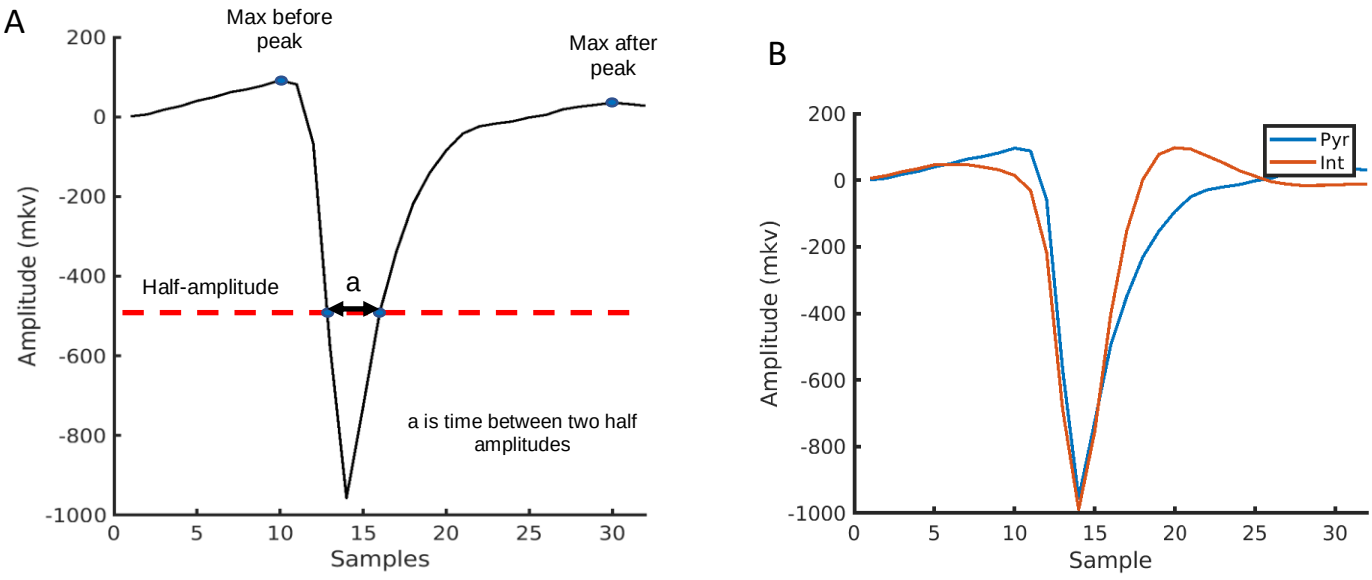

Figure M5. Classification of hippocampal neurons into putative pyramidal cells and putative interneurons. ***A.*** *Mean waveform from all recorded units. Waveform features used for classification are indicated.* ***B.*** *Mean waveforms for putative pyramidal cells and putative interneurons.*

Among all single units used in the analysis, we identified 94% putative pyramidal cells and 6% interneurons. Putative pyramidal cells had lower firing rate, wider waveforms and negative asymmetry index (1.57 ± 0.01 Hz vs. 18.1 Hz ± 1.33 Hz; 0.8 ± 0.008 ms vs 0.7 ± 0.01 ms; -0.42 ± 0.01 vs 0.27 ± 0.07, respectively; mean ± S.E.M.)

Place cell identification

Rate maps for all recorded single units with spatial bin of 0.8 cm were generated on the epochs where speed of the animal was higher than 3 cm/s, and then smoothed with 2D gaussian kernel (standard deviation is 2). Single unit was identified as a place cell if its spatial information was higher than 0.9 bits/s, average firing rate was higher than 0.3 Hz and place field covered more than 2% of the environment.

Place fields stability analysis

To assess stability of place fields, we calculated two measures: within-session stability for evaluation of baseline place field stability, and exploration-to-learning stability to assess how place fields changed during affective learning.

To calculate within-session stability, we concatenated all free exploration epochs before start of learning and split the resulting epoch in two equal parts. We have constructed rate maps using the same parameters as for place cells identification on both parts of free explorations, and correlated them. Resulting correlation coefficient served as the within-session stability index.

To calculate exploration-to-learning stability, we constructed rate maps using high-speed periods for free exploration before learning and learning periods. These rate maps were correlated to obtain the exploration-to-learning stability

Reactivation analysis

Explained variance. For more details, see Kudrimoti et al., 1999. Spike trains were binned in 100 ms long bins and z-scored to create spike time histograms for three types of epochs: PRESLEEP, WAKE, POSTSLEEP. PRESLEEP and POSTSLEEP epochs contained only NREM periods from respective recording sessions. WAKE epochs could be composed from different combinations of data from task period, I will indicate in the result section which data was used for WAKE epoch separately for each result. Binned spike time histograms for all hippocampal single units were correlated separately for each epoch. Resulting three correlation matrices were correlated between each other obtaining three correlation coefficients that were used to calculate explained variance (EV):

$$EV = \left(\frac{R_{task,post} - R_{task,pre} * R_{pre,post}}{\sqrt{\left(1 - R_{task,pre}^2\right) * \left(1 - R_{pre,post}^2\right)}} \right)^2$$

where R correspond to correlation coefficient of pairwise correlation matrices between periods of time indicated in lower index. Reversed explained variance (REV) was obtained by swapping PRESLEEP and POSTSLEEP epochs.

If EV > REV, it means that in POSTSLEEP epochs correlational structure is more similar to WAKE epoch than WAKE epoch is similar to PRESLEEP, and we detect reactivations.

PCA-based template matching. For more details, see Peyrache et al., 2010. Spike time histograms from periods containing PostSleep SWRs (±100 ms from the highest amplitude) binned at 40 ms and z-scored was used as a template epoch. Neuronal activity during template epochs was decomposed using principal component analysis (PCA) into components, each of which supposedly captures certain pattern of firing. We have used the two principal components among those ranged by the amount of variance explained. Once principal components are obtained, they were matched with binned spike-time histograms of target epochs, yielding a vector of reactivation strength measure which represents degree of similarity between principal component of template epoch and a particular time bin of target epoch. Thus, we obtained reactivation measure that has temporal resolution of a bin duration (40 ms). We matched activity recorded during sleep SWRs to investigate which activity from other periods of time (PreSleep, free exploration before learning, learning phase, etc.) contributes mostly to the potential reactivations during PostSleep SWRs.

Let *Q* be a z-scored spike-time histogram (dimensions *n* units x *b* bins). Correlation matrix of *Q* is

$$C = \frac{1}{b} Q Q^T$$

Each element in matrix *C* is a Person correlation coefficient. Matrix *C* is decomposed using PCA, which is expressed here as an eigenvector decomposition. Thus, matrix *C* can be represented as a sum of outer products of eigenvectors p^l (l=1…N) scaled by their respective eigenvalue λ_l:

$$C = \sum_{l=1}^{N} \lambda_l (p^l)^T p^l = \sum_{l=1}^{N} \lambda_l P^l$$

where P^l is a projector. Projectors can be viewed as a specific pattern of neuronal co-firing neurons. Reactivation strength measure $R_l(t)$ can be calculated by matching the projector P^l to a binned spike-time histograms for a target epoch:

$$R_l(t) = \sum_{i,j;i \neq j} Q_{it}^{TARGET} P_{ij}^l Q_{jt}^{TARGET}$$

where *i* and *j* are serial number of single units in *Q*.

Bayesian decoding. For more details, see Zhang et al., 1998. To calculate posterior probability of animal being in the position *pos* giving particular population vector *spikes*, we can apply Bayesian formula:

$$P(pos|spikes) = \frac{P(spikes|pos)\,P(pos)}{P(spikes)},$$

where *P(pos)* is the prior, or the probability of the animal to be found in certain spatial bin of environment, *P(spikes)* is the probability of certain number of spikes to occur in the temporal bin, P(spikes|pos) is the likelihood, or the conditional probability of certain number of spikes given certain position.

Applying two assumptions – that spikes of each neuron are drawn from the Poisson distribution, and that neurons are firing independently from each other, we can express likelihood as

$$P(spikes|pos) = \prod_{i=1}^{N} P(n|pos) = \prod_{i=1}^{N} \frac{(tf(pos))^{n_i}}{n_i!} * e^{-tf(pos)}$$

When inserting likelihood into Bayes formula, we have:

$$P(pos|spikes) = C(t, spikes)P(pos)\left(\prod_{i=1}^{N} \frac{(tf(pos))^{n_i}}{n_i!}\right) * e^{-t\sum_{i=1}^{N} f_i(pos)}$$

where where C(t, spikes) is the normalization constant such as sum of P(pos|spikes) across all spatial bins equals 1.

The latter formula was used to assess reactivations. We use 0.5 cm spatial bin to create priors and rate maps for Bayesian decoding. Inferred 2D coordinates of an animal could be linearized according to procedure described above (*Alignment and linearization of trajectories*).

Decoding using artificial neural networks

Feature extraction. Raw electrophysiological signal was high-pass filtered (> 350 Hz) using FIR filter. Deviations in the signal that exceed 3 STDs were detected, and waveforms of 32 samples (peak at sample 14) were extracted. STD were re-calculated for each 3.6 s of data. Extracted waveforms were organized in windows of *T* ms (in this study we used *T* of 36, 108, 256 and 504 ms). Waveforms were extracted separately for each spike group.

Network architecture. Full architecture of artificial neural network (ANN) that was used in this study to decode animal position based on the raw electrophysiological signal can be seen in *Fig M6*. We use a stack of convolutional neural network (CNN) and long short term

memory (LSTM) network. The latter one is a subtype of recurrent neuronal networks, a class of deep learning models that was designed specifically to detect and incorporate in itself temporal dependencies.

We trained N CNNs (where N is a number of spike groups) that were fed with input tensor of shape m x ch x 32 (where m is number of spikes, ch is number of channels in spike group). Each CNN had three layers with increasing number of filters (8, 16, 32) and a kernel size of 2 by 3. Last convolution layer is connected to dense layer with 128 output units, which is in turn followed by a 50% dropout layer. Two more dense layers with 128 units are applied before the resulting tensors are gathered back into windows of *T* ms and 128 output units from each CNN were pooled together.

Resulting tensor of l x (N x 128) where l is a number of temporal windows with spikes used for training is fed into four consecutive LSTM layers with 20% LSTM dropout between each of them. Last LSTM layer was followed by a dense layer with 2 output units, corresponding to x and y coordinates.

Training procedure. We used mean squared error of coordinates (Euclidean loss) as a loss function to train our ANN. The learning was done over 150 epochs, and we used RMSProp optimizer with a constant learning rate 0.0003 with a batch size of 52.

Algorithm was tested on 4 time windows: 36, 108, 252 and 504 ms. For each of the time window used, separate training starting from randow weights was performed. For 108, 252 and 504 ms long time windows, we overlapped windows due to the lack of data.

Confidence of decoding. In addition to position inference with Euclidean loss, our architecture was trained to predict Euclidean loss itself. To do so, we have constructed second loss function:

$$\mathcal{L}_{loss} = MSE(L_{pred} - \mathcal{L}_{Euclidean})$$

Online decoding of animal's position. OpenEphys software was used in online decoding experiment (Siegle et al., 2017). Raw data were processed in 36 ms-long packages. All processing of the data was performed either by native or customary written OpenEphys plugins. Each data package was high-pass (> 350 Hz) filtered, an individual threshold was placed on every channel of interest. Waveforms of 32 samples obtained using such thresholding procedure were processed using Tensorflow. Inferred position was used to trigger intracranial stimulation with an interface of custom-written Arduino and Matlab scripts. Latency between the end of the package (inference time) and stimulation artefact recorded from the brain was registered as a round-trip latency.

Statistical analysis

All data are represented as mean±SEM in the present manuscript.

Due to the fact that all distributions in the dataset failed Kolmogorov-Smirnov normality test, statistical comparisons were performed using Wilcoxon rank sum test.

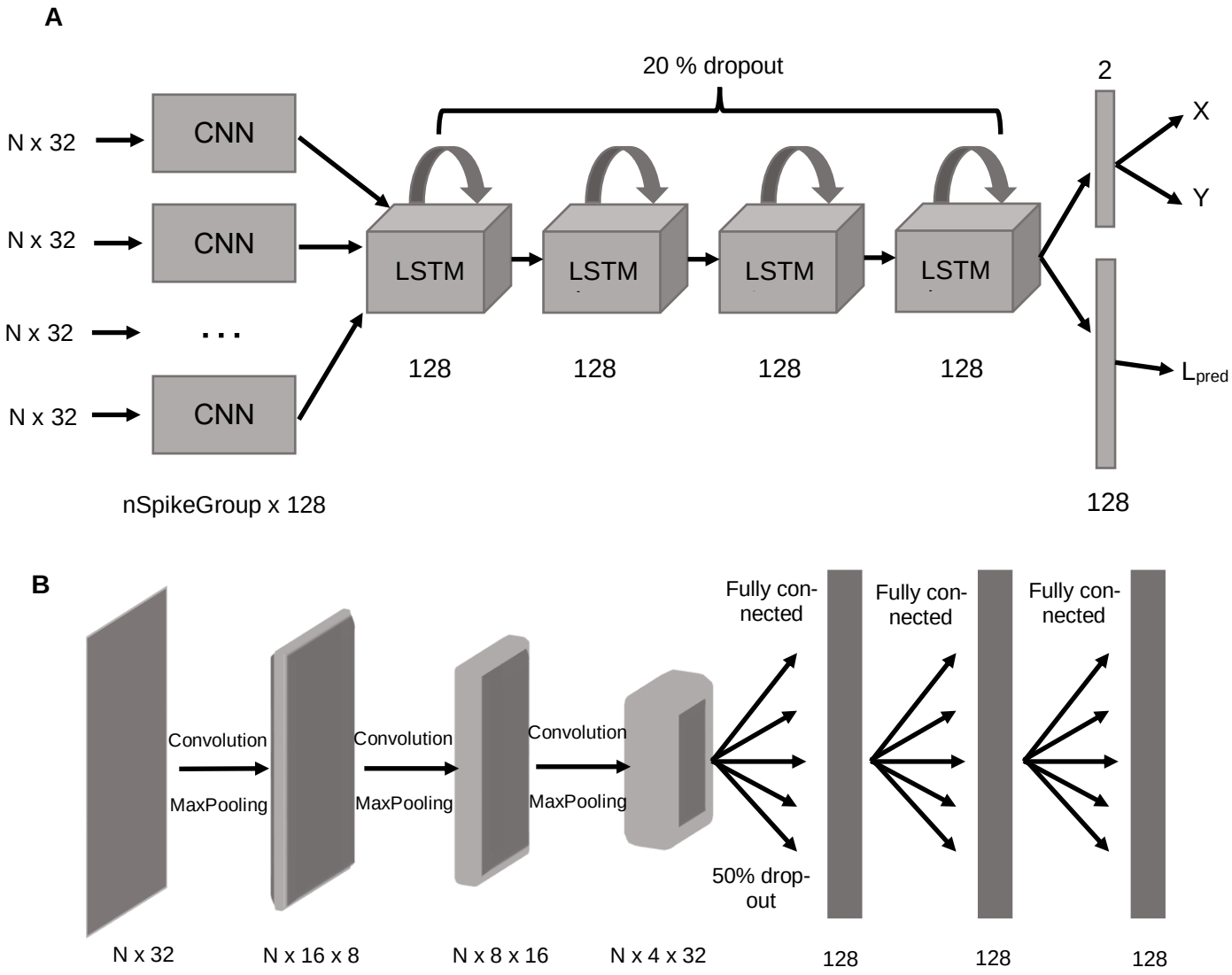

Figure M6. Architecture of position decoder artificial neural network used in the study. ***A.*** *Waveforms from N channels that compose one spike group are sent into separate convolutional network, each of which outputs 128 units. These units are pooled to four consecutive LSTM layer are applied to them. The last layer is connected to a dense layer which outputs 2 values: X and Y coordinate of an animal.* ***B.*** *Architecture of the CNN. Waveforms from N channels that compose one spike group are sent into 3 consecutive convolutional layers with kernel size of (2,3), and then into three dense layers of 128 units each.*

Students perspective on the thesis years

(for future generations)

This little section will describe my experience as a doctoral student outside of conventional thesis format. PhD is rarely a straightforward path, and manuscripts rarely report how winding it is and almost never acknowledge that the finishing point is very much different from the one envisioned in the beginning. I write it mostly for future generation of PhD students who will be hopefully more aware of how the whole process looks like from the inside. Karim told me that it could be also interesting for the researchers who evaluate this thesis, as it immerses them more in my path of becoming researcher (I don't know, honestly☺).

I came to the lab to launch a large project: my supervisor, Karim, had just won a prestigious ERC grant that aimed to prove that aversive associations can be neutralized by pairing reactivations of aversive experience with intracranial rewarding stimulations during sleep. Before approaching this experiment, one had to confirm that aversive experience **is** reactivated during sleep. Hippocampal reactivations after aversive spatial learning became the topic of my PhD; nonetheless, we always kept the ultimate goal in mind.

I did not have any preliminary results when I started my PhD, and little was ready in the lab for immediate production of the results. I had to establish massive neuronal recordings in the lab and aversive spatial protocol with intracranial aversive stimulation. At the start, I have adopted the UMaze protocol from another student of the lab, Sophie Bagur.

I began my PhD with setting up neuronal recordings from hippocampus. We decided to start with tetrodes: I used implantations of 3 tetrodes on self-made drive in each hemisphere. Around 4 months passed before I achieved stable recordings. We used tetrodes for two years, and I was steadily increasing number of neurons recorded per mouse but it still remained excruciatingly low, and in the beginning of my third year we have moved to using silicon probes that have boosted number of neurons and stability of surgery many-fold. It was definitely very late decision, which we should had made in the first year of my PhD.

6 months in the PhD, I started behavioral experimentation. After 3-4 months more passed we have realized that the protocol we used induce very mild and unstable avoidance behavior. It took me 2 more months to find right parameters for the protocol (we decreased surface of the aversive zone and interstimulus interval). Thus, all data reported in this thesis have been collected after the first year of my PhD has passed.

In addition, I had a feeling that my implantations in dlPAG are not stable enough. In the beginning of my second year, I've launched an experiment that aimed to find optimal brain coordinates of dlPAG. I've made 10 implantations with different coordinates and tested all of them in the UMaze experiment. The results were mixed. It helped me to realize that 10 implantations are not enough to make conclusions in such uncontrolled situation but also increased stability of my future surgery by a lot.

Thus, after the first year of my PhD I have only set up neuronal recordings and behavioral protocol and after the second year I have recorded almost 10 mice but average number of neurons per mouse remained very low. I felt that I have no data after half of my PhD is gone.

We adjusted by using silicon probes (the first mouse I've recorded had 100 neurons) but the pandemics kicked in and the experiments were halted for 6 months. When we came back to the lab, our idea was to force sleep counterconditioning experiment as the finishing time drew close. At the same time, I started to closely participate in developing of the ANN-based position decoder (initial design is credit of Thibault Balenbois). After spending 6 months more, trying and failing sleep counterconditioning, I have come to the official end of my PhD funding. This experiment proved to be very difficult for several reasons: first, it demanded relatively large number of place cells that cover at least one arm of the UMaze (and it is surprisingly hard to achieve) and fully tested position decoder (which was not the case as it was taking its final shape at these days).

After 3 years of my PhD, I was in the same point as after 2 years of it. Luckily, I have won one more year of funding. We have decided to concentrate on affective spatial learning protocols, as it could still yield novel results and become the basis of my PhD thesis. In this final year, we have recorded more than 10 mice and largely finished the development of the ANN-based position decoder. Only after 3 years of PhD, industrious data collection has begun. **More than a half of data that you see in this thesis was recorded in the last year.**

I believe that there are more questions to be tackled within our datasets and, more importantly, more data to be recorded. I think that more realistic time estimation to complete the project of such ambition, starting from scratch, is 5-6 years uninterrupted. Unfortunately, laws of France limit my PhD experience by 4 years.

If you are fresh PhD student and you somehow find yourself at the end of this boring tale, I would advise to work in small chunks and react immediately if something does not work. Also, in my case it was a bad idea to jump at high-gain-high-risk experiment. In case it does not work, you might lose so much of the precious time.

It was the most difficult journey of my life, and I hope I've learned a lot. Large thanks again to all who worked close with me on the similar topics: Sam, Thibault, Pierre and, of course, Karim.

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RÉSUMÉ

L'hippocampe est nécessaire à l'encodage et à la consolidation des souvenirs déclaratifs chez l'homme. Chez les animaux, les souvenirs dépendant de l'hippocampe sont souvent étudiés dans le paradigme de la navigation spatiale.

Les cellules de lieu sont des neurones de l'hippocampe qui présentent une excitation spécifique à un lieu. Dans les états de calme, tels que le sommeil et l'éveil à faible vitesse, les cellules de lieu rétablissent les schémas observés pendant l'exploration active - un phénomène appelé "réactivations". Il existe de nombreuses preuves que les réactivations hippocampiques sont le corrélat neuronal de la consolidation de la mémoire spatiale. Cependant, on ne sait toujours pas si l'hippocampe code pour des informations purement spatiales ou s'il code également pour des paramètres non spatiaux tels que la valence émotionnelle.

Il est connu dans la littérature que les lieux de récompense sont plus souvent réactivés dans le sommeil après un comportement actif que les autres. Deux hypothèses concurrentes pourraient expliquer ce fait. Selon l'une d'elles, les lieux associés à certaines valeurs motivationnelles et émotionnelles sont davantage réactivés. L'autre hypothèse postule que l'augmentation du taux de réactivation de l'environnement récompensé est simplement due à l'augmentation du temps passé dans l'emplacement de la récompense.

Pour répondre à cette question, nous avons réalisé deux expériences parallèles utilisant l'apprentissage par l'aversion et la récompense. Dans l'une d'elles, nous avons utilisé une stimulation intracrânienne aversive de la matière grise périaqueducale pour créer une association spatiale aversive, et dans la seconde, nous avons utilisé une stimulation récompensante du faisceau médian du cerveau antérieur pour créer une association spatiale appétitif.

D'un point de vue comportemental, les emplacements émotionnellement importants présentaient une différence massive en termes d'occupation après l'apprentissage. Cependant, dans le sommeil suivant les sessions d'apprentissage, nous avons trouvé des réactivations à la fois pour les associations aversives et pour les associations gratifiantes, ce qui confirme l'hypothèse selon laquelle les variables motivationnelles et émotionnelles de la tâche affectent le codage hippocampique. Nous avons démontré que les patterns neuronaux actifs pendant l'apprentissage aversif sont réactivés pendant le sommeil suivant la tâche plus fortement que l'activité enregistrée pendant l'exploration libre. Étant donné que les cartes cognitives dans notre tâche étaient principalement stables, nous avons conclu que l'activité hippocampique pendant la tâche et dans les réactivations enregistrées après la tâche ne code pas pour des informations spatiales pures mais aussi pour la saillance motivationnelle associée à l'espace. À l'appui de cette affirmation, nous avons constaté que la force des réactivations pendant le sommeil était en corrélation avec l'ampleur du comportement d'évitement. En outre, nous avons montré qu'après l'apprentissage de l'aversion, malgré une diminution significative du temps passé dans la zone d'aversion, les réactivations consistaient principalement en des représentations de la zone aversive et des emplacements adjacents, reflétant probablement un comportement d'évitement.

En outre, nous avons tenté d'inverser l'association spatiale aversive en utilisant une stimulation intracrânienne gratifiante dans le sommeil qui suit l'apprentissage aversif. Pour atteindre cet objectif, nous avons développé une interface cerveau-ordinateur qui repose sur l'empilement de réseaux de neurones artificiels convolutifs et récurrents. En d'autres termes, nous avons conçu le décodeur de position, qui sera capable de décoder quelle position est réactivée à partir de l'activité hippocampique en ligne. Ce décodeur ne nécessite pas de tri des pointes comme la plupart des méthodes de décodage publiées et, plus important encore, il possède une mesure de confiance qui permet à l'utilisateur de filtrer les fausses positions décodées.

MOTS CLÉS

Mémoire – Peur – Sommeil – Hippocampe – L'apprentissage profond – Réseaux neuronaux artificiels

ABSTRACT

Hippocampus is required for encoding and consolidation of declarative memories in humans. In animals, hippocampus-dependent memories are often studied in the paradigm of spatial navigation.

Place cells are hippocampal neurons that exhibit location-specific firing. In calm states, such as sleep and low-speed wakefulness, place cells are reinstating patterns that were observed during active exploration - a phenomenon termed 'reactivations'. There is ample evidence that hippocampal reactivations are neural correlate of spatial memory consolidation. However, it is still not clear whether hippocampus codes for purely spatial information, or it also encodes non-spatial parameters such as emotional valence.

It is known from the literature that rewarded locations are reactivated more often in sleep following active behavior than the others. There are two competing hypotheses that could explain this fact. According to one of them, locations that are associated with certain motivational and emotional values are reactivated more. Other hypothesis postulates that increase in reactivation rate of rewarded environment is caused simply by increased time spent in the reward location.

To tackle this question, we performed two parallel experiments employing aversive and rewarding learning. In one of them, we used aversive intracranial stimulation of periaqueductal gray matter to create aversive spatial association, and in the second one, we used rewarding stimulation of medial forebrain bundle to create appetitive spatial association.

Behaviorally, emotionally important locations had massive difference in terms of occupancy after learning. However, in sleep following learning sessions we have found reactivations both for aversive and for rewarding experience, which confirms the hypothesis that motivational and emotional variables of the task affect hippocampal coding. We have demonstrated that neuronal patterns active during aversive learning are reactivated during sleep following the task stronger than the activity recorded during free exploration. Given that cognitive maps in our task were predominantly stable, we concluded that hippocampal activity during the task and in reactivations registered after the task does not code for pure spatial information but also for motivational salience associated with space. Supporting this claim, we have found that strength of sleep reactivations correlated with the magnitude of avoidance behavior. In addition, we have shown that after aversive learning, despite significantly decreasing time spent in the aversive zone, reactivations mostly consisted of representations of the aversive zone and adjacent locations, possibly reflecting avoidance behavior.

In addition, we have attempted to reverse aversive spatial association by using rewarding intracranial stimulation in the sleep that follows aversive learning. To achieve this goal, we have developed brain-computer interface that is based on the stack of convolutional and recurrent artificial neural networks. In other words, we have designed the position decoder, that will be able to decode which position is reactivated from hippocampal activity online. This decoder does not require spike sorting as most published decoding methods, and more importantly has a confidence measure that allows the user to filter out spuriously decoded positions.

KEYWORDS

Memory – Fear – Sleep – Hippocampus – Deep learning – Artificial neural networks