Charles University in Prague
Faculty of Science

Study programme: Biology
Branch of study: Animal physiology

Bc. Daniela Černotová

Role of ventral hippocampus and medial prefrontal cortex in behavioral flexibility in rodents

Role ventrálního hipokampu a mediální prefrontální kůry v behaviorální flexibilitě u hlodavců

Diploma thesis

Supervisor: RNDr. Jan Svoboda, Ph.D.

Prague 2020

PROHLÁŠENÍ

Prohlašuji, že jsem svou diplomovou práci vypracovala samostatně a že jsem uvedla veškeré použité informační zdroje a literaturu. Na získání a vyhodnocení výsledků se podílel RNDr. Jan Svoboda, Ph.D. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze dne 30.5.2020 ……

Podpis

PODĚKOVÁNÍ

Ráda bych poděkovala svému školiteli RNDr. Janu Svobodovi, Ph.D. za spolupráci a trpělivost, s kterou mě provázel v průběhu celého magisterského studia. Zároveň děkuji za podněty ke zkvalitnění obsahu diplomové práce, pomoc při statistickém vyhodnocování a ochotu konzultovat veškerou problematiku. Chtěla bych poděkovat také Michaele Radostné za asistenci během perfuzí a všem kolegům z oddělení Neurofyziologie paměti za přátelské pracovní prostředí. V neposlední řadě mé poděkování patří všem, kteří mě během mých studií podporovali.

Tato práce byla podpořena granty GACR 17-04047S a AZV 17-30833A.

ABSTRAKT

Adaptace na neustále se měnící prostředí je vlastností nutnou pro přežití všech volně žijících živočichů, ale i zásadním předpokladem pro každodenní interakce s okolním světem. Hlavní neurální substráty zajišťující kognitivní a behaviorální flexibilitu jsou již dobře známy a spočívají především na vzájemné komunikaci mezi prefrontální kůrou (PFC) a striatem. Některé části PFC jsou hojně inervovány ventrálním hipokampem (vHPC), což může výrazně ovlivňovat její funkce. Odchylky v hipokampo-prefrontálním spojení se navíc ukazují být významným faktorem narušujícím kognitivní funkce u některých neuropsychiatrických onemocnění. To je také důvodem, proč je přesná funkce tohoto spojení podstatnou složkou současného výzkumu.

Cílem této práce bylo otestovat roli vHPC a mediální PFC (mPFC) v úloze aktivního vyhýbání se místu na rotující aréně 1) ve dvou variantách testujících flexibilní chování – reversalu a set-shiftingu – a 2) ve vybavení si naučené úlohy. Dané struktury jsme inaktivovali kombinací jednostranných, oboustranných nebo kombinovaných lokálních injekcí muscimolu (agonisty $GABA_A$ receptorů). V počátku reversalu se přizpůsobování novým podmínkám zhoršilo u zvířat s inaktivovaným vHPC, přičemž inaktivace mPFC neovlivnila žádnou z úloh. V úloze testující vybavení bylo pozorováno narušení u obou skupin jak oboustranně inaktivovaných, tak kombinovaně inaktivovaných zvířat, na rozdíl od jednostranných, kde se neprojevily žádné deficity. Výsledky potvrzují obecně přijímaný názor, že se vHPC podílí na behaviorální flexibilitě, a to především v úlohách, kde je k tomu potřebná navigace v prostoru. Překvapujícím bylo zjištění, že se mPFC v těchto situacích nejspíše neuplatňuje.

Klíčová slova: behaviorální flexibilita, muscimol, inaktivace, hipokampo-prefrontální spojení, kolotočové bludiště, rotující aréna, psychiatrické onemocnění

ABSTRACT

Behavioral adaptation to a continuously changing environment is critical for the survival of the animals, but also day-to-day interactions in the human world. The main components maintaining flexibility in cognition and behavior are well-established and depend mostly on proper intercommunication within the prefrontal cortex (PFC) and striatum. Some parts of the PFC are densely innervated by the ventral hippocampus (vHPC), which has a great impact on its functioning. Also, hippocampal-prefrontal circuit dysfunction has been shown to disrupt the integrity of flexible cognition in some neuropsychiatric diseases. Therefore, the exact functional role of this pathway is an indispensable part of the research.

The aim of this study was to test the role of the vHPC and the medial PFC (mPFC) in an active place avoidance task on a rotating arena in 1) two flexibility task variants – reversal learning and set-shifting – and 2) the spatial memory retrieval. We inactivated these structures by muscimol ($GABA_A$ receptor agonist) in a variety of unilateral, bilateral, and combined local injections. Disrupted performance was apparent in reversal learning in vHPC-inactivated rats. No effect was seen in mPFC-inactivated rats. Impairments after the task acquisition were observed in bilateral vHPC and mPFC inactivations and both ipsilateral and contralateral mPFC-vHPC inactivations, but not in unilateral vHPC/mPFC inactivations. These results confirm the notion that the vHPC participates in some forms of behavioral flexibility, especially when spatial cues are needed. Regarding flexibility, it seems rather unexpectedly that the mPFC is not taxed in this task.

Key words: behavioral flexibility, muscimol, inactivation, hippocampal-prefrontal pathway, Carousel, rotating arena, psychiatric illness

LIST OF ABBREVIATIONS

AAPA	allothetic active place avoidance
ACC	anterior cingulate cortex
aHPC	anterior hippocampus
ASST	attentional set-shifting task
AVP	arginine vasopressin
AP	anterior-posterior axis
BI	bilateral
CA	cornu Ammonis
CONTRA	contralateral
CRH	corticotropin-releasing hormone
DG	dentate gyrus
dHPC	dorsal hippocampus
dlPFC	dorsolateral prefrontal cortex
EC	entorhinal cortex
EDS	extradimensional set-shift
FF	fimbria/fornix
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
HPC	hippocampus
ID/ED	intradimensional/extradimensional
IDS	intradimensional set-shift
iHPC	intermediate hippocampus
IL	infralimbic cortex
IPSI	ipsilateral
MD	mediodorsal thalamus
ML	medial-lateral axis
mPFC	medial prefrontal cortex
MWM	Morris water maze
MUS	muscimol
NAc	nucleus accumbens
NVHL	neonatal ventral hippocampal lesion
OFC	orbitofrontal cortex
PBS	phosphate-buffered saline
PFC	prefrontal cortex
PL	prelimbic cortex
PTSD	posttraumatic stress disorder
PVN	paraventricular nucleus
RE	nucleus reuniens
SUB	subiculum
SAL	saline
UNI	unilateral
vHPC	ventral hippocampus
vlPFC	ventrolateral prefrontal cortex
vSUB	ventral subiculum
VTA	ventral tegmental area
WCST	Wisconsin card sorting test

TABLE OF CONTENTS

THEORETICAL PART

1 INTRODUCTION

For most people, it is natural to selectively pay attention to different objects, abandon old rules, search for new strategies, and adapt to day-to-day changes in the environment. However, it is not that natural for people with some psychiatric disorders, including schizophrenia or major depression. Many schizophrenic patients struggle with cognitive deficits that so far cannot be treated by any medication. The inability to think coherently and impaired flexibility can undoubtedly complicate the quality of life of an individual.

Over several decades of research, a huge step forward has been made in understanding the neural correlates of flexible cognition, interaction in subserving neural systems and physiological background, however, a lot yet remains to be elucidated. The main brain structures behind flexible thinking are the prefrontal cortex with striatum and specific thalamic nuclei. The dysfunction of these structures is apparent in psychiatric patients on physiological, anatomical, and functional levels. Moreover, aberrations in the ventral hippocampus, a subsection that is essential for the communication between the whole hippocampus and the prefrontal cortex, have also been abundantly described in these patients. Increasing evidence of impairments in communication between the ventral hippocampus and the prefrontal cortex indicates its inevitable role in cognitive flexibility.

2 BEHAVIORAL FLEXIBILITY

Cognitive flexibility is an important component of executive functioning. The term encompasses the ability to shift attention between different features of a stimulus, task sets, strategies, or behavioral responses. Flexible cognition allows for shaping our behavior or intentions in dynamically changing circumstances. Since most of the research has been conducted on animals, it is complicated to study cognitive processes, which are covert for the experimenter. The inner change in animals' cognition is, however, reflected in the modulation of their behavior, that is, on the other hand, well observable. Therefore, it is preferable to use the term behavioral flexibility, as for the ability to stop ongoing behavior and modify it or adopt a new one, depending on the specific conditions.

2.1 Forms of behavioral flexibility

Changes in cognitive and behavioral processes can be divided into several types, depending on the character of the task in which they are experimentally tested. The most assessed forms of flexibility are reversal learning and attentional or strategy set-shifting. **Reversal learning** involves a shift of relevance in one defined set of cues. The cue that was initially reinforced in simple discrimination

now becomes irrelevant, and vice versa, previously non-reinforced cue is newly of high value. The behavioral response usually remains the same – for example, an animal receiving a reward after selecting vanilla odor must now select mint odor, which was being ignored before. This type of flexibility requires an extinction of previously learned stimulus-response association and reinforcement of a new one. Sometimes, the term "reversal learning" is also used in tasks requiring a change of behavior. If an animal initially needs to go right to obtain a reward, in the new set, it must start going left. In both examples, the task rule or the cue type remains in the same dimension, and it is not required to switch attention between more dimensions. Therefore, it is a relatively simple form of flexibility (Brown & Tait, 2016).

In contrast, **set-shifting** is cognitively more difficult to perform, as it requires a combination of sensory perception, working memory and sustained attention. A change can be performed within a whole cue set (one dimension) or between different cue features, tasks, or mental sets (more different dimensions), in which the subject must redirect its attention. Based on this information, we define two subcategories of set-shifting. Firstly, an **intradimensional** set-shift (IDS) involves replacing a whole set of relevant and irrelevant cues for new ones within one dimension (*e.g.,* if one dimension is odor, the change is made by substituting lavender and mint scent for jasmine and rosemary), while an **extradimensional** set-shift (EDS) refers to an alternation between different cues, rules or strategies, depending on currently relevant contingencies (*e.g.,* switch of relevance from substrate texture to odor). The different features of presented cues can be olfactory, visual, tactile or spatial and can be variously combined, especially in challenging spatial tasks, which is one of the main reasons why set-shifting is a powerful tool in animal research – such experimental setup reliably mimics complex situations emerging in everyday human life (Popik & Nikiforuk, 2015).

Another usually assessed forms of flexible behavior are through **extinction**. This phenomenon is happening unconsciously if a conditioned behavior is no longer reinforced with a reward or punishment. A common use of testing this ability is in fear conditioning, when an aversive stimulus, *e.g.,* mild electric shock, is paired with a neutral context, *e.g.,* wall color in the room, or some stimulus, *e.g.,* tone (Milad & Quirk, 2002). If the aversive stimulus is not present anymore, the fear response slowly fades until it disappears entirely. Extinction-like processes are a component of complex behaviors observed in reversals and set-shifting tasks.

There is a considerable body of literature proving all the above-mentioned examples to be anatomically distinguishable. Reversal learning relies on reciprocal communication between the orbitofrontal cortex and striatum, whereas set-shifting is mediated predominantly by medial parts of the prefrontal cortex in rodents (or its dorsolateral homolog in humans) (for a comprehensive review, see Floresco et al., 2009). Parts of the medial prefrontal cortex and the orbitofrontal cortex

(ventral frontal regions in humans) are also the neural correlates of inhibitory control in rodents (Chudasama et al., 2003; Swick et al., 2008; Szatkowska et al., 2007), together with the subthalamic nucleus (Aron & Poldrack, 2006; Phillips & Brown, 2000). Extinction is a complex process dependent upon transfer of information between the prefrontal cortex and the limbic system (Quirk & Mueller, 2008). Despite the named brain structures preferentially driving some subsets of cognitive flexibility, it cannot be concluded that they depend solely on them – to some extent, all components related to flexible cognition overlap and interfere with each other.

2.2 Testing flexible behavior

2.2.1 Digging tasks

Digging tasks allow researchers to assess two forms of flexibility – reversal learning and attentional set-shifting. The original **Attentional Set-Shifting Task (ASST)** was designed by J. M. Birrell and V. J. Brown (Birrell & Brown, 2000). In this paradigm, rats must rely on tactile and olfactory senses, which are generally well developed in rodents. A rat must dig in two bowls, different in texture, odor, and digging medium, and learn to associate a certain rule with a hidden reward. The baited bowl is initially discriminated by a specific odor (*e.g.,* vanilla and cinnamon, with cinnamon being rewarded) and later filled with different mediums (*e.g.,* stone chips vs. wood beads), irrelevant in the meantime. The first reversal learning is presented, with vanilla being now rewarded instead of cinnamon. When the rat successfully learns this change in contingency, an intradimensional shift is made, in which the odors change completely (*e.g.,* from vanilla and cinnamon to mint and thyme, mint being rewarded). Next, the second reversal follows, thyme being rewarded now. Finally, an extradimensional shift is done, where the odor dimension is newly irrelevant, and the rat must suddenly switch attention to the medium, one of them being rewarded (*e.g.,* stone chips). This, again, is followed by the last, third reversal, where the rewarded medium changes. Every part of the task is repeated before getting to the next phase until rats reach six correct consecutive choices, and the number of errors and trials to reach the criterion are analyzed.

The ASST is considered to be the rat version of human **Wisconsin Card Sorting Test (WCST)** or **Intra-Extra Dimensional Set Shift (ID/ED) Task**, that are used to evaluate possible damage of the prefrontal cortex (Drewe, 1974), or eventual dysfunction, commonly observed in autism spectrum disorders (Prior & Hoffmann, 1990), dementias (Paolo et al., 1996) and schizophrenia (Abbruzzese et al., 1995). In the WCST, a person is asked to sort cards based on a previously non-defined rule and must learn to sort them either by number, color, or shape of objects presented on the cards. Several reversals or set-shifts can be performed. The sorting dimension is unpredictably changed, and the person needs to adjust grouping to variable contingencies. The ID/ED task is a digitalized

form of testing used to evaluate cognitive flexibility. The resemblance of the set-shifting paradigm for rodents with human task variants, especially the ID/ED task, posits the ASST to be a suitable way for assessing flexibility in rodents, and therefore, getting more detailed knowledge about the neural basis and the exact role of different components of flexibility (Tait et al., 2018).

2.2.2 Maze-based tasks

Stimuli presented in maze-based experiments are constant during the initial learning and the set-shift. Therefore, animals only need to switch in the discrimination within one set, or shift attention between different perceptual sets, without learning of new stimuli. **The operant chamber** is used to test cognitive abilities regardless of spatial navigation (Floresco et al., 2008). Rats learn to press a lever associated with a visual cue (always press light-signaled), or a specific response (always press left). Set-shifting is conducted simply by changing between visual-cue and response-based strategies.

In order to be successful in completing spatial maze tasks, the animal must integrate spatial navigation and distinct executive functions, along with a continual visual perception of the surrounding environment. Presumably, the most abundant spatial maze variant is **the cross maze**, alternatively its modified T- and Y-maze versions. In the simple discrimination task, an animal learns to go to a specific maze arm, based on visual cues (a star and a moon on the end walls of the to-go arms, the star being relevant), or an egocentric response (always going left). A series of alternations can be made to assess spatial behavioral flexibility. Reversals can be made after initial acquisition by a change in the correct response (always going right), or modification of cue value (a moon, previously ignored, now indicating the right direction). An alternation between strategies is also possible, which enables for testing set-shifting (Torres-Berrío et al., 2019).

The Morris water maze (MWM) is another widely used navigation task in rodents, especially when researching learning and memory. A rat is placed in a water-filled pool, where it must locate an invisible platform by using various cues. Several types of learning can be assessed based on the form of protocol, including discrimination learning, spatial, cued, and latent learning (Vorhees & Williams, 2006). Spatial working memory can be tested by two or more consecutive trials in one day separated by a 15-second pause, with a new starting position of the platform every day. Reversal learning in this paradigm is represented by relocating the platform to the opposite sector after a few days of learning one stable position of this platform, shifts can be made when the position is changed to a different than the opposite sector.

2.2.2.1 Evaluating flexible behavior

Despite each task having its own methods of evaluating, the main analyzed parameters are shared,

for instance, the number of trials to learn a new strategy (Birrell & Brown, 2000), or time to reach a particular criterion (Trivedi & Coover, 2004). Several types of errors can be observed in cognitively demanding tasks (Floresco et al., 2009). After the initial acquisition of the task, the conditions change, and an animal must rapidly adapt to a different situation. To do this mental/behavioral switch, the animal first abandon the currently used tactics. The animal initially continues using previously established, but now incorrect strategy. Afterward, it starts to modulate its ongoing behavior or starts to look for a completely new strategy to get a reward or avoid punishment again. If the animal keeps using an old strategy, then the behavior is scored as a **perseverative error**. This type of error shows us how able the animal is to inhibit the old behavior, and how willing it is to look for a new one. After finding a new strategy, a strengthening of the new behavior starts. If the animal uses a new strategy in 25–75% of trials, the errors are not scored as perseverative anymore, because the animal exerts the new response in more than one-fourth of trials. Instead, they are scored as **regressive,** because the animal is unable to maintain the newly-reinforced strategy, and it keeps returning to the previously-reinforced one. The last type of error occurs when the rat performs a behavior that was **never reinforced**; in other words, it is incorrect in both initial and consecutive training. These never-reinforced errors show us the ability of the animal to cast off ineffective strategies. Doing the mentioned types of errors points to dysfunction in different components of learning.

2.2.3 The Carousel maze

The Carousel is a place avoidance paradigm invented at the end of the 20th century in order to study the activity of hippocampal place cells in rats (Bures et al., 1997), but it can be used for mice as well (Cimadevilla et al., 2001). It consists of a circular arena platform bounded by a plexiglass wall, situated in a room with distal visual landmarks. Other cues can be placed on the wall, together indicating a position of an invisible and forbidden sector. A rat learns to move freely on the arena, except the forbidden sector, stepping into which is punished by a mild shock, delivered to the rat. The sector is usually at a 60° angle with its vertex in the center of the arena. Food pellets can be given through an automated feeder above the arena for increased motivation to move around on the platform. The arena is set to rotate slowly (1 rpm), which allows dissociation of two frames – the arena frame and the room frame. The invisible sector can be situated in one of these frames, which results in different strategy demands. For these purposes, **signs "+" and "-" can be used to define the importance of reference frames** in different tasks (Wesierska et al., 2005).

The most common task to test the cognitive abilities is the active allothetic place avoidance (AAPA) task. The arena rotates, but the to-be-avoided sector remains stationary, *i.e.* the room is the relevant frame, whereas the cues on the arena are irrelevant. The naming for this configuration

setup with respect to the frame relevance is the **Arena- Room+ task**. To avoid the sector successfully, the rat must enhance its locomotor activity and adjust its actions to stay in the "safe zone" on the arena. Under these conditions, only allothetic navigation (by distant landmarks) is being used by the rat. The animal is continuously monitoring its position by a relative distance to the room-frame cues and its position on the arena, while ignoring self-generated cues like excrements or scents (Bahník & Stuchlík, 2015). Developing passive strategies such as freezing or continual small movements after receiving the shock is incorrect, because rats keep getting shocks as the rotation of the arena moves the rat into the forbidden sector (Cimadevilla et al., 2000). With appropriate pretraining, rats are even able to avoid in complete darkness, depending solely on idiothetic orientation, which, as noted above, plays a negligible role in light. However, animals commit relatively high number of errors (Bahník & Stuchlík, 2015).

Alternatively, the configuration can be set to the **Arena+ Room- task** (or passive allothetic place avoidance task). In this task, the sector rotates with the arena. The cues placed on the plexiglass wall now become the relevant frame, while the distal room cues become irrelevant. The rat therefore learns to pay attention solely to the arena cues, one of which indicates the position of the to-be-avoided sector. Besides, the rat can also use self-generated scents to avoid the sector. In this task, the rat is not required to move to avoid the sector; the rat can stay in one place, away from the sector, to avoid getting shocks. Therefore, the rats are usually food-deprived, meaning appetitively motivated to enhance their locomotor activity and collect food pellets that are being dispersed on the arena (Cimadevilla et al., 2000). The active and passive place avoidance tasks can be used simultaneously, as well. At that moment, the rats must pay attention to both reference frames at once (Kelemen & Fenton, 2010).

The rotating arena tasks generate considerable cognitive load in rodents, however, they are very fast in learning the position of the sector; they can acquire the task in a single session and continue to avoid the area even when there is no shock delivered anymore (Bures et al., 1997; Cimadevilla et al., 2000). The next advantage of this task is that both relevant and irrelevant frames are presented from the beginning throughout the whole experiment, so the animal naturally learns to perceive only the currently relevant dimension. Two forms of flexibility can be tested after acquiring the rules of the task. The first is reversal learning, in which the position of the sector is changed to the opposite location, but the task demands remain the same (usually in the Arena-Room+ task). Cardinal directions are used to simplify navigation in the room, thus, if the sector is initially positioned to "north" and is stable relative to the room, in the next phase, it is moved to "south". Set-shifting is the second eventual form of testing behavioral flexibility, in which the frame relevance changes; the animal must switch between navigation objects from the room cues to the

arena cues (or vice versa). The possibility to test this flexibility makes the rotating arena a great tool for researching deficits in cognitive coordination, for example after administration of pharmacological drugs or when modelling neuropsychiatric diseases (Bubenikova-Valesova et al., 2008; Svoboda et al., 2015).

2.3 Neural substrates

Successful adaptation to changing contingencies depends on overlapping circuitries connecting the prefrontal cortex (PFC) with other subcortical structures. Many cognitive functions are dissociated in different regions of the PFC, which together select relevant stimuli, integrate them with experience and emotions, and modify resultant behavior. In humans, the dorsolateral PFC (dlPFC) relates to executive functions, like attention, planning, working memory, or problem-solving, whereas ventral parts of the PFC allow for motor inhibition and verbal communication (Szczepanski & Knight, 2014). The anterior cingulate cortex (ACC) modulates emotional regulation and monitors error and conflict responses, while the orbitofrontal cortex (OFC) mostly mediates motivational processes, and both contribute to decision-making (Carter et al., 1998; Khani, 2014; Szczepanski & Knight, 2014). The PFC as a whole is the key structure involved in cognitive control, that is, the ability to actively use relevant information and guide behavior while ignoring irrelevant stimuli in the presented task, because it functions as the main information processing center, receiving inputs from many subcortical and sensory systems and transferring processed information to other, especially motor behavior driving structures. The most important role in this process has the well-established **thalamofrontostriatal loop**, forming an interconnected network subserving the cognitive flexibility (Cummings, 1995; Tekin & Cummings, 2002). This loop consists of:

1. The frontal lobe and its projections to the striatum – distinct parts of the PFC, including OFC and medial PFC (mPFC), project to the caudate nucleus, putamen and nucleus accumbens, which is followed by projections to globus pallidus or substantia nigra.
2. The basal ganglia and its projections to the thalamus – globus pallidus and substantia nigra target the mediodorsal and ventral anterior thalamus.
3. The thalamus and its projections to the frontal lobe – thalamus sends projections back to the PFC, providing feedback and closing this functional loop.

The thalamofrontostriatal loop has some other open loops that integrate information from distinct brain structures, including the parietal cortex, tegmentum, and limbic system, and that can modulate performance in various circumstances. Frontal and thalamocortical projections are all excitatory glutamatergic, while striatal projections are mostly inhibitory, mediated by γ-aminobutyric acid (GABA) (Tekin & Cummings, 2002). Additionally, the interaction of the

thalamofrontostriatal loop structures is modulated by other neurotransmitters, most notably dopamine, serotonin, and acetylcholine (Cummings, 1995).

Most of the knowledge regarding behavioral flexibility comes from studies on rats and mice. In a general view, when the currently used strategy becomes inappropriate, the PFC, predominantly orbitofrontal and dorsolateral subdivisions, allows for abandoning of the irrelevant strategy and searching for a new one. The dorsomedial striatum discriminates stimuli based on the motivational outcome, and regarding the information provided from the PFC, modulates behavior while the PFC is still searching for new strategies. When a new strategy is found, the striatum facilitates its maintaining (Ragozzino, 2007). Furthermore, the mediodorsal thalamus and the ventral striatum eliminate inappropriate strategies, and, with the help of the dopaminergic system, guide the PFC to hold on a new, correct strategy (see Fig. 1) (Floresco et al., 2009).

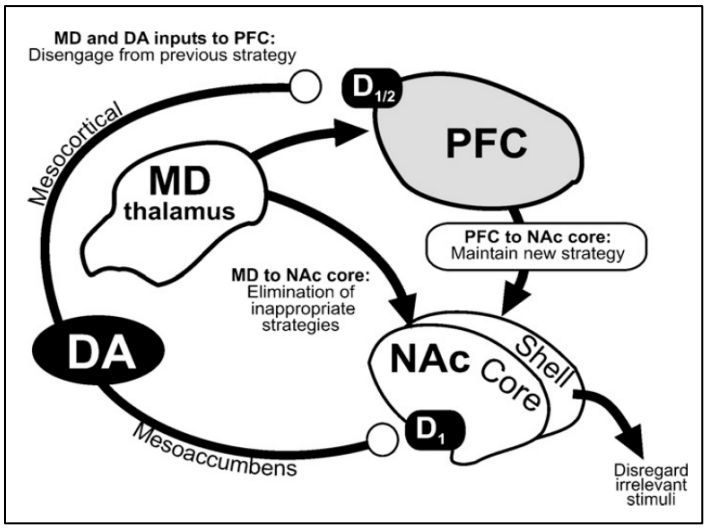

Fig. 1: Schematic of the thalamofrontostriatal loop and its complementary role in behavioral flexibility. Different components are mediated by different neural structures: The PFC is important for abandoning old strategies and searching for a new one, while the NAc and the MD eliminate irrelevant strategies and stimuli, with dopamine modulating this circuitry. **PFC**, prefrontal cortex; **MD**, mediodorsal; **NAc**, nucleus accumbens; **DA**, dopamine; **$D_{1/2}$**, dopamine receptors 1 or 2. From Floresco et al. (2009).

2.3.1 Prefrontal cortex

The PFC is naming for the anterior part of frontal lobes in mammals, critically involved in emotional processing, control of behavior, and mainly in most cognitive functions, including working memory, selective attention, or abstract thinking. The complex of these and other functions, in the end, has a significant impact on our thoughts, modulates our decisions, character, and enables cognitive control. Therefore, even slight structural and physiological changes in the PFC can have serious consequences on its whole functioning.

The PFC is further divided into functionally distinct segments that mediate different forms of flexible behavior. In rats, possibly the most important region is **the medial prefrontal cortex (mPFC)**, which is further divided into three subdivisions, from the ventral to the dorsal direction: the infralimbic cortex (IL), the prelimbic cortex (PL), and the anterior cingulate (ACC) (Hamilton & Brigman, 2015). A notable amount of studies showed that lesioning or inactivating the mPFC

pharmacologically in rats impairs performance in experiments, where an attentional set-shift between strategies, sets, or rules is needed, without impairing reversal learning (for example Boulougouris et al.; Floresco et al., 2008; Birrell & Brown, 2000; de Bruin et al., 1994; Malá et al., 2015; Ragozzino et al., 1999). On the other hand, some results regarding the participation of the mPFC in reversal learning differ from others. Several studies have pointed out that performance in mPFC-lesioned rats is affected in reversal learning by increasing the number of errors before achieving success in the task (Bussey et al., 1997a; Li & Shao, 1998). The authors explain this observation by potential attentional problems of the animals because this cognitive function is crucial for the tasks. Next, lesions of the mPFC can also enhance perseverative errors in reversal learning, proving its importance in inhibiting previously reinforced responses (Kosaki & Watanabe, 2012). Such perseverative errors were also observed in set-shifting tasks (Floresco et al., 2008; Ragozzino et al., 1999). Impairments in reversal learning were also observed in mPFC-lesioned rats in situations when the presented stimuli were hard to discriminate due to their similar appearance, or in later stages of reversal learning, indicating problems with maintaining attention (Bussey et al., 1997a).

As mentioned before, the mPFC plays an indispensable role in switching attention between multiple sets. Evidence attributes attention-switching to the PL and IL cortices (Delatour & Gisquet-Verrier, 2000; Rich & Shapiro, 2007, 2009). These findings are consistent with human studies. Functional magnetic resonance imaging (fMRI) showed increased activation in the ventrolateral PFC (vlPFC) and dlPFC during extradimensional shifts (Rogers et al., 2000; Shafritz et al., 2005), which are considered to be homologous to the rat IL and PL, respectively (Granon, 2000; Seamans et al., 2008). What stays in further research is the exact contribution of the PFC to cognitive control. A recent study by Park et al. (2019) has suggested that mPFC is not necessarily critical for cognitive control, despite the prevailing opinion that the mPFC significantly contributes to information processing and related cognitive functions. They did not observe any impairment in either learning or memory in the AAPA task on the rotating arena. Thus, the spatial representation might be more dependent on other structures, possibly the hippocampus (Kelemen & Fenton, 2010; Lee et al., 2012).

The dorsal part of rodent mPFC, **the anterior cingulate cortex (ACC)**, contributes to set-shifting as well, but differently from the mPFC. Previous research showed that damage to this area leads to more consecutive errors after the task-switching (Rushworth et al., 2002), and general impairments in discrimination, but without perseveration to the previously relevant stimuli (Kosaki & Watanabe, 2012), as seen after damage to the mPFC. Another study, conducted on ACC-lesioned rats, found deficits in the IDS task (Ng et al., 2007). Authors of this study propose that there are two types of attentional processing. First, the ACC is important in guiding attention toward closely

related stimuli within one dimension in combination with suppressing irrelevant information, whereas the mPFC guides attention when the perceptual sets are distinct. The ACC lesions can also disrupt appetitive Pavlovian conditioning — in studies comparing mPFC and ACC, it was observed that only the ACC lesions impaired stimulus-reward learning *per se* (Bussey et al., 1997a; Bussey et al., 1997b). Based on these studies, the ACC might play a more general role in modulating attention, in contrast with the mPFC, which is the main system for paying attention to the relevant set of stimuli. However, as the authors noted earlier, more work is necessary to determine the exact role of these structures in forming new associations, detecting correct choices, and attentional processing.

In contrast with the mPFC and its predominant role in mediating set-shifting, **the orbitofrontal cortex (OFC)** is the main brain region contributing to reversal learning, predominantly by inhibiting previously reinforced stimulus-response associations and allowing for strengthening new ones, which was broadly reported in literature in both rodents (Bissonette et al., 2008; Boulougouris et al., 2007; Ghods-Sharifi et al., 2008; McAlonan & Brown, 2003) and humans (Fellows & Farah, 2003; Ghahremani et al., 2010; O'Doherty et al., 2001). According to the fMRI studies conducted on human participants, the OFC guides behavior by evaluating the rewarding or punishing outcomes. Rats with impaired function of the OFC need a higher number of trials to reach the criterion of successful task completing, albeit they do not incline to perseverative errors, but rather regressive (Ghods-Sharifi et al., 2008; McAlonan & Brown, 2003). When reversing cue-outcome associations in Pavlovian conditioning, the rats with inactivated OFC stopped responding to the previously rewarded cue, but did not start to favor the newly rewarded cue (Burke et al., 2009). A more recent study used atypical reversal learning in an operant chamber; the choices were designed to get a reward with 80% probability if chosen lever A, and 20% probability if chosen lever B (Amodeo et al., 2017). The activity of the OFC neurons was measured during the task. Increased activity of the OFC correlated with selecting the high-probability choice. Thus, the OFC is likely important for using previous experience in subsequent behavior and increasing preference for high-probability option, or growing desire to the newly rewarded cues.

2.3.2 Striatum

The striatum is a subcortical area belonging to basal ganglia, involved mostly in the initiation and planning of movements and behavior (Rolls, 1994), and cognitive flexibility (Floresco et al., 2006). **Dorsal striatum** (consisting of the caudate nucleus and putamen) contributes to flexibility by initiating the need for change and facilitating decision-making based on motivation (Balleine et al., 2007), and maintaining a new strategy in set-shifting (Ragozzino et al., 2002). In a study comparing activation of different brain regions during the WCST, activity in dorsal striatum correlated with

exposure to negative feedback (Monchi et al., 2001). Rats with lesions of dorsomedial striatum were also impaired in reversal learning (Castañé et al., 2010), consistent with primates (Clarke et al., 2008), both showing higher perseveration to the previous task. Dorsal parts of striatum are also critical in extinction learning; extinction of conditioned response was much faster in rats with lesions of dorsal striatum than in controls (Castañé et al., 2010).

Nucleus accumbens (NAc), representing ventral striatum, helps in maintaining novel strategies and eliminating inappropriate ones, especially in set-shifting. This evidence was reported in an inactivation study that was examining the communication between the PFC, NAc and mediodorsal thalamus; inactivation of the NAc-thalamic pathway led to an increase in never-reinforced errors, while inactivation of the PFC-NAc pathway led to more perseverative errors (Block et al., 2007). The NAc can be further divided into core and shell, both having their unique roles in set-shifting. Inactivation of the NAc core resulted in disrupted set-shifting in the cross maze, while inactivation of the NAc shell did not show any impairment (Floresco et al., 2006). Furthermore, if the NAc shell was inactivated before the initial discrimination learning, the rats had better results in the subsequent set shift. Consistent with this study, the NAc core facilitates set-shifting and maintaining of the new strategy, while the NAc shell generally mediates learning about stimuli relevance. Connections of distinct parts of the PFC with the ventral striatum and their implications on cognitive functions were confirmed in humans as well (Morris et al., 2016), and there is a common notion that dopamine release is crucial for balanced frontostriatal communication. Dopamine is being released from striatum when planning of set-shift (Monchi et al., 2006) and positively modulates set-shifting by enhancing frontostriatal communication (Nagano-Saito et al., 2008). Communication within the NAc neurons is also critically dependent on dopamine transmission and balanced functioning of dopamine receptors (Haluk & Floresco, 2009).

2.3.3 Mediodorsal thalamus

The mediodorsal thalamus (MD) has dense reciprocal connections with the PFC (Condé, 1995) and shares some projections with the striatum (Berendse & Groenewegen, 1990). The MD notably supports frontostriatal communication and affects performance in set-shifting tasks. Lesions resulted mostly in increasing perseverative tendencies (Hunt & Aggleton, 1998) and enhancing the number of never-reinforced errors (Block et al., 2007). Bilateral inactivations of the MD disrupted ED set-shifting comparably to combined contralateral inactivation of the MD/PFC or the MD/NAc (Block et al., 2007). Regarding this study, the MD facilitates the abandoning of a previously relevant strategy and helps with eliminating inappropriate strategies.

3 VENTRAL HIPPOCAMPUS

3.1 Basic anatomy

The hippocampus (HPC) is a bilateral brain structure situated in the temporal part of the mammalian brain (Insausti, 1993). It varies in size, but the functions remain relatively the same across species and include memory, learning, spatial navigation, and emotional regulation. Based on different hippocampal inputs and outputs and the size of pyramidal neurons, the hippocampus can be divided into four subfields, CA1–4 (CA is an abbreviation of c*ornu Ammonis*, an earlier name of the hippocampus). The CA4 is often considered to be a part of the dentate gyrus (DG), which is closely attached to the hippocampus. The DG, together with the rest of CA subfields and other adjacent parts – the subiculum (SUB) and the entorhinal cortex (EC) – form a strongly interconnected unitary complex, called **the hippocampal formation**. The EC is the main relay transferring information from the association cortices to the hippocampus and the DG (Witter & Amaral, 1991). In contrast, the CA1 sends afferents via the SUB back to the EC, which sends processed information through fimbria/fornix to other brain structures (Naber et al., 2001). Hippocampal CA consists of pyramidal glutamatergic cells, clustering their bodies in one dense layer, and about 10% of all hippocampal cells are local GABAergic interneurons (Bezaire & Soltesz, 2013; Woodson et al., 1989). The coordinated oscillatory activity of hippocampal cells depends on well-tuned interactions between these inhibitory interneurons and other excitatory cells (Csicsvari et al., 1999; Klausberger et al., 2003).

The hippocampus lies on a dorsal-ventral axis in rodents and other mammals, and posterior-anterior axis in primates. The rodent dorsal part corresponds to the primate posterior, whereas the ventral part to the primate anterior sections. Following these axes, the hippocampus can be anatomically and functionally divided into separate segments that have different gene expression, connectivity with other brain structures, and behavioral functions (see Fig. 2) (Fanselow & Dong, 2010). A general notion is that **the dorsal hippocampus (dHPC, posterior in primates)** is more involved in spatial navigation, learning and episodic memory processing (Moser et al., 1995; Potvin et al., 2006), whereas **the ventral hippocampus (vHPC, anterior in primates)** is mainly implicated in regulation of emotions and motivational behavior (Bannerman et al., 2003; Kjelstrup et al., 2002). Little is known about a third mid-section, **the intermediate hippocampus (iHPC)**. A few studies suggested it might have a distinct role in spatial processing and cognitive functioning (Bast et al., 2009; Kenney & Manahan-Vaughan, 2013). However, it is important to note that the iHPC as a separate unit deserves more attention in the research. There is certain inconsistency in the literature in actual distinguishing between the intermediate and the ventral hippocampus,

which might interfere with some results and interpretations (Fanselow & Dong, 2010).

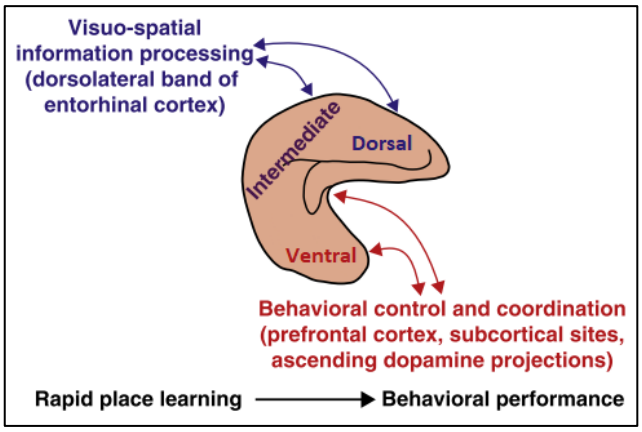

Fig. 2: Functional connectivity of the hippocampal subdivisions. Dorsal hippocampus processes visuo-spatial information by interacting with the entorhinal cortex and enables rapid place learning, whereas ventral hippocampus coordinates behavior by connections with the prefrontal cortex and subcortical structures, and by modulation of dopaminergic mesocortical pathway. Intermediate hippocampus helps in translating acquired spatial information into behavior. Adapted from Bast (2011).

3.2 Mediated functions

3.2.1 Stress, fear, and anxiety

The hippocampus, as a part of the limbic system, shares abundant reciprocal projections with the amygdala (Henke, 1990; Pikkarainen et al., 1999). The vHPC is often mentioned in the context of innate **behavior related to fear and anxiety**, especially in new or potentially dangerous situations. Selective lesions or inactivations of the vHPC have anxiolytic effects and cause disinhibition in behavior. Rats with lesioned vHPC spent more time in open arms of an elevated plus-maze than controls (Kjelstrup et al., 2002). Moreover, they had reduced latency to start eating in tests of hyponeophagia or in entering anxiogenic environments, and they were overall more socially active (McHugh et al., 2004). The vHPC also modulates **conditioned fear expression**. It was reported many times that lesions of the vHPC or the ventral subiculum (vSUB) correlated with reduced freezing in the presence of conditioned tone, or context stimuli (Bannerman et al., 2003; Maren, 1999; Richmond et al., 1999; Trivedi & Coover, 2004). Inactivations of the vHPC also impair renewal of contextual fear after extinction (Hobin et al., 2006). **Reactivating contextual fear expression** was showed to be reliant on active communication between the vHPC and the parvalbumin-expressing interneurons in the mPFC (Marek et al., 2018).

Another situation in which the vHPC comes to the fore is when **coping with stress**. Some individuals are more vulnerable to chronic stress, but others resist more steadily, without any traces of anxious or depressive conditions. The integrity of the vHPC is considered to have an eminent role in stress resilience, not only because of its connections with the amygdala, but also with the hypothalamus (Herman et al., 1992). The vSUB and the ventral CA1 regions can **regulate the hypothalamic-pituitary axis by the tonic inhibition** of neurons containing corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) in the paraventricular nucleus (PVN) of the

hypothalamus (Herman et al., 1992). This was supported by lesion studies of the vHPC, in which lesioned rats had significantly higher CRH-mRNA levels in the PVN after exposure to stress, and an increased concentration of plasma CRH was significantly prolonged compared to the control group (Chrapusta et al., 2003; Herman et al., 1995). Moreover, selective lesions of the vHPC resulted in higher susceptibility of developing stress ulcers under stressful conditions (Henke, 1990). An interesting study was made recently by Pearson-Leary et al. (2017), which revealed clear differences in the vHPC activity between stress-resilient and stress-vulnerable rats. **Higher activity of the vHPC in stress-vulnerable rats** resulted in higher local vascularization and occurrence of pro-inflammatory processes. Additionally, the authors suggest that anti-inflammatory agents reduce vulnerability to stress, which could relieve some individuals suffering from trauma or stressful conditions.

3.2.2 Spatial representation

Spatial processing in the vHPC remains a subject of discussion. The vHPC was initially not considered to be critically involved in spatial learning. Partial lesions of the vHPC in rats did not impair performance in a MWM spatial learning task until the lesion was done to the whole vHPC (in comparison, the dHPC showed first impairments from 20% lesions, and increased with higher volume lesioned) (Moser et al., 1993; Moser et al., 1995). It was reported in other studies that the vHPC was not involved in spatial reference and working memories (Bannerman et al., 2003; Pothuizen et al., 2004; Potvin et al., 2006). In 2003, de Hoz et al. modified the experimental protocol used by Moser et al. (1995). They have shown that the vHPC can affect spatial learning equally to the dHPC if the training protocol is slightly different (4 trials a day for 8 days, compared to previous 8 trials a day for 6 days). Subsequent studies have confirmed that lesions of the vHPC impair spatial learning (Chambers et al., 1996; Ferbinteanu et al., 2003) and spatial memory retrieval (Broadbent et al., 2004; Loureiro et al., 2012). Lidocaine inactivations of the vHPC showed impairment in spatial memory retrieval as well (Floresco et al., 1997; Seamans et al., 1998). More to that, place cells were also observed in the vHPC, but in smaller amount than in the dHPC (Jung et al., 1994). These cells are similar to the dHPC place cells – they code position of the rat in specific place fields, but these place fields are bigger and less specific, and they are considered to encode context, while the dHPC place cells encode more detailed position (de Hoz et al., 2003; Poucet et al., 1994).

A more recent study investigated intercommunication between the dHPC and the vHPC during the MWM task learning in rats. The activity of the hippocampi was locally inhibited by muscimol in bilateral or combined inactivations. The spatial performance was significantly impaired when silencing the whole hippocampi and similarly impaired in the bilateral dHPC and the vHPC inactivations. Ipsilateral inactivations (in one hemisphere) showed only little impairment, but

inactivation of one dHPC and contralateral vHPC resulted in remarkable deficits, resembling bilateral inactivations (Lee et al., 2019). Overall, despite the initial opinion that the vHPC is not involved in spatial learning, newer studies have shown that the vHPC is needed for **spatial learning** and **representation of spatial memories**, and together with the above-mentioned findings, it can be concluded that the whole hippocampus, acting as a unitary structure, is needed for spatial processing.

3.2.3 Cognitive functions

Lately, several studies have started to emphasize the contribution of the vHPC to distinct cognitive functions and cognitive flexibility. The vSUB is the predominant section providing dense innervation of the striatum and projects primarily to the caudate nucleus and putamen (dorsal striatum). A generous number of projections are also sent to medial parts of the NAc (Groenewegen et al., 1987). The vHPC further innervates the mPFC monosynaptically (Jay & Witter, 1991) or via different brain circuits; these connections are described in detail in the next chapter. Floresco et al. (1997) examined functional interconnectivity between these structures in a spatially-cued radial arm maze task in a delayed and nondelayed version by using combinations of contralateral inactivations of vHPC-mPFC or vHPC-NAc. Rats had to collect food pellets in 4 baited arms, the position of which was changed every day (nondelayed task), or in 4 baited arms, which were blocked 30 minutes before the task during the initial training phase (delayed task). Errors were scored as re-entries to the previously baited arms. In the results, the authors concluded that inactivation of vHPC-NAc impaired foraging in the nondelayed version of the task, whereas vHPC-mPFC inactivations impaired performance in the delayed version. Thus, the vHPC is implicated in **short-term memory** and **exploratory and goal-directed behavior**. Brady et al. (2010) followed up this finding and, in the same paradigm, tested adult rats with neonatal lesions of the vHPC (NVHL rats). The performance of these rats was not compromised in the spatial nondelayed task, but the rats were impaired in the delayed task, which similarly points to the disrupted hippocampal-prefrontal pathway. Together with other studies, there is an increasingly accepted view that the vHPC is exceptionally important in the neonatal development of the PFC. Neonatal lesions of the vHPC lead to **working memory deficits** in adult rats, while lesions done to adult rats do not leave an effect (Chambers et al., 1996; Lipska, 2002). The NVHL rats further show deficits in set-shifting, but not in reversal learning or acquisition in the operant chamber, and it generally takes them longer to perform a lever response (Placek et al., 2013). Various studies showed impairments in either **attentional set-shifting or spatial strategy set-shifting** in animals with dysfunctional vHPC (Brady, 2009; Brooks et al., 2012; Torres-Berrío et al., 2019). These impairments were manifested mainly by perseverative errors, indicating problems with abandoning previous strategies and holding on to learned rules,

habits, and behavior. Anatomical alterations supporting the vHPC-mPFC deficits in NVHL rats were also found – the PFC pyramidal neurons have much shorter dendrites and reduced arborization, and the density of dendritic spines is much lower in the PFC, but also in the NAc, compared to healthy rats (Flores et al., 2005; Marquis et al., 2008). The NVHL adult rats resemble many behavioral and neurobiological aberrations that are seen in schizophrenia patients, therefore, it is a valid and often used animal model in research of this illness (O'Donnell, 2012).

3.3 Connections with the PFC

3.3.1 Monosynaptic interconnectivity

A direct monosynaptic vHPC-mPFC pathway was described in both rodents (Jay et al., 1989; Swanson, 1981) and primates (Barbas & Blatt, 1995). The projections lead ipsilaterally through the fimbria-fornix complex. The pathway consists of excitatory glutamatergic pyramidal neurons, projecting from the **ventral and intermediate CA1 and the vSUB to the PL, IL,** and medial orbital parts of the mPFC in rodents (Hoover & Vertes, 2007; Jay et al., 1992; Jay & Witter, 1991). Moreover, some of the vHPC neurons send simultaneous projections to the amygdala (Ishikawa & Nakamura, 2006). Hippocampal axon terminals target pyramidal excitatory neurons (Carr & Sesack, 1996; Jay & Witter, 1991) and parvalbumin-expressing inhibitory interneurons (Gabbott et al., 2002; Tierney et al., 2004) in the mPFC. Stimulation of neurons in the vHPC can evoke responses in both interneurons and pyramidal neurons of the PFC. The interneurons respond faster and with higher action potential frequency than pyramidal neurons, indicating feedforward inhibition in pyramidal cells of the PFC (Tierney et al., 2004). Repeated stimulation of the ventral CA1 can elicit long-term potentiation or long-term depression on synapses with the mPFC *in vivo* (Laroche et al., 1990; Takita et al., 1999).

A more detailed view of synaptic connections between the vHPC and mPFC neurons has been described recently. It has been shown in mice that the vHPC densely innervates neurons in superficial and deep layers of the IL, but only in deep layers of the PL. These projections target cortico-amygdala and cortico-cortical neurons in the IL and only cortico-cortical neurons in the PL (see Fig. 3). They can evoke an action potential in deep cortico-cortical neurons of IL, suggesting this region to be especially sensitive to hippocampal inputs (Liu & Carter, 2018). These anatomical findings confirm that the vHPC could modulate the integrity of the mPFC and fear-related behaviors. Moreover, synaptic plasticity of this circuit **modulates activity in the mPFC**, *i.e.*, learning, emotions, and the final cognitive output.

Whereas the majority of research has been focusing on the vHPC-mPFC pathway as the main direct flow of information from the hippocampus to the PFC, a recent study reported a direct

projection **from the dHPC to the PL** as well (Ye et al., 2017). Few evidences of direct PFC-to-HPC projections have been reported so far. A direct pathway goes from the ACC to the dHPC (Rajasethupathy et al., 2015), or the vHPC (Bian et al., 2019). All these connections seem to modulate retrieval of fear memories and fear generalization (a phenomena where conditioned fear extends the fear response to similar contexts or stimuli), but the exact function and the extent to what it modulates the behavioral flexibility, if at all, remains in further research.

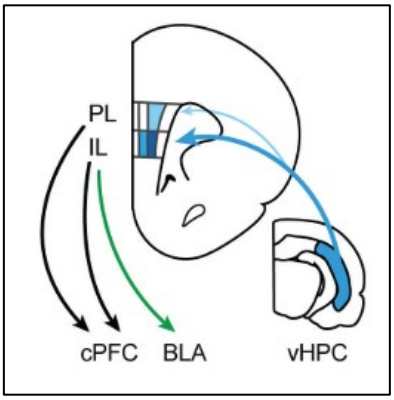

Fig. 3: Direct hippocampal-prefrontal connections. The ventral hippocampus innervates neurons in the prelimbic and infralimbic cortices, which send projections to the contralateral prefrontal cortex (both) and the basolateral amygdala (only the infralimbic cortical neurons). **PL**, prelimbic cortex; **IL**, infralimbic cortex; **vHPC**, ventral hippocampus; **cPFC**, contralateral prefrontal cortex; **BLA**, basolateral amygdala. From Liu & Carter (2018).

3.3.2 Multisynaptic connectivity

Some cells in the mPFC receive convergent inputs from both the MD and the vHPC (Gigg et al., 1994). The lateral EC sends some projections to the MD (Groenewegen, 1988), however, the hippocampus itself does not project to the MD. In this context, more attention is paid to the medial thalamic nucleus reuniens (RE), mainly because 1) most projections from the thalamus to the HPC come from the RE (Bokor et al., 2002), 2) the RE targets the ventral CA1 and the vSUB (Wouterlood et al., 1990), the two subdivisions that project to the mPFC. Lastly, the RE further receives dense projections from all the subdivisions of the mPFC (Vertes, 2002). This creates an **interconnected circuit, with the RE being the mediator** when transferring information from the mPFC to the vHPC (see Fig. 4). A newly published study suggested that the RE might play a critical role in memory consolidation during sleep, as it coordinates the oscillatory activity between the hippocampus and the neocortex (Hauer et al., 2019). Lesioning of the RE showed impairment in flexibility tasks relying on both the HPC and the mPFC, which confirms its involvement in **attentional processes and spatial working memory** (Linley et al., 2016; Viena et al., 2018; Davoodi et al., 2009).

Another circuitry interconnecting the HPC with the PFC goes through the midbrain ventral tegmental area (VTA) and the NAc. The VTA is reciprocally connected with the PFC and the NAc (Beckstead et al., 1979; Carr & Sesack, 2000; Fuxe et al., 1974; Xia et al., 2011) and concurrently sends projections to the hippocampal formation (Scatton et al., 1980). The vSUB can elicit higher activity of dopamine-releasing neurons in the VTA and regulate their firing properties indirectly through connections with the NAc (Floresco et al., 2001). It has been repeatedly shown that the

VTA acts as a modulator of various cognitive functions, including working memory (Martig et al., 2009; Romanides et al., 1999) and learning (Martig & Mizumori, 2011; Takahashi et al., 2009), along with long-term memory consolidation (Ghanbarian & Motamedi, 2013). **Dopamine** is the main neurotransmitter of this circuit, and the modulation of dopaminergic D_1 receptors in the vHPC-mPFC structures is necessary for information transfer and flexible behavior. Delayed recall of information, which is dependent on the vHPC-mPFC circuit (Floresco et al., 1997), requires activation of these D_1 receptors (Seamans et al., 1998). Moreover, infusing D_1 receptor antagonists into the PL/IL enhances perseveration and disables shifting between visual-cue and response cue discriminations (Ragozzino, 2002).

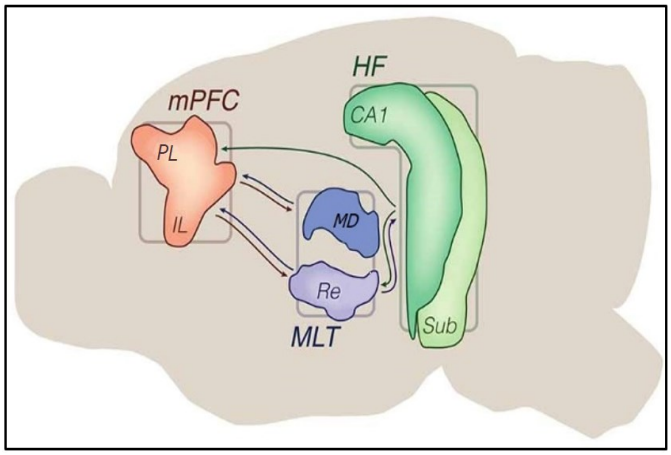

Fig. 4: Interconnectivity between thalamus, hippocampus, and medial prefrontal cortex. The hippocampus innervates the medial prefrontal cortex, while the opposite direction of information transfer goes via nucleus reuniens. **mPFC**, medial prefrontal cortex; **PL**, prelimbic cortex; **IL**, infralimbic cortex; **MLT**, midline thalamus; **Re**, nucleus reuniens; **MD,** mediodorsal thalamus; **HF**, hippocampal formation; **CA1**, cornu Ammonis 1; **Sub**, subiculum. Adapted from Bueno-Junior & Leite (2018).

3.4 Establishing the role of the hippocampal-prefrontal connection in rat studies

The exact functions of the hippocampal-prefrontal circuit are still not well-established. So far, it has been found that pathways between the hippocampus and the PFC are implicated in memory retrieval, spatial working memory, emotional regulation, and flexible behavior. The hippocampus transfers information to the PFC, which converts them into cognitive and motor processes. Floresco et al. (1997) first used a model of asymmetric HPC-PFC inhibition – they pharmacologically inactivated the vHPC and the PL unilaterally in the opposite hemispheres, and, therefore, blocked the information transmission between them (see Fig. 5). As I already mentioned earlier, rats with inactivated vHPC-PL were notably impaired in **memory retrieval** during the delayed task, which indicates impairments in the ability to retain acquired knowledge about the spatial location. In contrast, the nondelayed task did not show any impairment in working memory of vHPC-PL inactivated rats. Wang & Cai (2006) performed similar inactivations in the delayed spatial alternation task in a T-maze. They observed impairments after the vHPC-PL inactivations, which mimicked the performance after doing only bilateral vHPC or PL inactivations. This circuit is, therefore, crucial for **spatial working memory**. Another series of experiments were conducted in rats in order to establish the

role of the HPC-PFC circuit in **recognition memory**. Cooperation between these structures is needed for remembering spatial, temporal and contextual details of the presented objects (Barker & Warburton, 2011). An increased coherence in the activity of the PFC and HPC neurons was observed during a Y-maze rule acquisition (Benchenane et al., 2010). When animals had to make the right choice to obtain a reward, the PFC neurons adjusted their firing activity and synchronized with hippocampal theta rhythm. The use of asymmetric inactivations also disrupted **reward learning** in operant boxes (Izaki et al., 2000). This experiment is supported by findings in humans, showing that the hippocampus is involved in detecting novel stimuli and facilitating subsequent learning (Knight & Nakada, 1998). This indicates that the vHPC also transfers **non-spatial information** to the PFC.

Lastly, modulation of the mPFC by the vHPC is also needed for processing **emotions and behavior related to fear and anxiety**. Given the facts that the mPFC is implicated in fear expression and fear extinction (Corcoran & Quirk, 2007; Milad & Quirk, 2002; Vidal-Gonzalez et al., 2006), and that the hippocampus can dissociate between different contexts (Place et al., 2016), the vHPC could inform the mPFC about dangerous situations and initiate fear expression, or fear inhibition inside the extinction context, respectively. This has been confirmed by asymmetric disconnections of the vHPC-mPFC structures, which disrupted fear renewal outside the extinction context (Orsini et al., 2011), and similar results were obtained by the optogenetically inhibited vHPC-IL pathway (Marek et al., 2018). Additionally, the activity of the mPFC neurons is synchronized with hippocampal theta rhythm in anxiogenic environments (Adhikari et al., 2010, 2011).

Only a few experiments have so far investigated the function of the vHPC-mPFC pathway in **behavioral flexibility**. Impaired set-shifting became a subject of interest in adult NVHL rats, which indicated a significant role of this pathway on the mPFC development (Brady, 2009; Placek et al., 2013). Malá et al. (2015) conducted reversal learning and set-shifting experiments in rats using a T-maze. They examined the effect of independent bilateral or combined bilateral fimbria/fornix (FF)-mPFC lesions on performance in rats. While there was no significant effect of bilateral lesions of the mPFC or the FF in reversal learning, combined lesions resulted in a higher number of errors and more sessions needed to acquire a new rule. In set-shifting, all the groups showed impairment in these criteria (Malá et al., 2015). Other authors investigated behavioral flexibility employing a cross-maze (Torres-Berrío et al., 2019). Tetrodotoxin, a voltage-gated sodium channel blocker, was used for bilateral inactivations of the vHPC or the mPFC in an allocentric/egocentric spatial strategy switching task. The results showed significant impairment in **shifting between navigational strategies** when the vHPC was inactivated, but not in the initial learning. The impairment was manifested as a higher number of perseverative errors. The results resembled deficits that were observed after the inactivation solely of the mPFC.

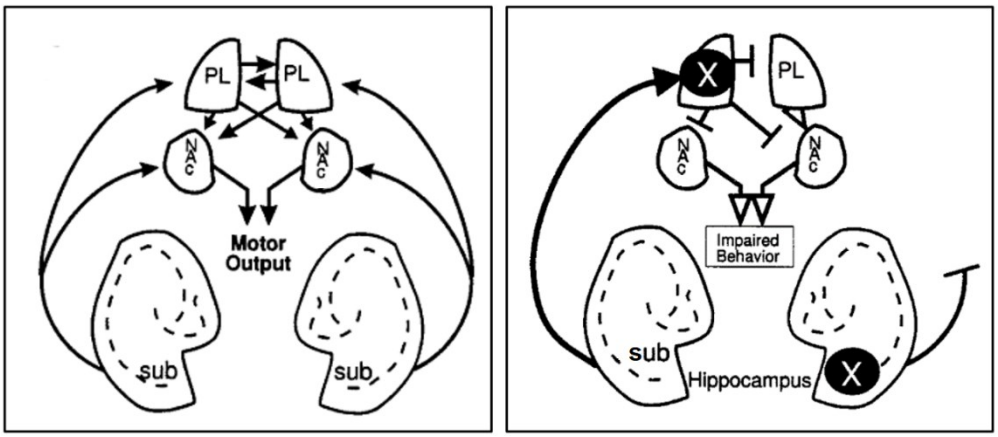

Fig. 5: Diagram of anatomical connections and functional impairment after asymmetric inactivations between the hippocampus, prefrontal cortex, and striatum during the delayed spatial win-shift task. Hippocampus transfers spatial information about food location to the nucleus accumbens and the prelimbic cortex. The hippocampal-prefrontal pathway helps in recalling the information after a delay, and the prelimbic cortex transfers the information to nucleus accumbens, which converts them into proper behavioral response (left). If the information transfer between the hippocampus and the prefrontal cortex is blocked (inactivation in one ventral hippocampus and contralateral prefrontal cortex), knowledge about previous food location cannot be processed and performance during the task is impaired (right). **PL**, prelimbic cortex; **NAc**, nucleus accumbens; **sub**, subiculum. Adapted from Floresco et al. (1997).

4 DEFICITS CAUSED BY ABERRANT HPC-PFC INTERACTION

Getting older is an inevitable process of every long-living organism and is naturally accompanied by worsening of cognitive flexibility. Nevertheless, it is important to dissociate this well-founded decline from pathophysiological conditions, often co-occurring with other features, given the specific psychiatric illness. The hippocampus is a brain structure sensitive to external and internal environmental changes and, considering its projections with the PFC, alterations in this pathway are unavoidable and lead to deficits in attentional processes, working memory, episodic memory, and emotional regulation. On top of that, disruption of the hippocampal-prefrontal pathway is likely the symptomatic basis for some psychiatric illnesses. In the following lines, I will describe some of these impaired functions, lying upon the dysfunctional state of the HPC-PFC pathway.

4.1 Schizophrenia

Developing schizophrenia is dependent on genetic predispositions in combination with sociological and environmental factors, for example, prenatal exposure to viral infection, immune dysregulation, or facing stressful events during the lifetime. All these factors sum up and lead to a higher risk of evolving schizophrenia, however, the main trigger for the illness onset is unknown. The symptoms are classified into three groups: positive (*e.g.,* hallucinations), negative (*e.g.,* apathy), and cognitive, which cover a great variety of deficits. They manifest as impaired attention, working memory,

planning, and decision making, but also as disrupted processing of verbal information and deficient verbal expression of thoughts (Bowie & Harvey, 2006). Cognitive symptoms can be observed even before the illness onset (Fusar-Poli et al., 2012) and are present in different degrees in most patients (Heinrichs & Zakzanis, 1998; Keefe et al., 2005).

Due to disorganized thinking and disrupted coherence in brain state, schizophrenia patients are significantly impaired in **cognitive flexibility**. They have perseverative tendencies in the WCST and are slower in completing the card sorting, compared to healthy subjects (Everett et al., 2001). The performance in the WCST resembles the performance of people with frontal cortical damage, pointing to physiological or structural changes in frontal lobes (Haut et al., 1996). Patients are also considerably impaired in the ID/ED task. More trials are needed to reach the criterion in the ID/ED task in simple discrimination, reversal learning, IDS and EDS, and they make more errors in IDS stages than the control group, albeit the range of impairment is variable across patients (Pantelis et al., 1999). Some are successful in learning the IDS task, showing only mild perseverative deficits, but they may not be able to set-shift in the EDS task, while others are not even able to perform the IDS task, pointing to a more general impairment in cognitive functioning. Impulsivity observed during the tasks also indicates the failure of inhibitory mechanisms. Moreover, schizophrenia patients are impaired in most tests sensitive not only to frontal, but also temporal lobe damage (Kolb & Whishaw, 1983).

Ample evidence links the primary features of schizophrenia with hippocampal-prefrontal pathophysiology. **Anatomical and structural changes** are observed in their frontal lobes and hippocampi. People with schizophrenia have reduced volume of the anterior hippocampus (aHPC, corresponding to the vHPC in rodents) and the OFC (Schobel et al., 2009) and reduced density of gray matter in the dlPFC (corresponding to mPFC in rodents) (Cannon et al., 2002). *Postmortem* studies revealed a significant reduction of dendritic spines in pyramidal cortical neurons (Garey et al., 1998). These could also be the reason for lower gray matter density, rather than loss of neurons. Moreover, schizophrenic patients have disrupted GABA signaling. They have a lower number of parvalbumin-containing interneurons in the PFC and the HPC (Beasley & Reynolds, 1997; Zhang & Reynolds, 2002), and metabolic alterations in the GABA synthesis and reuptake have been observed (Volk et al., 2001). Besides, **abnormal activity of the HPC itself can disrupt the integrity of the PFC.** Excessive dopamine release is one of the theories explaining behavioral and cognitive abnormalities in schizophrenia. This can be partly because of dysfunction in the vSUB, whose activation leads to the spontaneous firing of dopaminergic VTA neurons, which project to the PFC (Floresco et al., 2001). Furthermore, early and prodromal stages of schizophrenia show local hyperactivity in excitatory HPC and PFC neurons, which can be the cause of impaired GABAergic signalization (Anticevic et al.,

2015; Heckers & Konradi, 2015). There is also a theory that hippocampal disinhibition leads to compensational mechanisms, excitotoxicity and eventual atrophy and hypoactivity, characteristic for chronic stages of schizophrenia (Chan et al., 2011; Schobel et al., 2013). The notion that the hippocampal pathology functionally modulates the activity of the PFC is well demonstrated in a study of 9 monozygotic twins, one of each suffering from schizophrenia (Weinberger et al. , 1992). The volume of the aHPC positively correlated with the rate of blood flow and activation of the dlPFC in the WCST task – the more the schizophrenia-affected twin differed from its healthy twin in the aHPC volume, the more hypoactivation of the dlPFC was observed during the task. Aberrant functional HPC-PFC connectivity can be observed in working memory tests as well (Wolf et al., 2009).

Given the fact that schizophrenia patients have problems with **emotional regulation**, one study showed that not all of them can acquire an autonomic fear response to conditioned stimuli. They also show higher autonomic responses to unconditioned stimuli in comparison with healthy subjects (Maren & Holt, 2004). Moreover, they are not impaired in extinction learning but show deficits in context-dependent recall in the following day, which, once again, relates to disruption in the hippocampal-prefrontal connectivity (Milad & Quirk, 2002).

4.2 Other diseases

More psychiatric diseases have been linked with aberrant hippocampal-prefrontal functioning. For example, abnormal emotional regulation is apparent in **major depression** and **posttraumatic stress disorder (PTSD)**. People suffering from these conditions have significantly smaller volume of the HPC (Bearden et al., 2009; Karl et al., 2006), and they show deficits in working memory (Christopher & MacDonald, 2005; Honzel et al., 2014) and functional connectivity during sleep memory consolidation (Genzel et al., 2015). In a study conducted on a depression model in rats, it was suggested that desynchronization of the PFC with hippocampal theta rhythm might be the result of this impairment and disrupted neural plasticity in depressed patients (Zheng & Zhang, 2015). Similarly, PTSD patients show abnormalities in this pathway, especially in fear-related tasks. During extinction recall, the HPC-PFC structures showed decreased activation, which could lead to impaired contextual processing and hypersensitive emotional response (Milad et al., 2009). These abnormalities might arise from a prior stressful situation. Both the mPFC and the HPC are sensitive to increased concentrations of corticosterone, a hormone that is released during stressful situations (Dominguez et al., 2014). Exposure to stress can inhibit long-term potentiation (Rocher et al., 2004), induction of which is critical for extinction (Farinelli et al., 2006). Disruptions in flexible cognition, including abnormalities in the HPC-PFC structures, are also seen in neurodegenerative diseases, especially **Alzheimer's disease**. Decreased hippocampal volume (Shi et al., 2009) and cognitive rigidity are common hallmarks of this condition (Johnstone et al., 2002).

Cognitive flexibility is very sensitive to the morphological and physiological changes and damage of its subserving neural systems. **The given evidence links frontotemporal aberrations to a variety of disorders and illnesses**. Therefore, it is important to elucidate the exact functional role of this circuitry. Extensive knowledge of functional connectivity and physiological mechanisms can help us detect pathologies in the early stages of these illnesses and may facilitate searching for better-designed therapies, leading to amelioration of distinct symptoms, or slowing the further progress of the specific disease.

EXPERIMENTAL PART

5 AIMS OF WORK

The first aim of this work is to establish the role of the mPFC and the vHPC in behavioral flexibility with the use of two cognitively demanding tasks – reversal learning and set-shifting. The second aim is to test the role of these structures in spatial memory retrieval. The main hypotheses are that 1) inactivations of the vHPC will disrupt flexible behavior and impair performance on the arena, 2) the results will be similar with the inactivation of the mPFC, 3) inactivations of either the mPFC or the vHPC will not disrupt performance in the spatial memory retrieval.

6 MATERIALS AND METHODS

6.1 Subjects

Male Long-Evans rats (n=126) in the age of 3–4 months acquired from the Institute of Physiology's breeding colony were used for the experiments. Rats were housed in plastic cages by two and kept at 12/12 h light/dark cycle (lights on at 7 am), with food and water available *ad libitum*. At least a 10-day acclimatization period was given to the animals before surgery. 3 days before the experiment initiation, the animals were food-restricted, and during the experiments weighted daily and maintained at 85–90% of their *ad libitum* body weight. A day before the first learning session, they were implanted a needle through a skin fold between shoulders, which was twisted on the tip to prevent it from slipping out. This procedure was painless (comparable to subcutaneous injections in humans) and did not require anesthesia.

Twenty rats had problems with the initial Arena- Room+ task acquisition (more than 10 entrances to the shock sector on the 5th day or manifesting inappropriate behavioral strategies) and were excluded from the experiments. Seven rats were excluded because of repetitive motor behavior after the application of muscimol during the inactivation days. Five rats had their implants detached during the experiments and were euthanased immediately. Two rats were excluded additionally due to inaccurate cannula localization. None of these rats was included in the data analysis. All animal treatment complied with the Czech Animal Protection Act and EU directive 2010/63/EC.

6.2 Surgical procedures

14 days before the start of behavioral training, the rats were anesthetized with continuous-flow isoflurane (5% for induction, 2–2.5% for maintenance) and mounted in a stereotaxic apparatus. An incision was made to expose the skull, and two holes (1 mm in diameter) were drilled (relative to bregma) at +3.2 AP and ± 1.3 ML for the prelimbic mPFC, or -5.2 AP and ± 5.4 ML for the vHPC-

aiming cannulas. The guide cannulas were custom-made from stainless steel (22-gauge, 11 mm in length), and implanted below the skull surface, 3 mm for the mPFC (at 10° angle from the midline) or 6 mm for the vHPC (vertically). Dummy cannulas were inserted into the guide cannulas and remained there until the injections were initiated. Two (for the mPFC or the vHPC groups) or four (for the combined groups) anchoring skull screws were mounted to the skull, and, together with the cannulas, embedded with dental cement. The wound was sutured, and a local antiseptic and anesthetic were applied to prevent infection and pain. Postoperative care was provided by adding antibiotics and analgesics to the drinking water. The rats were checked daily and were left to recover from the surgery for at least 14 days.

6.3 Inactivation procedures

Muscimol (1 μg/μl, diluted in 0.9% saline, stored at -20 °C; Sigma Aldrich) was used as the inactivation agent in the experiments. It is a $GABA_A$ agonist that enhances inhibitory activity in the brain, which in result temporarily inactivates neurons, with a duration of several hours (Martin, 1991). For the infusion, a 5 μl Hamilton syringe was used, connected by a polyethylene tube with an injection cannula (27-gauge, 12 mm in length; protruding 1 mm below the guide cannulas). One infusion of muscimol was administered to the rats 1 day before the onset of behavioral training to habituate them to the drug exposure. Other injections were given in the inactivation days, 20 mins prior to each session. A volume of 0.5 μl of muscimol was injected slowly (1 min duration) into the appropriate structures, dependent on the experimental group (described below in “Experimental design and groups”), and the injection cannula was removed after another 30 secs to avoid reflux of the solution. Dummy cannulas were placed back inside the guide cannulas when the injection was completed.

6.4 The Carousel maze

The apparatus consists of a circular metallic platform (82 cm in diameter with a 5 cm high outer border, elevated 100 cm above the ground), circumscribed by a 50 cm high transparent plexiglass wall, and a motor providing the arena rotation (1 rpm, clockwise). An infrared LED is attached to a harness laced on the rats' back, and another infrared LED is mounted on the outer edge of the arena. These allow a camera (mounted above the arena) to monitor the position of the rat relative to the room and the arena. A tracking system (Track Analysis, Biosignal Group, USA) was used to record the location and deliver mild shocks to rats during sessions if they entered a forbidden 60-degree sector. The shocks (50 Hz, 500-ms intervals, 0.3–0.6 mA) were delivered via an electric cable ending with a clip attached to the needle implanted in rat’s back. The intensity of shocks was adjusted individually by an experimenter, dependent on the rat’s response to the aversive stimulus. A feeder

with barley grains was rotating slowly above the arena and let grains fall on the platform. All recorded data were stored to the computer for further analysis.

6.5 Experimental design and groups

Both experiments were conducted during the light phase of the day in a room with dimmed light. Navigation in the room was guided by various room cues (a table, a cabinet, a door), whereas position on the arena was indicated by distinct cue cards placed on the plexiglass arena wall, different in color, shape, and pattern. The initial configuration of the room and arena cues was the same for all animals. Before the initial training, the animals were given 2 days of 5-minute handling and 5 minutes of arena acclimation (no shocks or rotation) with grains falling from the feeder to avoid hyponeophagia during the behavioral testing. Then, the Arena- Room+ task training followed (arena rotating with the shocked sector placed in "north"), and it consisted of 5 sessions (1/day, 20 mins in duration). After each session, the arena was wiped with alcohol to eliminate odor marks made by animals. The feeder was active during the experiments to enhance locomotion of the animals on the arena. After the initial 5 days of acquisition, the conditions differed for two separate experiments (see Fig. 6 for an illustration).

Experiment 1 – Behavioral flexibility

This experiment tested the ability to coordinate behavior with inactivated vHPC or mPFC in two cognitively demanding tasks, in which the conditions for successful performance changed. Following 5 days of acquisition of the Arena- Room+ task, the position of the shock sector was reversed from "north" to "south" and rats had to learn to avoid the new location. Rats typically try to avoid both sectors first, but they gradually stop avoiding "north" and avoid only "south". This phase consisted of 5 reversal learning days. After the reversal learning, the Arena+ Room- task followed, in which the reference frames were changed for another 5 days. The sector was newly set to rotate with the arena, and the rat had to set-shift its attention – ignore newly irrelevant room cues, and mind its position relative to the arena cue cards, which indicated the location of the newly forbidden sector. In this task, rats have two options of behavior: they can either learn the position of the sector and move along the arena while collecting grains outside the sector, or, they can stay in one location without moving at all, which frees them from delivering any shocks, albeit they will not eat any grains. The latter strategy makes this task more complicated to analyze – the rat does not give the experimenter feedback about the actual learning of the task. Therefore, our rats were food-deprived and motivated to move and feed on the grains.

43 rats with bilateral cannula implants in the mPFC (n=23) or the vHPC (n=20) were used in this experiment. They were randomly divided into muscimol and saline groups before the experiment initiation. In the first 2 days of reversal learning and set-shifting, muscimol or saline injections were administered to these rats (0.5 µl to each cannula depending on the group). The next 3 days of learning in both phases were used to balance the eventual group differences.

Experiment 2 – Spatial memory retrieval

This experiment tested the ability to retrieve learned behavior in the Arena- Room+ task. 49 rats with bilateral cannula implants in the mPFC (n=18), vHPC (n=17) or both structures (n=14) were used in this experiment. They were randomly divided into unilateral and bilateral (for the mPFC and vHPC inactivations) or into contralateral and ipsilateral (for the combined inactivations) groups before the experiment initiation. We assumed that the information was transferred ipsilaterally via both hemispheric pathways of the brain. By the contralateral inactivations, we disconnected the functional pathways in both hemispheres. The inactivated vHPC could not transfer information to the mPFC on one side, while the attenuated activity in the mPFC on another side could not process this information. Ipsilateral inactivations disconnect both structures in one hemisphere, resulting in one dysfunctional pathway, but the pathway in the other hemisphere is enabled to compensate for the impairment. Muscimol injections (0.5 µl to each cannula depending on the group) were administered to the rats one day after the 5-day Arena- Room+ learning, on day 6. One more session followed the day after (day 7), with the injection of saline, and was used to compare the performance with the inactivation day.

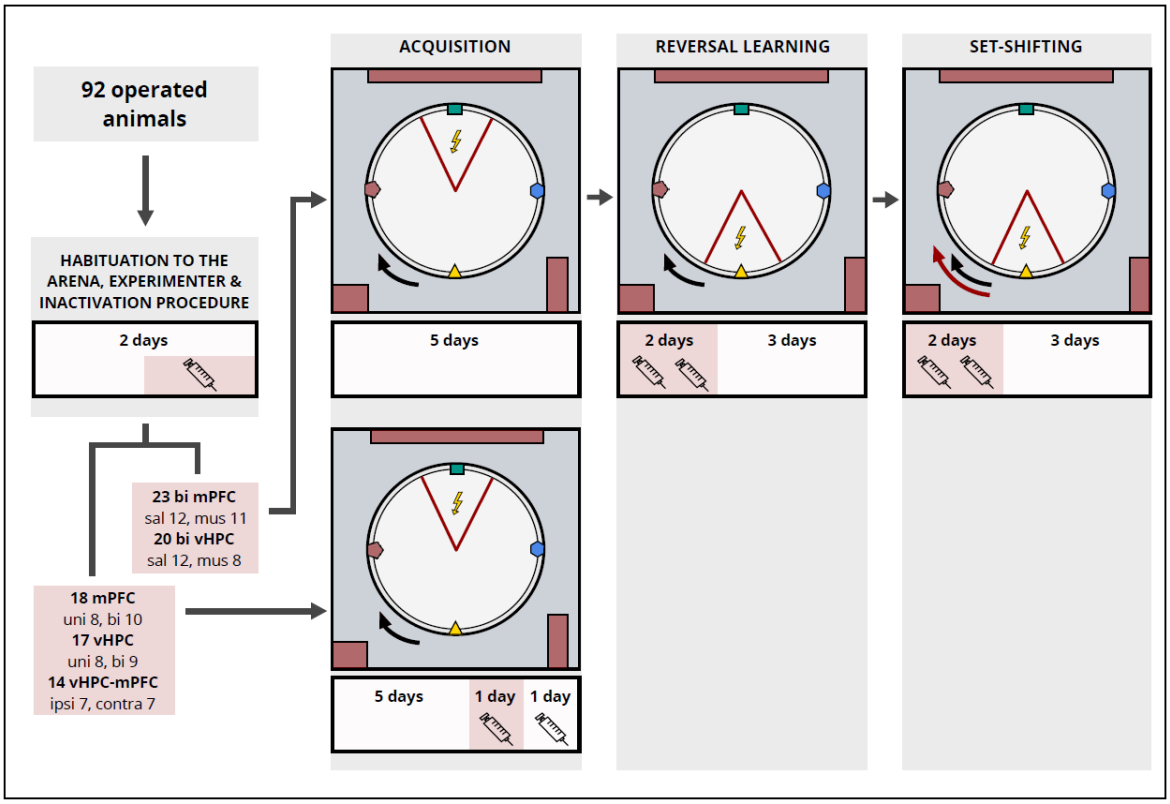

Fig. 6: Experimental design. Operated rats (n=92) were habituated to the arena and the experimenter for two consecutive days. On the second day, they were divided into specific groups based on the experiment and they were given one habituation injection. Then, they were trained for 5 days in the Arena- Room+ task to avoid a specific sector located in "north" (ACQUISITION). In the first experiment, the position of the sector was reversed to "south" for another 5 days and the rats were given muscimol or saline in the first 2 inactivation days of the experiment (REVERSAL LEARNING). The task was then changed to Arena+ Room-, where the sector rotated with the arena for the last 5 days, and the inactivations were administered again in the first 2 days of the new conditions (SET-SHIFTING). In the second experiment, muscimol was infused into the specific brain structures on day 6 in the Arena- Room+ task, with one more session of saline injections on day 7 (ACQUISITION). **sal**, saline; **mus**, muscimol; **uni,** unilateral; **bi,** bilateral; **ipsi,** ipsilateral; **contra**, contralateral.

6.6 Cannula placement verification

Once the rats finished the behavioral testing, they were deeply anesthetized with ketamine and xylazine and perfused intracardially with 0.1 M PBS (pH 7.4), followed by 300–400 ml of 4% paraformaldehyde diluted in 0.1 M PBS. A 1 µl of ink diluted in PBS was infused with a 5 µl Hamilton syringe and an infusion cannula by an infusion pump (200 nl/min) into each cannula to label the sites of injection. The brains were fixed in 4% paraformaldehyde for 24 hours, then placed in 30% sucrose solution until they sunk, and were stored at -80 °C for further processing. The brains were either cut coronally by blade in the cannula locations to verify the site of ink injection or cut to 50 µl coronal sections using a cryostat. Every 5th section was collected, let dry on gelatin-coated slides and Nissl stained using a standard protocol. The slides were coverslipped using Permount and cannula location was verified in a light microscope.

6.7 Evaluated parameters and statistical analysis

A generous number of parameters can be measured from the Carousel sessions. The primary parameters are the **number of entrances** to the punished sector and the **number of shocks** delivered (1 second lasting shocks are repeated every 500 milliseconds if the animal stays in the sector). **Time to the first entrance** reflects the ability to remember the position from the previous session; **the path to the first entrance** is an analog usable in the arena frame avoidance when the sector rotates with the arena. A higher value in these parameters indicates that the animal remembers to avoid the sector from the previous sessions while moving on the arena. The **maximum time avoided** represents the longest sustained time interval between two entrances, for which the animal was avoiding the sector; it measures if the animal can concentrate and maintain avoidance throughout a session. The **total path** is another important parameter measured in the Carousel. The position of the rat is recorded every 40 ms. The total path is calculated as the sum of distances between each two positions recorded 1 second apart. Locomotion is essential for avoiding the sector in the active Arena- Room+ task, but in the passive versions, staying in one location is a possible strategy. Because low locomotion makes further analysis hard to interpret, we motivate the animals to enhance their locomotion by giving them grains throughout the session. In contrast, hyperlocomotion can lead to higher entrances, despite the animal knowing the position of the sector. **Thigmotaxis** is a reliable index of anxiety, analyzed from the position of the rat on the arena during the session. It is calculated as a proportion of time spent within the annulus of 20% radius width from walls. A higher value means that the rat stays mostly near walls and does not explore the center of the arena. This can be observed commonly at the beginning of behavioral testing. We decreased thigmotaxis by dispersing grains on the platform, which motivated the rat to move across the whole arena. The proportion of **time spent in specific sectors** can also be computed. The values are described in the interval of 0–1; 0 meaning the rat was not in the sector during the session, 1 meaning the rat moved only in the analyzed sector. Because the arena was divided into 6 quadrants, 1 of which forbidden, the mean time that the animal would spend in one sector randomly is 0.167 (approximately 3 minutes and 20 seconds). The proportion of **time spent in the previously forbidden sector** is especially helpful in flexibility tasks as it indicates perseveration.

All data were analyzed by an open-source program Carousel Maze Manager (version 0.4.0.) (Bahník, 2014). Statistical analysis was performed using GraphPad Prism 8 software. Level of significance in the analyses was set at alpha = 0.05. All data were tested for normal distribution and analyzed with repeated measures two-way ANOVA (factors of days or time x groups). Data that were not normally distributed were transformed mathematically to meet the conditions. Sidak's multiple comparison

post-hoc test was used to compare the groups within the individual sessions. Data in graphs show group means and error bars indicate the standard error of mean.

7 RESULTS

From 126 animals, 92 finished the experiments and were included in the statistical analysis. Main parameters tested were the number of entrances and shocks, time to the first entrance, maximum time avoided, time spent in the forbidden sector, thigmotaxis and total path. During the reversal learning and set-shifting, time in the previously forbidden sector was analyzed to evaluate perseveration. In the set-shifting task, distance to the first entrance was measured instead of time to evaluate if the animal remembered the sector position from the previous day.

7.1 Experiment 1 – Behavioral flexibility

The goal of this experiment was to examine the adaptation of bilaterally inactivated vHPC or mPFC rats to changing requirements in avoidance-related tasks. Prior to these tasks, 5 days of acquisition of the initial sector avoidance were performed. All the animals included in the analyses responded to the mild shocks and learned the task with no significant differences between the groups (data not shown). Following the acquisition, the requirements changed twice in consecutive 5-day tasks – reversal learning and set-shifting. The inactivations were performed during the first two days of each phase.

7.1.1 Reversal learning (Arena- Room+)

Animals from both saline and muscimol mPFC groups demonstrated successful reversal learning, as evidenced by the significant effect of days in the majority of avoidance-related parameters when conducting two-way ANOVA on the whole 5-day phase (Tab. 1A). The groups learned the reversed sector location at a similar pace, as confirmed by no significance in the effects of group or interaction in any of the analyzed parameters. The animals gradually lowered the number of entrances and shocks (Fig. 7) and increased the maximum time of avoiding the sector (Fig. 11). As the training proceeded, they increased the time spent in the previously forbidden sector (Fig. 10) and reduced the time in the newly forbidden sector (Fig. 9). Furthermore, thigmotaxis was lowering over the sessions and the total path slightly increased (Fig. 11). No significant effect was seen regarding time to the first entrance (Fig. 11). Most importantly, muscimol inactivations of the mPFC did not have any effect on abandoning the previous position of the sector (Fig. 10) and learning the new one (Fig. 9).

Animals from both control and muscimol vHPC groups learned the task during the 5 days, which is documented in the significant effect of days in most of the avoidance-related parameters (Tab. 1B). Inter-group differences in learning were, however, detected by the significant effects of group in some parameters and one effect of interaction in the maximum time avoided (Tab. 1B). The differences between groups in individual days were analyzed by post-hoc tests and are demonstrated in the graphs below (Fig. 7–11). Compared to the saline-injected rats, the muscimol-inactivated vHPC rats had a much higher number of entrances and shocks on day 2 (Fig. 7). Moreover, they had lower maximum time of avoiding the sector and kept relatively low levels on both inactivation days (Fig. 11), and they spent more time in the newly forbidden sector on day 2 (Fig. 9). The muscimol-inactivated vHPC rats spent less time in the previously forbidden sector, as seen by the significant effect of group, but they did not show any higher or lower rate of perseveration during the inactivation sessions when analyzed by a post-hoc test (Fig. 10). A significant effect of interaction was revealed for thigmotaxis, but post-hoc tests did not show any significant differences (Fig. 11). The inactivations did not affect the total path or time to the first entrance (Fig. 11).

To investigate the dynamics of learning and adaptation to the new forbidden location of the sector in the vHPC groups, we further analyzed 5-minute intervals of the inactivation days 1 and 2, respectively. A significant effect of time was revealed by two-way ANOVA for each of these days, demonstrating within-session learning, *i.e.* the animals reduced the number of entrances and shocks within one session, similarly as the time spent in the forbidden sector (Tab. $1b_{1+2}$). Furthermore, the effects of group and interaction on both days confirmed differences between the saline- and muscimol-treated rats. Compared to the saline group, the muscimol group was significantly worse in the last 5 minutes on day 1 and most of the time on day 2 regarding the number of shocks and entrances (Fig. 8), and the animals spent more time in the forbidden sector (Fig. 9). A significant effect of interaction was revealed for day 1 regarding time spent in the previously forbidden sector (Tab. $1b_1$). Post-hoc tests showed that there was a difference in the last 5 minutes of the day 1 session, where the saline group increased the time spent in the previously forbidden sector, while the muscimol group continued to avoid the sector (Fig. 10).

A) mPFC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	**F (4, 84) = 18.92, p < 0.0001**	F (1, 21) = 0.8523, p = 0.3664	F (4, 84) = 0.9839, p = 0.4209	-
Entrances	**F (4, 84) = 37.34, p < 0.0001**	F (1, 21) = 1.742, p = 0.2011	F (4, 84) = 0.4341, p = 0.7837	ln(y+1)
Shocks	**F (4, 84) = 62.77, p < 0.0001**	F (1, 21) = 3.61, p = 0.0713	F (4, 84) = 0.7291, p = 0.5746	ln(y+1)
Time to the first entrance	F (4, 84) = 1.341, p = 0.6528	F (1, 21) = 0.2268, p = 0.6388	F (4, 84) = 0.6154, p = 0.6528	ln(y+0.1)
Maximum time avoided	**F (4, 84) = 44.02, p < 0.0001**	F (1, 21) = 3.64, p = 0.0702	F (4, 84) = 1.393, p = 0.2434	ln(y+1)
Time in the newly forbidden sector	**F (4,84) = 75.77, p < 0.0001**	F (1, 21) = 4.192, p = 0.0533	F (4, 84) = 0.4372, p = 0.7814	ln(y+0.001)
Time in the previously forbidden sector	**F (4, 84) = 4.868, p = 0.0014**	F (1, 21) = 0.01315, p = 0.9098	F (4, 84) = 0.3959, p = 0.8111	-
Thigmotaxis	**F (4, 84) = 7.012, p < 0.0001**	F (1, 21) = 1.329, p = 0.262	F (4, 84) = 0.5277, p = 0.7156	sqrt(1-y+0.01)

B) vHPC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	F (4, 72) = 2.443, p = 0.0543	F (1, 18) = 1.687, p = 0.2104	F (4, 72) = 0.8206, p = 0.5163	ln(y)
Entrances	**F (4, 72) = 35.79, p < 0.0001**	**F (1, 18) = 7.728, p = 0.0124**	F (4, 72) = 1.21, p = 0.0.3139	ln(y+1)
Shocks	**F (4, 72) = 43.94, p < 0.0001**	**F (1, 18) = 8.977, p = 0.0078**	F (4, 72) = 1.506, p = 0.2095	ln(y+1)
Time to the first entrance	**F (4, 72) = 3.41, p = 0.013**	F (1, 18) = 0.2158, p = 0.6479	F (4, 72) = 0.9983, p = 0.4142	ln(y+1)
Maximum time avoided	**F (4, 72) = 22.95, p < 0.0001**	**F (1, 18) = 11.57, p = 0.0032**	**F (4, 72) = 3.685, p = 0.0087**	ln(y+1)
Time in the newly forbidden sector	**F (4, 72) = 41.47, p < 0.0001**	**F (1, 18) = 7.033, p = 0.0162**	F (4, 72) = 1.708, p = 0.1576	ln(y+0.001)
Time in the previously forbidden sector	F (4, 72) = 1.941, p = 0.1128	**F (1, 18) = 6.421, p = 0.0208**	F (4, 72) = 0.1866, p = 0.9447	-
Thigmotaxis	F (4, 72) = 2.203, p = 0.0772	F (1, 18) = 0.9026, p = 0.3547	**F (4, 72) = 3.691, p = 0.0086**	-

b1) vHPC	Effect of time	Effect of group	Effect of interaction	Transformation
Entrances	**F (3, 54) = 5.433, p = 0.0024**	F (1, 18) = 3.596, p = 0.0741	**F (3, 54) = 4.04, p = 0.0115**	ln(y+1)
Shocks	**F (3, 54) = 8.732, p < 0.0001**	**F (1, 18) = 4.545, p = 0.0471**	**F (3, 54) = 6.152, p = 0.0011**	ln(y+1)
Time in the newly forbidden sector	**F (3, 54) = 6.291, p = 0.001**	F (1, 18) = 3.746, p = 0.0688	**F (3, 54) = 3.238, p = 0.0291**	ln(y+0.001)
Time in the previously forbidden sector	F (3, 54) = 0.5516, p = 0.6493	F (1, 18) = 1.388, p = 0.254	**F (3, 54) = 3.368, p = 0.025**	-
b2) vHPC	**Effect of time**	**Effect of group**	**Effect of interaction**	**Transformation**
Entrances	**F (3, 54) = 5.181, p = 0.0032**	**F (1, 18) = 18.52, p = 0.0004**	F (3, 54) = 1.378, p = 0.2593	ln(y+1)
Shocks	**F (3, 54) = 4.809, p = 0.0049**	**F (1, 18) = 16.42, p = 0.0007**	**F (3, 54) = 2.804, p = 0.0483**	ln(y+1)
Time in the newly forbidden sector	**F (3, 54) = 4.858, p = 0.0046**	**F (1, 18) = 13.8, p = 0.0016**	**F (3, 54) = 3.166, p = 0.0317**	ln(y+0.001)
Time in the previously forbidden sector	F (3, 54) = 0.2871, p = 0.8345	F (1, 18) = 2.493, p = 0.1318	F (3, 54) = 1.320, p = 0.2773	ln(y+1)

Tab. 1: Two-way ANOVA results of the 5-day reversal sessions for the mPFC (A) and the vHPC (B) groups and the detailed 5-minute intervals on days 1 (b1) and 2 (b2) for the vHPC groups. ln = natural logarithm, **sqrt** = square root

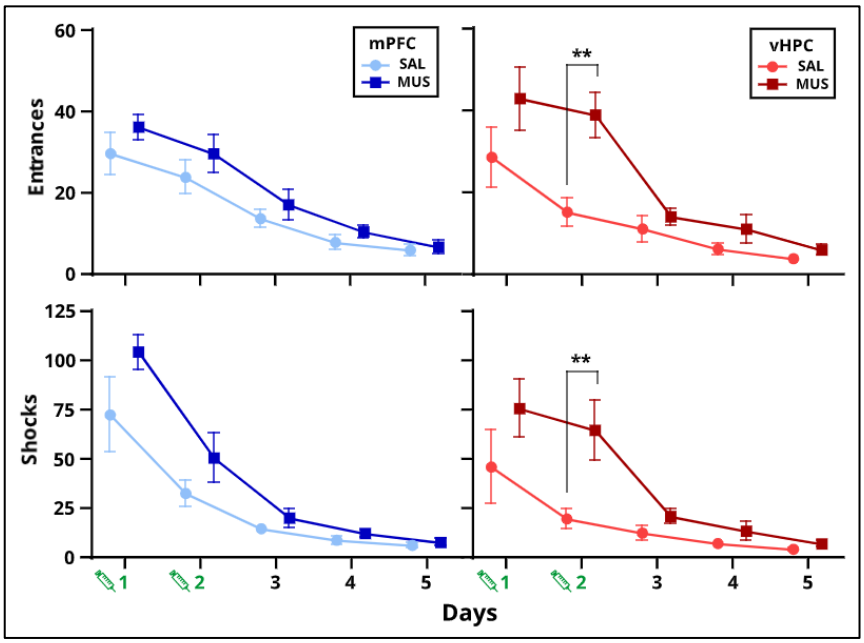

Fig. 7: Number of entrances (top) and shocks (bottom) during the 5-day reversal learning. Animals with the inactivated vHPC had a much higher number of entrances [p = 0.004] to the forbidden sector and were getting more shocks [p = 0.0017] on inactivation day 2. No effect of inactivations was observed in the mPFC group.

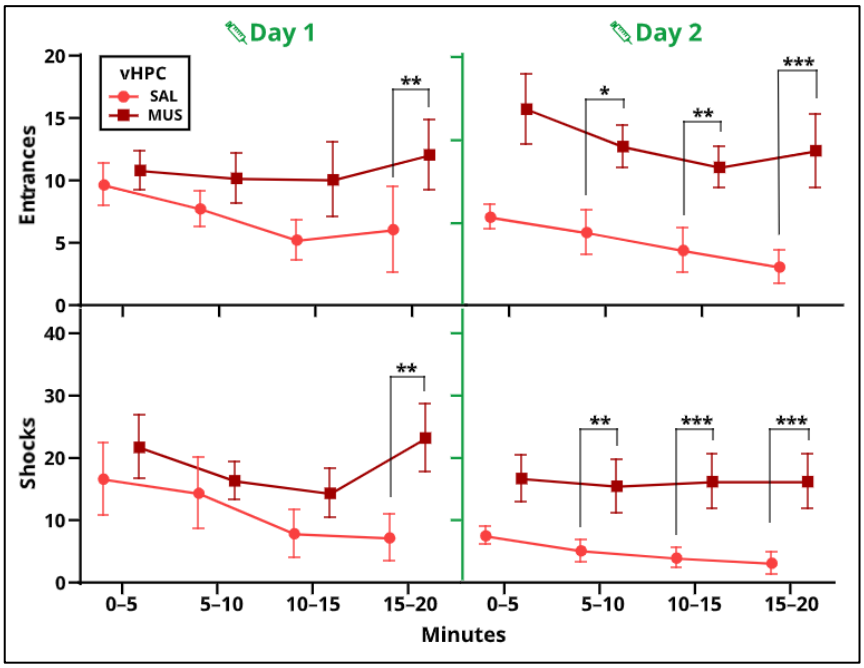

Fig. 8: Number of entrances (top) and shocks (bottom) in 5-minute intervals on days 1 and 2 of reversal learning for the vHPC groups. Saline-treated animals slowly reduced the number of entrances and shocks and learned to avoid the new sector. In contrast, muscimol-treated animals maintained high number entrances and shocks, significantly in the last five minutes on day 1 [entrances: p = 0.0054; shocks: p = 0.0012], and on day 2 in minutes 5–10 [p = 0.0167], 10–15 [p = 0.002] and 15–20 [p = 0.0003] for entrances, or minutes 5–10 [p = 0.01], 10–15 [p = 0.0006] and 15–20 [p = 0.0002] for shocks.

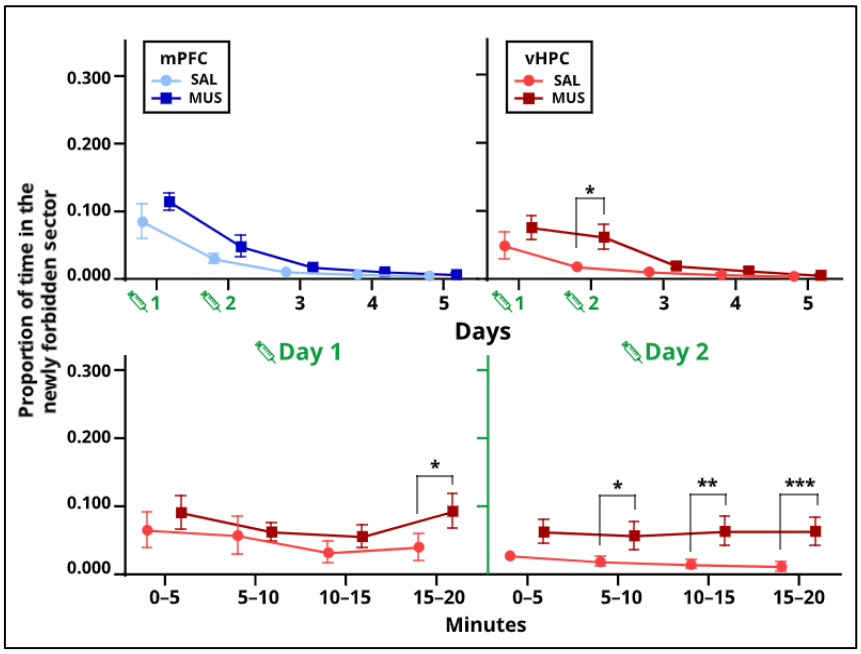

Fig. 9: Time spent in the newly forbidden sector during the 5-day reversal learning (top) and 5-minute intervals on days 1 and 2 (bottom) for the vHPC groups. No differences were observed over days in the mPFC groups. Significant impairment was revealed in the muscimol vHPC group during the last 5 minutes on day 1 [p = 0.0078]. The animals also spent more time in the sector on day 2, in minutes 5–10 [p = 0.0288], 10–15 [p = 0.0011] and 15–20 [p = 0.0005], apart from the saline group.

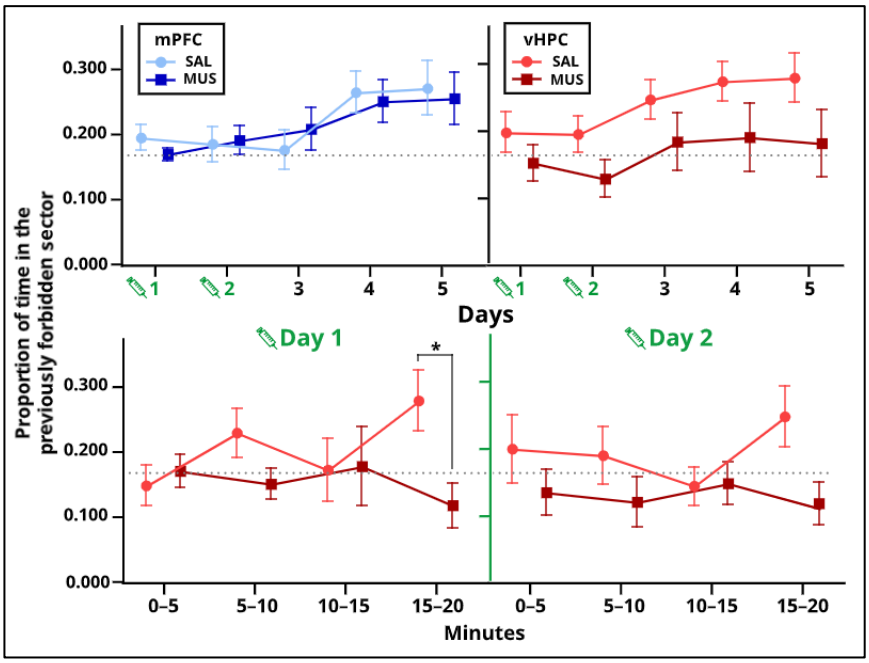

Fig. 10: Time spent in the previously forbidden sector during the 5-day reversal learning (top) and 5-minute intervals on days 1 and 2 (bottom) for the vHPC groups. The gray line illustrates the proportion of time that the animals would spend in the sector randomly (= 0.167). No differences were observed over days in either the mPFC or the vHPC groups. Detailed analysis for the vHPC groups showed that in the last 5 minutes of the session on day 1, the muscimol-inactivated rats spent less time in the previously forbidden sector than the saline-treated rats [p = 0.0361].

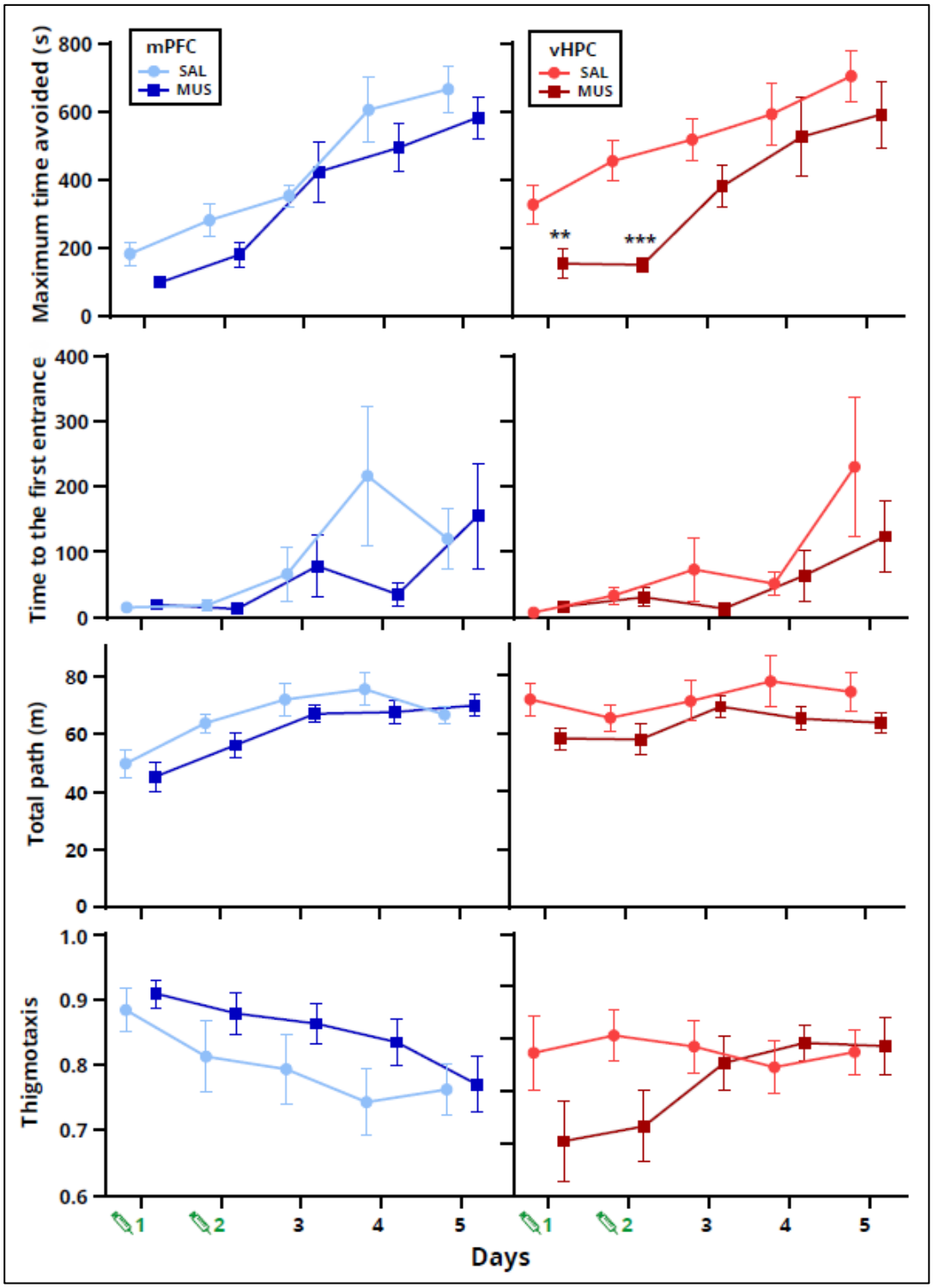

Fig. 11, from top to bottom: Maximum time avoided, time to the first entrance, thigmotaxis and total path during the 5-day reversal learning. The mPFC groups and the vHPC saline group increased the amount of time between the single entrances over the sessions, but the muscimol vHPC group could not avoid the punished sector for a long period of time neither on inactivation day 1 [p = 0.0077] nor day 2 [p = 0.0001]. There were no significant differences in either group in any of the last three parameters.

7.1.2 Set-shifting (Arena+ Room-)

A significant effect of days, as shown by two-way ANOVA for most parameters, indicates that both the mPFC and the vHPC groups learned the set-shifting task during the 5-day learning (Tab. 2). Two-way ANOVA did not, however, reveal any significance in effects of group or interaction in the avoidance-related parameters, showing that the saline-treated and muscimol-inactivated rats from both mPFC and vHPC groups did not differ in performance – they all gradually lowered the number of entrances and shocks (Fig. 12) and extended the maximum time of avoiding (Fig. 14). The mPFC groups increased the path to the first entrance as the days followed (Fig. 14). An effect of interaction was showed in thigmotaxis and total path (Tab. 2A). Post-hoc tests revealed higher thigmotaxis in the muscimol mPFC group on day 2 (Fig. 14). Lower thigmotaxis was observed in the first two days in the muscimol vHPC group as well but without any significance.

In the vHPC animals, the significant effect of interaction regarding time in the previously forbidden sector and the significant effect of group for time spent in each of the newly and previously forbidden sectors demonstrate differences between the muscimol and saline-treated rats (Tab 2B). Compared to the saline-treated rats, the muscimol-inactivated vHPC rats spent more time in the newly forbidden sector (significantly more on day 3), and in the previously forbidden sector (significantly more on days 1 and 4) (Fig. 13). Path to the first entrance was slightly increasing over days in both groups (Fig. 14). Overall, muscimol inactivations did not affect the learning of the Arena+ Room- task.

A) mPFC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	**F (4, 84) = 7.051, p < 0.0001**	F (1, 21) = 0.1225, p = 0.7298	**F (4, 84) = 2.738, p = 0.0339**	-
Entrances	**F (4, 84) = 10.68, p < 0.0001**	F (1, 21) = 1.367, p = 0.2555	F (4, 84) = 1.929, p = 0.1131	ln(y+1)
Shocks	**F (4, 84) = 11.69, p < 0.0001**	F (1, 21) = 1.356, p = 0.2573	F (4, 84) = 1.784, p = 0.1397	ln(y+1)
Path to the first entrance	**F (4, 84) = 5.563, p = 0.0005**	F (1, 21) = 1.578, p = 0.2229	F (4, 84) = 1.391, p = 1.391	ln(y+0.1)
Maximum time avoided	**F (4, 84) = 4.438, p = 0.0027**	F (1, 21) = 1.899, p= 0.1826	F (4, 84) = 1.497, p = 0.2103	ln(y+1)
Time in the newly forbidden sector	**F (4, 84) = 13.94, p < 0.0001**	F (1, 21) = 1.977, p = 0.1744	F (4, 84) = 1.268, p = 0.2891	ln(y+0.001)
Time in the previously forbidden sector	F (4, 84) = 0.509, p = 0.7293	F (1, 21) = 2.745, p = 0.1124	F (4, 84) = 0.9262, p = 0.4528	-
Thigmotaxis	**F (4, 84) = 5.569, p = 0.0005**	F (1, 21) = 4.299, p = 0.0506	**F (4, 84) = 2.801, p = 0.0309**	y squared

B) vHPC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	**F (4, 72) = 3.687, p = 0.0087**	F (1, 18) = 0.7994, p = 0.3831	F (4, 72) = 0.08916, p = 0.9856	-
Entrances	**F (4, 72) = 11.43, p < 0.0001**	F (1, 18) = 1.332, p = 0.2635	F (4, 72) = 1.437, p = 0.2305	ln(y+1)
Shocks	**F (4, 72) = 13.18, p < 0.0001**	F (1, 18) = 4.293, p = 0.0529	F (4, 72) = 0.9933, p = 0.4169	ln(y+1)
Path to the first entrance	F (4, 72) = 0.5262, p = 0.7168	F (1, 18) = 0.3079, p = 0.5858	F (4, 72) = 1.282, p = 0.285	ln(y+0.1)
Maximum time avoided	**F (4, 72) = 10.17, p < 0.0001**	F (1, 18) = 1.101, p = 0.308	F (4, 72) = 0.577, p = 0.6802	ln(y+1)
Time in the newly forbidden sector	**F (4, 72) = 17.51, p < 0.0001**	**F (1, 18) = 7.261, p = 0.0148**	F (4, 72) = 1.018, p = 0.4039	ln(y+0.001)
Time in the previously forbidden sector	F (4, 72) = 0.6259, p = 0.6456	**F (1, 18) = 8.8881, p = 0.008**	**F (4, 72) = 2.655, p = 0.0398**	-
Thigmotaxis	**F (4, 72) = 2.799, p = 0.0321**	F (1, 18) = 0.1917, p = 0.6668	F (4, 72) = 2.208, p = 0.0766	-

Tab. 2: Two-way ANOVA results of the 5-day set-shifting sessions of the mPFC (A) and the vHPC (B) groups. ln = natural logarithm

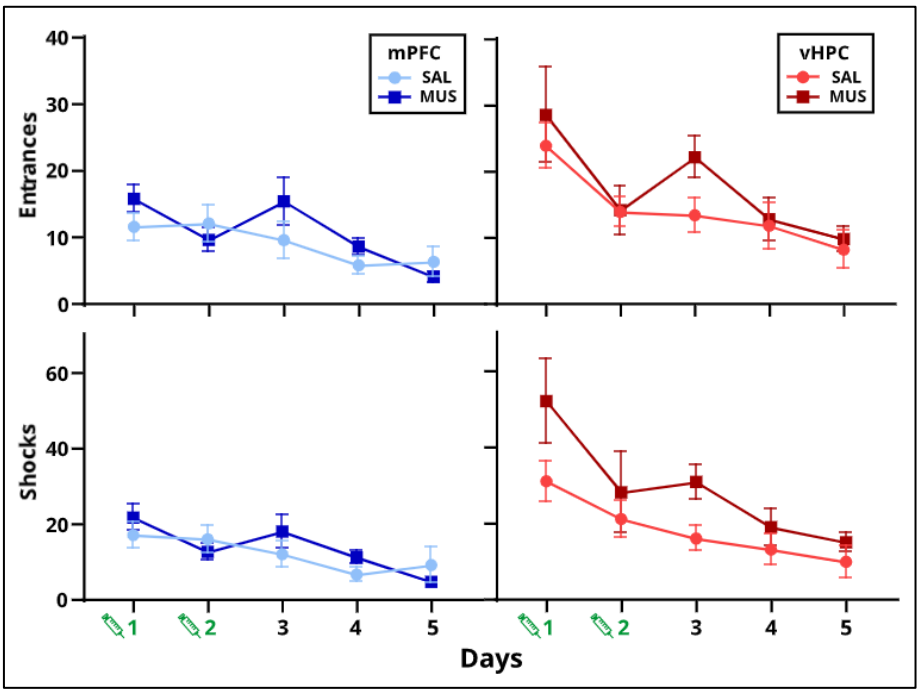

Fig. 12: The number of entrances (top) and shocks (bottom) during the 5-day set-shifting. Muscimol did not compromise the learning of the new strategy in either group; no significant difference between groups was observed.

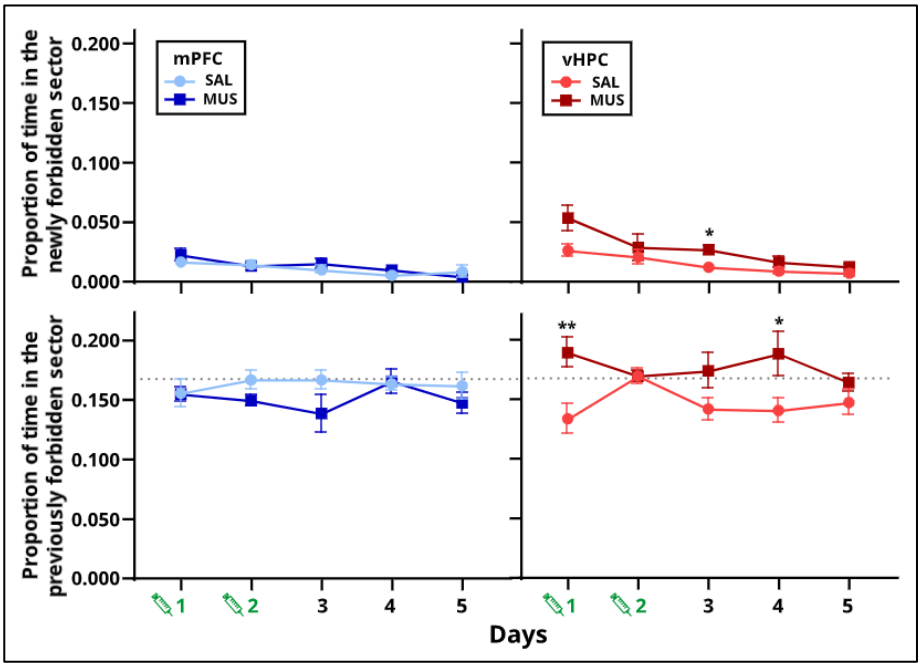

Fig. 13: Proportions of time spent in the newly (top) and previously (bottom) forbidden sectors during the 5-day set-shifting. The gray line illustrates the proportion of time that the animals would spend in the sector randomly (= 0.167). The vHPC-inactivated animals perseverated less on day 1 [p = 0.0042] and spent more time in the previously forbidden sector on day 4 [p = 0.0189] as well. Furthermore, they spent more time in the forbidden sector on day 3 [p = 0.0285].

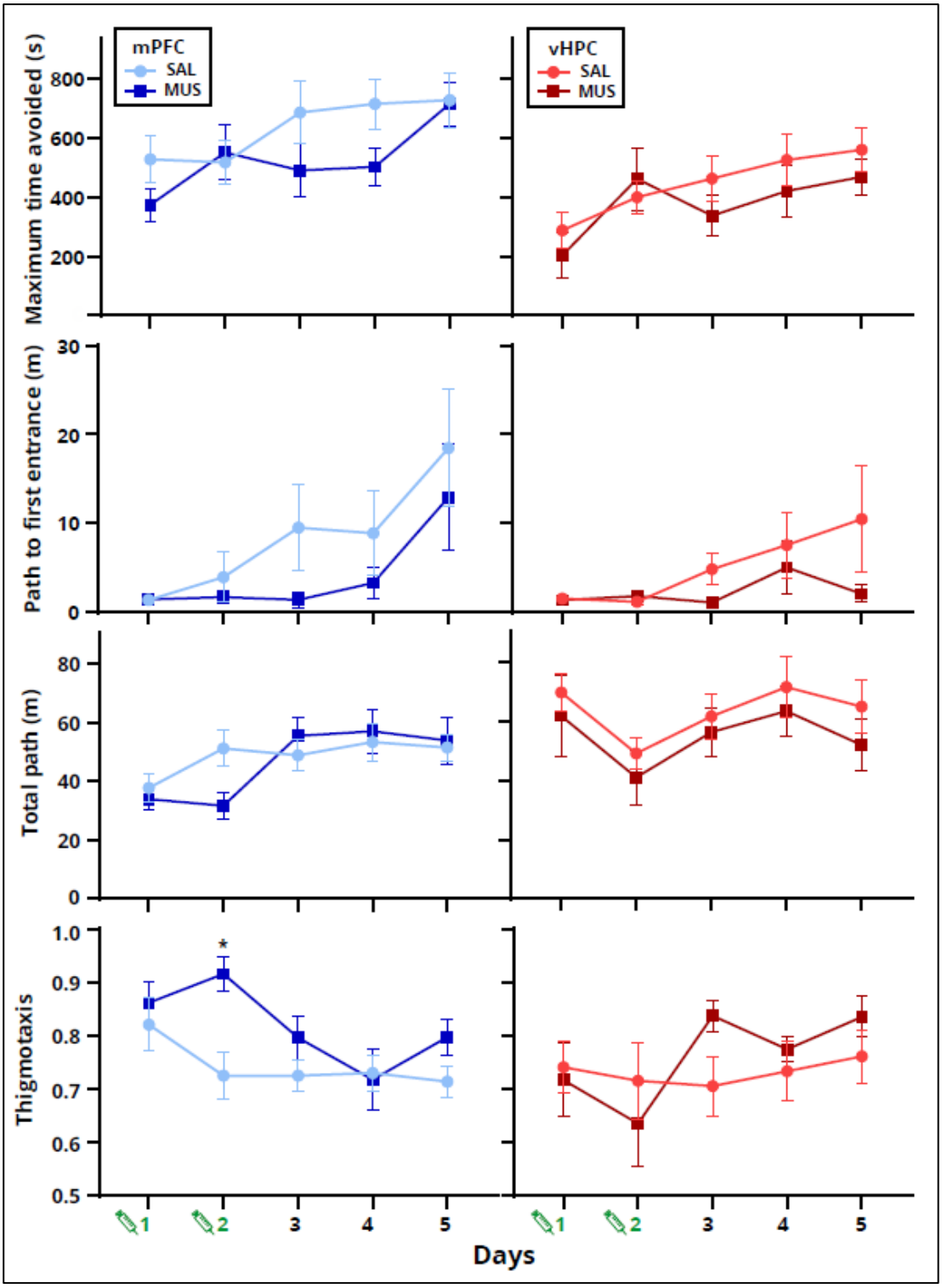

Fig. 14, from top to bottom: Maximum time avoided, path to the first entrance, total path and thigmotaxis during the 5-day set-shifting. Thigmotaxis was higher on day 2 for the muscimol mPFC group [p = 0.0025], but not on day 1. Thigmotaxis in the vHPC group was not affected by muscimol. No differences were observed in other parameters between sessions in either group; muscimol had no effect on these parameters.

7.2 Experiment 2 – Spatial memory retrieval

The aim of the second experiment was to test spatial memory retrieval and compare the performance when the mPFC or the vHPC were inactivated unilaterally (UNI) or bilaterally (BI), and after the contralateral or ipsilateral inactivations of combined mPFC-vHPC structures. There was no significant difference between the animal groups during the first 5 days of the acquisition (data not shown). All the animals included in the analyses responded to the mild shocks and were able to learn the task. The muscimol was applied on the 6th day, followed by saline control injections on the 7th day. These two days were statistically analyzed. One rat from the UNI/vHPC group was identified as an outlier and was excluded from the analysis of entrances, shocks, and time spent in the forbidden sector; the mentioned parameters in the vHPC groups were then analyzed using mixed-effects model as recommended by GraphPad Prism 8.0. The 6th day was further analyzed in 5-minute intervals to obtain more details about the dynamics of the inactivation effects on performance.

7.2.1 The mPFC inactivations

Statistical analysis revealed significant effects of days in all the avoidance-related parameters, but no effects of group, and a significant interaction effect was shown regarding maximum time avoided (Tab. 3A). Both groups had the first entrance to the sector much earlier on day 6 than the day after (Fig. 18). The UNI/mPFC group received more shocks but the number of entrances was not affected by the inactivations on day 6 (Fig. 15). In contrast, the BI/mPFC group was significantly impaired in performance – they could not avoid the sector for a long time (Fig. 18), which resulted in a high number of entrances and shocks (Fig. 15), and they spent much more time in the sector (Fig. 16), compared to day 7.

The groups did not show any differences in the number of entrances within the 5-minute intervals on day 6, but the effects of time were shown for the number of shocks and time spent in the sector (Tab. 3a). The BI/mPFC group kept the number of shocks at higher values (Fig. 15), similarly as the time spent in the forbidden sector (Fig. 16), while the UNI/mPFC group showed a tendency to reduce these values (not significantly). An effect of group was observed for total path and thigmotaxis between the days (Tab. 3A) – the BI/mPFC group walked less (Fig. 17) and showed more anxious behavior (Fig. 18). Because this behavior was observed on both days, it seems unlikely that this effect was associated with the muscimol application. Regarding the total path, both groups decreased the locomotor activity over time on day 6 (Fig. 17), as confirmed by the significant effects of group and time when conducting two-way ANOVA on the of 5-minute intervals (Tab. 3a).

7.2.2 The vHPC inactivations

The rats with inactivated vHPC showed problems with avoiding the sector. Two-way ANOVA revealed a significant effect of days in maximum time avoided (Tab. $3B_1$) – both groups were avoiding the sector for shorter intervals on day 6, compared to day 7, significantly for the BI/vHPC group only (Fig. 18). Mixed-effect models revealed a significant effect of days in the number of entrances, shocks and time spent in the forbidden sector (Tab. $3B_2$). While the UNI/vHPC group did not show any differences in behavior – they were avoiding the sector and spent short time in the sector – the BI/vHPC group was not able to avoid the forbidden location efficiently. On day 6, they had a significantly higher number of entrances (Fig. 15) and spent much more time in this sector (Fig. 16), which resulted in more shocks delivered (Fig. 15). Time to the first entrance, thigmotaxis, and total path were not affected by the muscimol inactivation (Fig. 17–18).

The detailed 5-minute analysis of day 6 revealed a significant effect of time in all the analyzed parameters (Tab. 3b). Both groups demonstrated intra-session learning; the animals were able to decrease the number of entrances over time, but apart from the UNI/vHPC group, the BI/vHPC group did not reduce the number of shocks delivered (Fig. 15). A significant effect of group was confirmed in the number of entrances and time in the forbidden sector – these parameters were at significantly higher values for the BI/vHPC group (Fig. 15–16). The total path was lowering within the session in both groups (Fig. 17).

7.2.3 The combined inactivations

Two-way ANOVA revealed an effect of days in all parameters except time to the first entrance (Tab. 3C). Both groups had significantly more entrances to the sector and a higher number of shocks delivered on day 6 (Fig. 15), and they could not avoid the sector for long intervals (Fig. 18). The proportion of time spent in the forbidden sector was therefore bigger in both groups (Fig. 16). A significant effect of group was observed regarding time to the first entrance but post-hoc tests did not show any differences between the groups (Fig. 18). Muscimol did not affect the total path (Fig. 17) but thigmotaxis was decreased in both groups (Fig. 18).

Statistical analysis of 5-minute intervals on day 6 revealed a significant effect of time in all the analyzed parameters (Tab. 3c). The intra-session learning did not occur regarding the number of entrances – they were consistently high throughout the inactivation session in both groups – but the number of shocks was, together with time spent in the sector, decreasing over time (significantly for the ipsilateral group), pointing to procedural, rather than spatial learning (Fig. 15).

A) mPFC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	F (1, 16) = 0.05427, p = 0.8187	**F (1, 16) = 14.28, p = 0.0016**	F (1, 16) = 2.459, p = 0.1364	ln(y-35)
Entrances	**F (1, 16) = 24.81, p = 0.0001**	F (1, 16) = 1.729, p = 0.2071	F (1, 16) = 2.715, p = 0.1189	-
Shocks	**F (1, 16) = 36.25, p < 0.0001**	F (1, 16) = 0.4276, p = 0.5225	F (1, 16) = 2.098, p = 0.1668	ln(y+1)
Time to the first entrance	**F (1, 16) = 17.43, p = 0.0007**	F (1, 16) = 0.1619, p = 0.6927	F (1, 16) = 0.03751, p = 0.8489	-
Maximum time avoided	**F (1, 16) = 28.41, p < 0.0001**	F (1, 16) = 0.01584, p = 0.9014	**F (1, 16) = 5.367, p = 0.0341**	-
Time in the forbidden sector	**F (1, 16) = 38.06, p < 0.0001**	F (1, 16) = 0.4163, p = 0.5279	F (1, 16) = 2.13, p = 0.1638	ln(y+0.001)
Thigmotaxis	F (1, 16) = 0.5925, p = 0.4527	**F (1, 16) = 14.88, p = 0.0014**	F (1, 16) = 0.07339, p = 0.7899	-

a) mPFC	Effect of time	Effect of group	Effect of interaction	Transformation
Total path	**F (3, 48) = 0.6182, p = 0.0016**	**F (1, 16) = 6.177, p = 0.0244**	F (3, 48) = 0.6182, p = 0.6066	ln(y-8)
Entrances	F (3, 48) = 2.765, p = 0.0519	F (1, 16) = 3.659, p = 0.0738	F (3, 48) = 0.2237, p = 0.8795	ln(y+1)
Shocks	**F (3, 48) = 3.231, p = 0.0304**	F (1, 16) = 2.566, p = 0.1288	F (3, 48) = 1.108, p = 0.3549	ln(y+1)
Time in the forbidden sector	**F (3, 48) = 3.002, p = 0.0395**	F (1, 16) = 2.405, p = 0.1405	F (3, 48) = 0.9893, p = 0.4058	ln(y+0.001)

B1) vHPC	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	F (1, 15) = 0.0129, p = 0.9111	F (1, 15) = 0.2139, p = 0.6504	F (1, 15) = 0.001971, p = 0.9652	-
Time to the first entrance	F (1, 15) = 1.31, p = 0.2704	F (1, 15) = 0.6726, p = 0.425	F (1, 15) = 0.05935, p = 0.8108	ln(y+1)
Maximum time avoided	**F (1, 15) = 12.57, p = 0.0029**	F (1, 15) = 1.123, p = 0.306	F (1, 15) = 0.1857, p = 0.6726	-
Thigmotaxis	F (1, 15) = 2.436, p = 0.1395	F (1, 15) = 0.01769, p = 0.8959	F (1, 15) = 1.532, p = 0.2349	y squared
B2) vHPC	**Effect of days**	**Effect of group**	**Effect of interaction**	**Transformation**
Time in the forbidden sector	**F (1, 14) = 12.52, p = 0.0033**	F (1, 15) = 4.03, p = 0.0631	**F (1, 14) = 6.208, p = 0.0259**	sqrt(y)
Entrances	**F (1, 14) = 16.15, p = 0.0013**	F (1, 15) = 2.435, p = 0.1395	F (1, 14) = 3.496, p = 0.0826	ln(y+1)
Shocks	**F (1, 14) = 15.38, p = 0.0015**	F (1, 15) = 3.203, p = 0.0937	F (1, 14) = 4.341, p = 0.056	ln(y+1)

b) vHPC	Effect of time	Effect of group	Effect of interaction	Transformation
Total path	**F (3, 45) = 19.11, p < 0.0001**	F (1, 15) = 0.1368, p = 0.7167	F (3, 45) = 0.05478, p = 0.9829	-
Entrances	**F (3, 42) = 7.067, p = 0.0006**	**F (1, 14) = 4.684, p = 0.0482**	F (3, 48) = 0.2237, p = 0.6191	ln(y+1)
Shocks	**F (3, 42) = 5.653, p = 0.0024**	F (1, 14) = 4.377, p = 0.0551	F (3, 42) = 1.321, p = 0.2802	ln(y+1)
Time in the forbidden sector	**F (3, 42) = 3.669, p = 0.0195**	**F (1, 14) = 4.759, p = 0.0467**	F (3, 42) = 1.003, p = 0.4011	sqrt(y)

C) combined	Effect of days	Effect of group	Effect of interaction	Transformation
Total path	**F (1, 12) = 6.534, p = 0.0252**	F (1, 12) = 0.6342, p = 0.4413	F (1, 12) = 0.1975, p = 0.6647	-
Entrances	**F (1, 12) = 26.13, p = 0.0003**	F (1, 12) = 0.03442, p = 0.8559	F (1, 12) = 0.02016, p = 0.8894	-
Shocks	**F (1, 12) = 30.74, p = 0.0001**	F (1, 12) = 4.28e-005, p = 0.9949	F (1, 12) = 0.00716, p = 0.934	ln(y+1)
Time to the first entrance	F (1, 12) = 0.5818, p = 0.4603	**F (1, 12) = 5.35, p = 0.0393**	F (1, 12) = 0.9045, p = 0.3603	ln(y+1)
Maximum time avoided	**F (1, 12) = 21.56, p = 0.0006**	F (1, 12) = 0.1685, p = 0.6887	F (1, 12) = 0.005707, p = 0.941	-
Time in the forbidden sector	**F (1, 12) = 23.17, p = 0.0004**	F (1, 12) = 0.01393, p = 0.908	F (1, 12) = 0.05429, p = 0.8197	ln(y+0.001)
Thigmotaxis	**F (1, 12) = 19.44, p = 0.0009**	F (12, 12) = 1.58, p = 0.22	F (1, 12) = 0.02302, p = 0.8819	-

c) combined	Effect of time	Effect of group	Effect of interaction	Transformation
Total path	**F (3, 36) = 3.811, p = 0.018**	F (1, 12) = 0.548, p = 0.4734	F (3, 36) = 0.4077, p = 0.7484	-
Entrances	**F (3, 36) = 3.057, p = 0.0406**	F (1, 12) = 0.04412, p = 0.8372	F (3, 36) = 0.5988, p = 0.62	-
Shocks	**F (3, 36) = 7.051, p = 0.0008**	F (1, 12) = 0.009952, p = 0.9222	F (3, 36) = 0.3766, p = 0.7704	ln(y+1)
Time in the forbidden sector	**F (3, 36) = 6.555, p = 0.0012**	F (1, 12) = 0.001964, p = 0.9654	F (3, 36) = 0.2881, p = 0.8336	ln(y+0.001)

Tab. 3: Results of the analysis between day 6 and 7. Two-way ANOVA shows differences for the mPFC groups (A), the vHPC groups (B1) and the combined groups (C), mixed-effect models were used for some parameters in the vHPC groups (B2). Two-way ANOVA results of 5-minute intervals for specific groups are showed in tables a-c. **ln** = natural logarithm; **sqrt** = square root

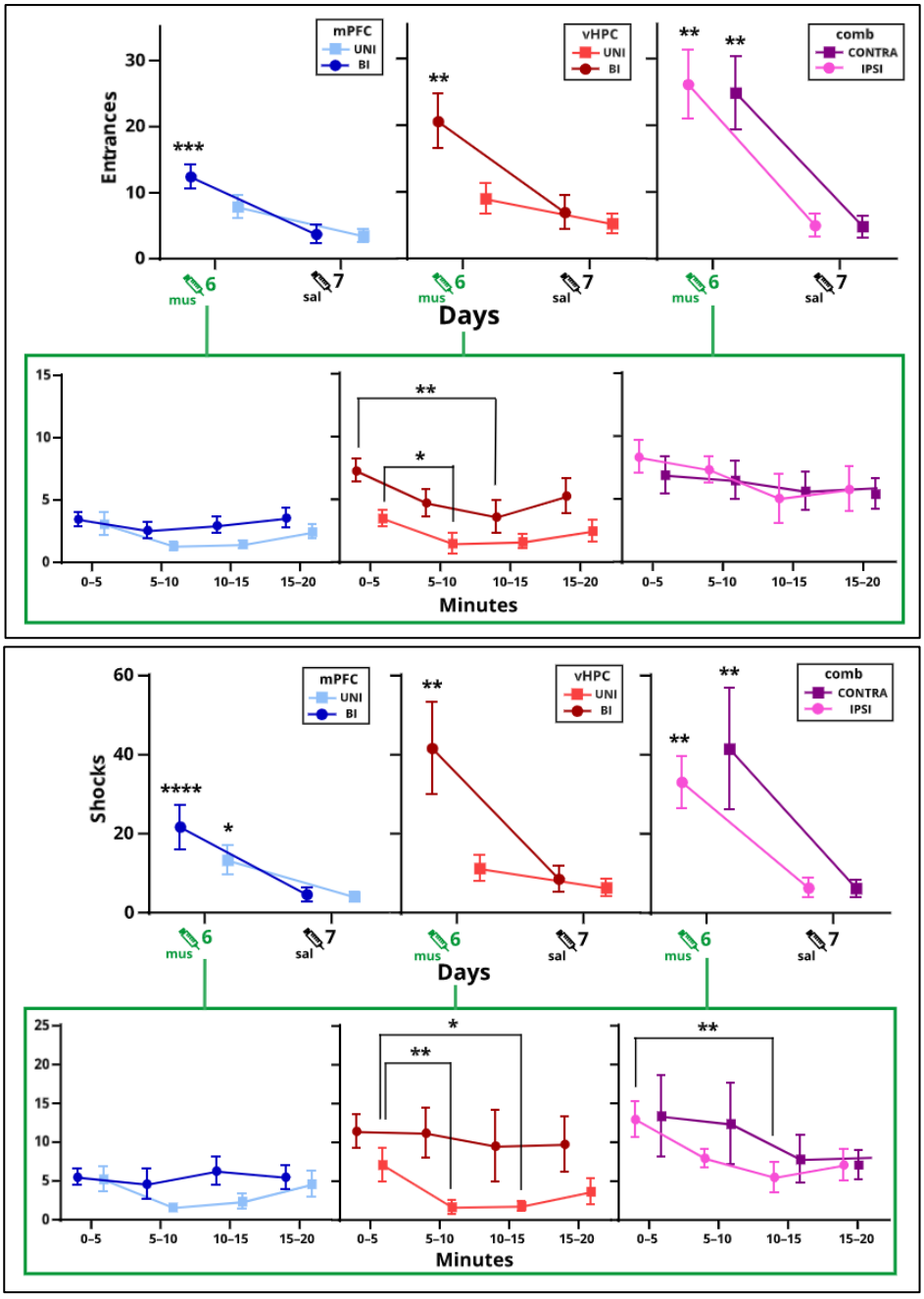

Fig. 15: Number of entrances (top) and shocks (bottom) in the individual groups and detailed 5-minute interval graphs of day 6 (green frames). The BI/mPFC group increased the number of entrances [p = 0.0003] and shocks [p < 0.0001] on the inactivation day; the performance remained stable over time. The UNI/mPFC group increased the number of shocks as well [p = 0.0147]. For the vHPC groups, only the bilaterally inactivated rats showed significant problems with not entering the sector [p = 0.0012] and got a bigger amount of shocks [p = 0.001]. Yet, they lowered the number of entrances over time [p = 0.0202 for intervals 0–5 vs. 5–10 for the UNI/vHPC group; p = 0.0069 for intervals 0–5 vs. 10–15 for the BI/vHPC group], but the number of shocks reduced markedly in the unilateral group only [p = 0.0061 for intervals 0–5 vs. 5–10; p = 0.1471 for intervals 0–5 vs. 10–15]. Both the combined groups were impaired in the number of entrances [p = 0.0059 for the ipsilateral group; p = 0.0085 for the contralateral group] and shocks [p = 0.0036 for the ipsilateral group; p = 0.0045 for the contralateral group]. The number of entrances was high during the whole session without any significant changes. The number of shocks was lowering, significantly for the ipsilateral group between the minute intervals 0–5 vs. 10–15 [p = 0.0034].

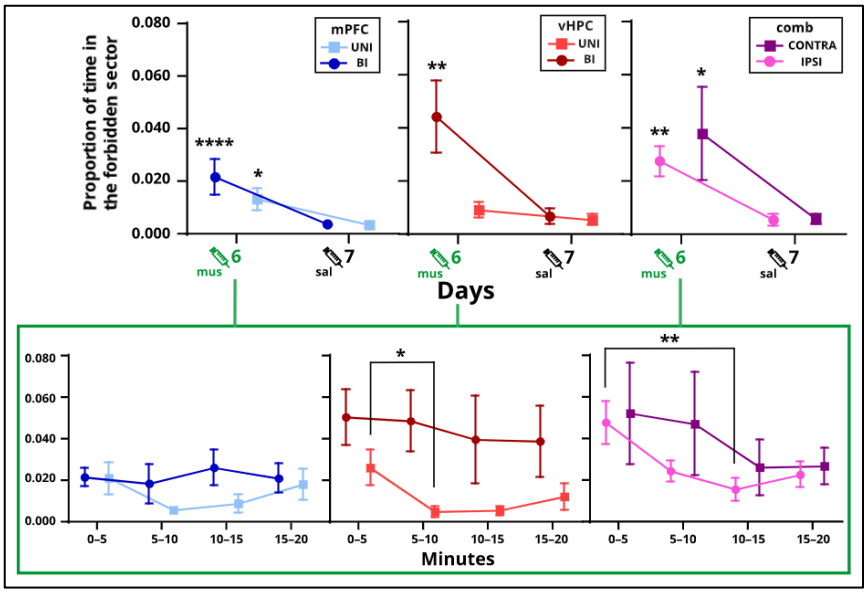

Fig. 16: Proportion of time spent in the forbidden sector in the individual groups and detailed 5-minute interval graphs of day 6 (green frame). Both the mPFC groups spent more time in the forbidden sector [UNI/mPFC: p = 0.0121, BI/mPFC: p < 0.0001]. Much greater proportion was also observed in the BI/vHPC group [p = 0.001], and in the ipsilateral [p = 0.0077] and contralateral [p = 0.0142] groups. Both bilateral groups were impaired within the whole session, whereas the unilateral groups had tendency to decrease the proportion, significantly in the UNI/vHPC group in the intervals 0–5 vs. 5–10 [p = 0.0434]. The ipsilateral group similarly reduced the time spent in the sector [p = 0.0077 for the intervals 0–5 vs. 10–15]. No significance was observed for the contralateral group.

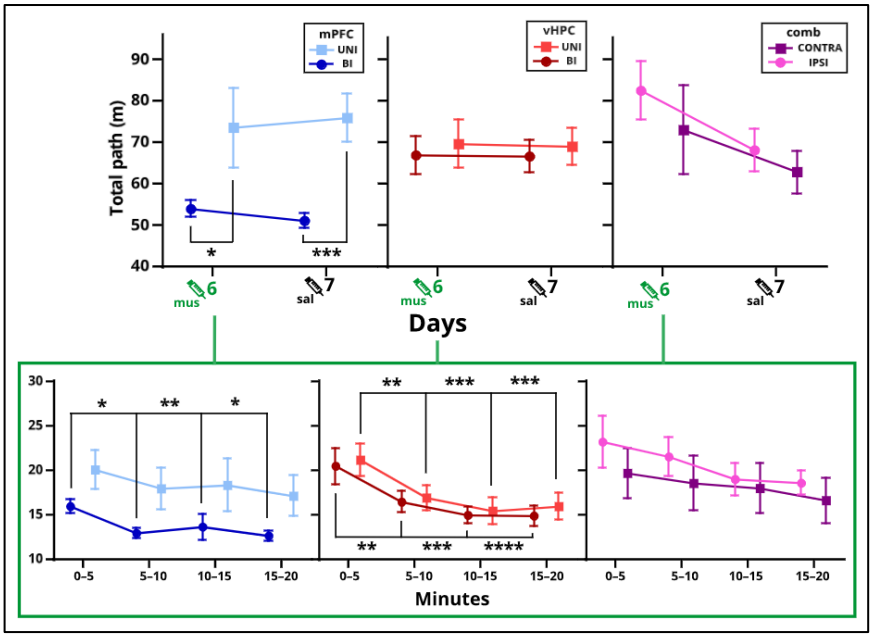

Fig. 17: Total path in the individual groups and detailed 5-minute interval graphs of day 6 (green frame). Group differences were observed in total path for the mPFC groups [p = 0.0261 on day 6; p = 0.0006 on day 7], but muscimol had no effect on either group. Detailed analysis of the 6th day showed tendency to move less for the BI/mPFC group [p = 0.0371 between the intervals 0–5 vs. 5–10; p = 0.0029 vs. 10–15; p = 0.0126 vs. 15–20], but also for the UNI/vHPC [minutes 0–5: p = 0.0074 vs. 5–10; p = 0.0002 vs. 10–15; p = 0.0007 vs. 15–20] and BIL/vHPC groups [minutes 0–5: p = 0.0067 vs. 5–10; p = 0.0001 vs. 10–15; p < 0.0001 vs. 15–20]. No difference was observed in the combined groups.

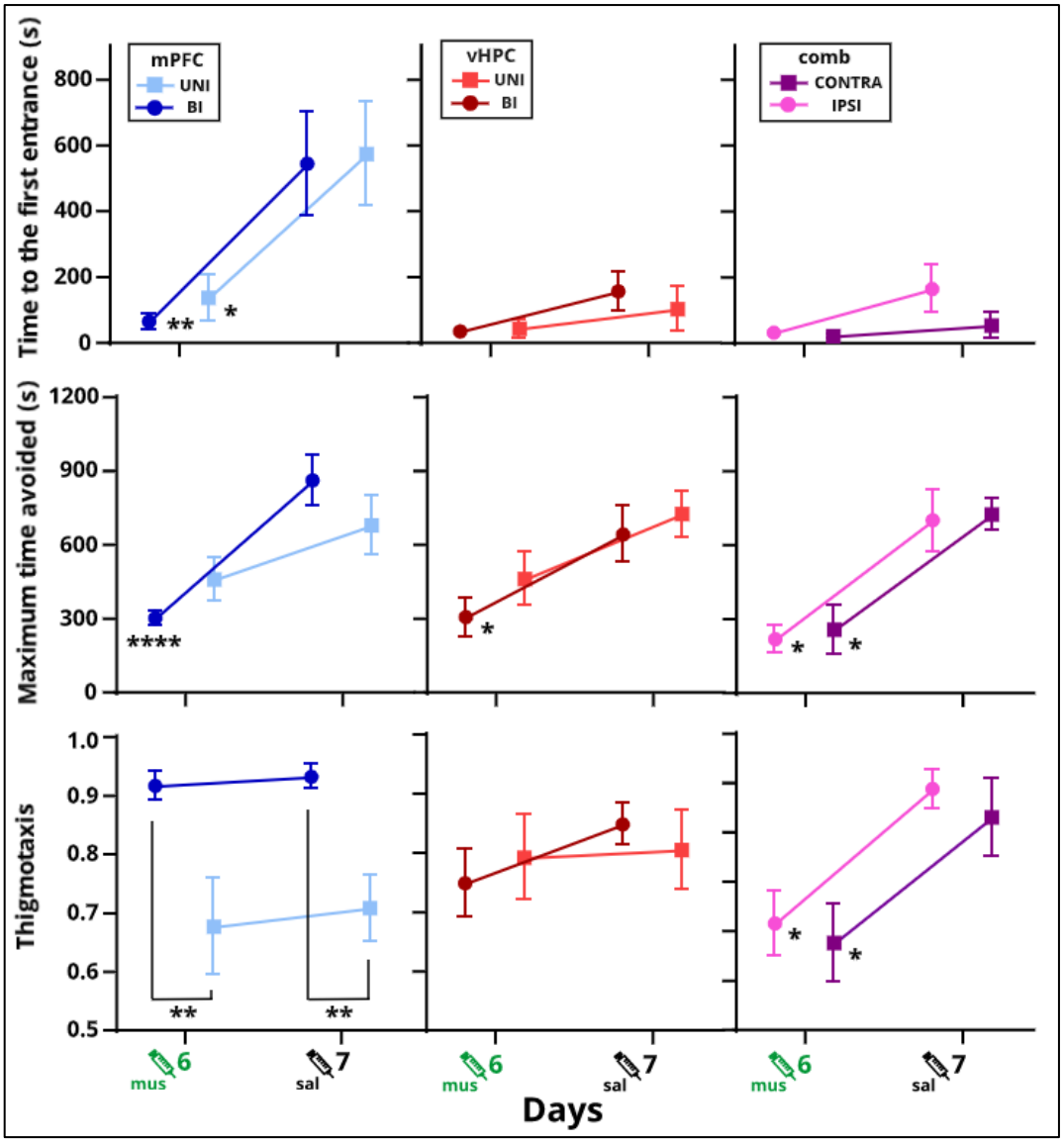

Fig. 18, from top to bottom: Time to the first entrance, maximum time avoided, and thigmotaxis in the individual groups on day 6. Time to the first entrance was lower in both mPFC groups [p = 0.0332 for the unilateral group, p = 0.0095 for the bilateral group]. Both the BI/mPFC [p < 0.0001] and the BI/vHPC [p = 0.002] groups had troubles with avoiding the sector for longer periods of time, similarly the combined groups [p = 0.0118 for the ipsilateral group, p = 0.0144 for the contralateral group]. Thigmotaxis differed between the mPFC groups on both analyzed days; UNI/mPFC rats were less anxious on the arena [p = 0.0023 for day 6; p = 0.0045 for day 7]. Inactivation decreased thigmotaxis in the ipsilateral [p = 0.0145] and contralateral [p = 0.0216] groups.

7.3 Verification of cannula implants

All rats whose performance was used for further analysis had cannulas in the mPFC or the vHPC, with ± 0.2 mm AP variance. Some hippocampal cannulas were placed more dorsally, aiming rather at the iHPC. These animals were not different in performance from the rats with cannulas at the vHPC and were included in the results.

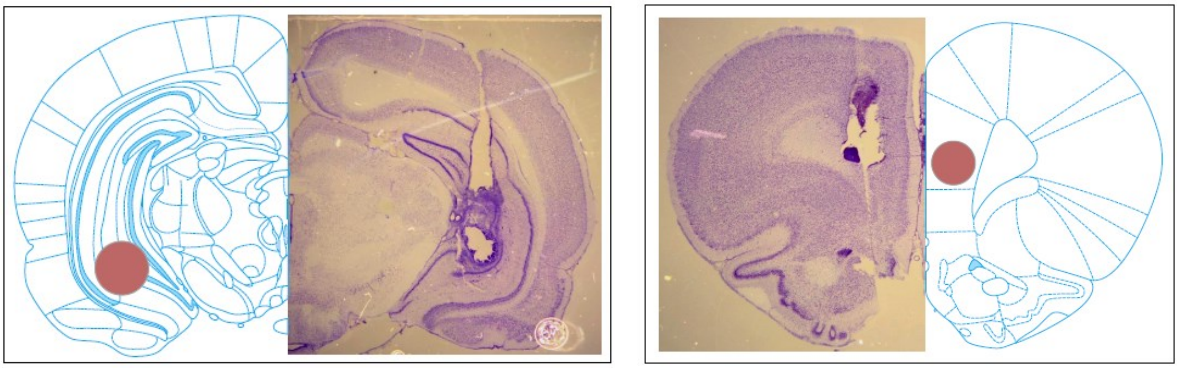

Fig. 19: An illustration of cannula placement. The left picture shows the direction of cannula (right half) and the location of the vHPC (red dot on the left half). The right picture shows the direction of cannula (left half) and the location of PL in the mPFC (red dot on the right half).

8 DISCUSSION

8.1 Experiment 1 – Behavioral flexibility

In this experiment, we investigated the role of the mPFC and the vHPC in behavioral flexibility on a rotating arena using local muscimol inactivations. After 5 days of the initial Arena- Room+ task acquisition, the rats performed 5 days of reversal learning of the Arena- Room+ task and another 5 days of set-shifting (Arena+ Room- task). We evaluated the effect of inactivations in the first 2 days of learning after the conditions changed.

Significant impairment was revealed in reversal learning in the vHPC-inactivated group. The animals entered the punished sector more on both inactivation days. From the session recordings, we could confirm that all the animals responded to shocks, and in the vHPC-inactivated group, there were two different ways of responding. First, the rats made a few steps against the arena rotation rather than running further away, therefore, the rotation moved them to the forbidden sector again, which resulted in several walk-shock repetitions until they finally ran to a greater distance. The second type of behavior after getting a shock was an apparent movement response in the punished sector, but an inability to escape from it, resulting in more shocks until the rat successfully escaped from the sector. The vHPC-inactivated rats did not exhibit any form of intra-session learning, as the number of entrances or shocks were not lowering throughout the session and the animals did not

decrease the time spent in the forbidden sector.

Despite the mentioned avoidance problems during the inactivation days and absence of intra-session learning, the animals were probably still processing information about the new sector location. As seen from day 3 of reversal learning (the first session with functional hippocampi), the muscimol-inactivated group equalled the number of entrances and shocks with the saline-treated group. It was previously documented that the hippocampus was involved in long-term memory formation but not in the acquisition of spatial information itself (Parron et al., 2001; Poucet et al., 1991). The authors suggest that the temporal storage of new spatial information relies on other cortical or subcortical structures, and that the information is translated into long-term memory when the hippocampal functioning returns to normal. Moreover, dHPC inactivations (the study of Parron et al., 2001) resembled effects of vHPC inactivations (Poucet et al., 1991). Consistent with these studies, our rats could process spatial information after the vHPC function restored.

The mPFC inactivations did not affect reversal learning in any of the evaluated parameters, which corresponds to other studies showing that the mPFC lesions or inactivations do not disrupt flexibility in this type of learning (for instance Birrell & Brown, 2000; Boulougouris et al., 2007; Floresco et al., 2008).

One limitation of this experiment is that none of the saline-treated groups showed any signs of perseveration at the beginning of reversal learning. Perseveration during the first half of the new task session was reported before in rats at similar experimental conditions (5 days of acquisition, 5 days of reversal learning, 20-minute sessions) (Svoboda et al., 2015). Since the Long-Evans rats used in our experiments came from a different breeding line, lack of perseveration might have been caused by a distinct behavioral profile. Our rats could either be excellent learners and adapt rapidly to the absence of being shocked on the “north”, or deficient in remembering the sector location from previous sessions. Because the rats were not learning the new sector location any quickly, we favour the latter option.

Regarding set-shifting, all the animals learned the task without complications, and muscimol inactivations did not impair performance in any of the groups. These findings were especially surprising for the mPFC-inactivated group, given the rich amount of literature showing clear evidence of the mPFC contribution in extradimensional set-shifting tasks, where lesions or inactivations resulted in more perseverative errors or increased trials to reach the task-specific criterion (for review, see Floresco et al., 2009 and Hamilton & Brigman, 2015). Similarly to reversal learning, the findings could be limited by little perseveration of the saline-treated mPFC group at the beginning of the set-shifting task. On the other hand, the saline-treated vHPC rats perseverated

on day 1, as the proportion of time in the previously forbidden sector was reliably under the value of random locomotion and was also lower than in the muscimol-inactivated vHPC rats. Since the saline-treated mPFC rats were not perseverating, the finding regarding the vHPC groups could be a simple coincidence, and these results are thus not conclusive.

8.2 Experiment 2 – Spatial memory retrieval

In this experiment, we searched for the role of these structures during the learned Arena- Room+ task. We tested rats in a combination of separate unilateral and bilateral inactivations of the vHPC or the mPFC, and a combination of contralateral or ipsilateral inactivations of both structures, to investigate the importance of the hippocampal-prefrontal pathway on spatial retrieval. After 5 days of learning the initial Arena- Room+ task, the rats had one day of muscimol inactivation and one day of saline injections. We analyzed the effect of inactivation on day 6 and compared it with the saline application on day 7.

Unilateral inactivations of the vHPC did not impair spatial retrieval, but bilateral inactivations led to more entrances to the sector and a higher number of shocks, similarly as in rats during reversal learning. Although the animals managed to lower the number of entrances throughout the inactivation session, they had clear difficulties with escaping from the sector. All the rats from the BI/vHPC group responded to shocks, some of them showing problems with escaping from the sector if once stepped inside, however, they did not develop the walk-shock strategy that was reported in the reversal learning rats. Both groups decreased their walked path during the inactivation session, but the total path was comparable with day 7 and did not affect performance.

A similar experiment (5 days of acquisition, tetrodotoxin inactivations on day 6, 20-minute sessions) showed that unilateral inactivation of the dHPC in rats was enough to impair learned avoidance (Cimadevilla et al., 2001). The retrieval thus seems to be more sensitive to the dHPC disruptions, but as demonstrated in our findings, the importance of the vHPC should not be omitted.

The mPFC-inactivated animals showed impairment in this task as well. The BI/mPFC rats had more entrances to the sector and were impaired throughout the whole inactivation session. The BI/mPFC group did not have sufficient locomotor activity that would keep them further from the punished sector, which could play a part in deficient avoidance on the inactivation day. From the session recordings, it seems that the animals struggled with concentrating on the avoidance. They did not run away as they approached the sector, but when they were delivered the first shock, they moved rapidly to the opposite side of the arena. Inactivations in both groups decreased time to the first entrance, which might indicate disrupted memory retrieval of the sector location.

Despite unimpaired behavior in the UNI/vHPC and UNI/mPFC groups, combined inactivations of these structures showed quite the opposite. Both the ipsilaterally and contralaterally inactivated groups were impaired in avoidance, and the results had similar features for both groups. The animals did not lower the entrances over the session, but procedural learning was observed in both groups – the animals lowered the number of shocks, and although significance was revealed for the contralateral group only, the ipsilateral group tended to lower the shocks as well. In conclusion, the hippocampal-prefrontal interaction is critical in learned avoidance on the arena, and the functioning pathway of one hemisphere does not compensate for their inactivated counterparts.

In previous studies, the vHPC lesions had anxiolytic effects on behavior (Kjelstrup et al., 2002; McHugh et al., 2004). Similar results were achieved by selective inactivations of the vHPC-to-mPFC projections, which impaired anxiety-related behavior (Padilla-Coreano et al., 2016). The vHPC and mPFC structures have been shown to cooperate and synchronize their activity when guiding anxiety-related behavior (Adhikari et al., 2010, 2011), which is consistent with our findings of significantly decreased thigmotaxis after the vHPC-mPFC intercommunication was disrupted. However, inactivating only the vHPC did not affect thigmotaxis in any of our experiments, and we did not observe any related anxiolytic effect of inactivations on performance, which interferes with available literature and our results.

The second experiment was conducted mainly to examine the validity of the way of testing behavioral flexibility in the first experiment. Because our results show impaired spatial retrieval in animals with bilateral inactivations of the vHPC and the mPFC groups, the cause of the observed impairment in reversal learning of vHPC-inactivated animals remains inconclusive. There might, however, be a dissociation between the impact of inactivations on retrieval and flexibility tasks. The performance of the BI/mPFC group was affected in the spatial retrieval task, but not in reversal learning or set-shifting tasks. Similarly, the results for BI/vHPC group showed impaired spatial retrieval and reversal learning, but not set-shifting. The impaired ability to retrieve the learned task in the bilaterally inactivated animals might also possibly be the cause of diminished perseveration in the flexibility tasks.

9 LIMITATIONS OF INACTIVATION TECHNIQUES

Local brain inactivations allow extensive silencing of specific brain areas and can alter brain functioning, including learning and memory, which could complicate the interpretation of

behavioral results. This represents a big disadvantage compared to other techniques that can selectively target neuron populations. Another disadvantage may result from the procedure itself – the exact volume and the rate of injection rely on subjective observation of the experimenter(s), and the dose infused may differ between subjects. Despite the mentioned disadvantages, inactivation studies are still effective in investigating general mechanisms and getting knowledge about the functions of brain structures. Moreover, the technique does not require any genetic modifications, and is relatively simple and fast to perform.

10 CONCLUSIONS

In this study, we confirmed an indispensable role of the vHPC in reversal learning, and we described the importance of both structures and their intercommunication in spatial memory retrieval. Based on our results, we conclude that:

1) The vHPC is critical for behavioral coordination in reversal learning, but not in set-shifting. The vHPC is needed for the reversal learning and coordination of escaping from a forbidden sector. The results have confirmed the hypothesis that the vHPC is required for behavioral flexibility on the rotating arena.

2) The mPFC is not needed for behavioral flexibility on the rotating arena in either reversal learning or set-shifting. No impairments were observed in rats with bilaterally inactivated mPFC. The hypothesis that the mPFC participates in flexible behavior has been proven wrong.

3) The mPFC and the vHPC are needed in spatial memory retrieval, and the hippocampal-prefrontal connection is critical for successful avoidance. At least one of these structures is needed intact for avoiding the learned sector location. The unilateral inactivation of mPFC-vHPC either contra- or ipsilaterally impairs avoidance as well. Although we assumed that the structures would not be critical for spatial memory retrieval, the second experiment showed quite the opposite.

Lack of flexible behavior is a common feature of various neuropsychiatric diseases, including schizophrenia, depression, or posttraumatic stress disorder. These diseases show complex morphological, physiological, and functional aberrations in the hippocampus and prefrontal cortex, together with impairments in their intercommunication. Our research has brought clear evidence about the importance of the vHPC in behavioral flexibility and revealed that both structures critically participate in spatial memory retrieval. Because the vHPC abnormalities have been observed in many diseases, it certainly deserves more attention in future research.

11 REFERENCES

Abbruzzese, M., Bellodi, L., Ferri, S., & Scarone, S. (1995). Frontal-lobe dysfunction in schizophrenia and obsessive-compulsive disorder - a neuropsychological study. Brain and Cognition, Vol. 27, pp. 202–212. https://doi.org/10.1006/brcg.1995.1017

Adhikari, A., Topiwala, M. A., & Gordon, J. A. (2010). Synchronized Activity between the Ventral Hippocampus and the Medial Prefrontal Cortex during Anxiety. Neuron, 65(2), 257–269. https://doi.org/10.1016/j.neuron.2009.12.002

Adhikari, A., Topiwala, M. A., & Gordon, J. A. (2011). Single Units in the Medial Prefrontal Cortex with Anxiety-Related Firing Patterns Are Preferentially Influenced by Ventral Hippocampal Activity. Neuron, 71(5), 898–910. https://doi.org/10.1016/j.neuron.2011.07.027

Amodeo, L. R., McMurray, M. S., & Roitman, J. D. (2017). Orbitofrontal cortex reflects changes in response–outcome contingencies during probabilistic reversal learning. Neuroscience, 345, 27–37. https://doi.org/10.1016/j.neuroscience.2016.03.034

Anticevic, A., Hu, X., Xiao, Y., Hu, J., Li, F., Bi, F., … Gong, Q. (2015). Early-Course Unmedicated Schizophrenia Patients Exhibit Elevated Prefrontal Connectivity Associated with Longitudinal Change. Journal of Neuroscience, 35(1), 267–286. https://doi.org/10.1523/JNEUROSCI.2310-14.2015

Aron, A. R., & Poldrack, R. A. (2006). Cortical and subcortical contributions to stop signal response inhibition: Role of the subthalamic nucleus. Journal of Neuroscience, 26(9), 2424–2433. https://doi.org/10.1523/JNEUROSCI.4682-05.2006

Bahník, Š. (2014). Carousel Maze Manager (Version 0.4.0) [Software]. Available from https://github.com/bahniks/CM_Manager_0_4_0

Bahník, Š., & Stuchlík, A. (2015). Temporal and spatial strategies in an active place avoidance task on Carousel: a study of e ff ects of stability of arena rotation speed in rats. PeerJ 3:E1257. https://doi.org/10.7717/peerj.1257

Balleine, B. W., Delgado, M. R., & Hikosaka, O. (2007). The Role of the Dorsal Striatum in Reward and Decision-Making. Journal of Neuroscience, 27(31), 8161–8165. https://doi.org/10.1523/JNEUROSCI.1554-07.2007

Bannerman, D. M., Good, M. A., Yee, B. K., Heupel, M. J., Iversen, S. D., & Rawlins, J. N. P. (1999). Double dissociation of function within the hippocampus: A comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. Behavioral Neuroscience, 113(6), 1170–1188. https://doi.org/10.1037/0735-7044.113.6.1170

Bannerman, D. M., Grubb, M., Deacon, R. M. J., Yee, B. K., Feldon, J., & Rawlins, J. N. P. (2003). Ventral hippocampal lesions affect anxiety but not spatial learning. Behavioural Brain Research, 139(1–2), 197–213. https://doi.org/10.1016/S0166-4328(02)00268-1

Barbas, H., & Blatt, G. J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. Hippocampus, 5(6), 511–533. https://doi.org/10.1002/hipo.450050604

Barker, G. R. I., & Warburton, E. C. (2011). When Is the Hippocampus Involved in Recognition Memory? Journal of Neuroscience, 31(29), 10721–10731. https://doi.org/10.1523/JNEUROSCI.6413-10.2011

Bast, T. (2011). The hippocampal learning-behavior translation and the functional significance of hippocampal dysfunction in schizophrenia. Current Opinion in Neurobiology, 21(3), 492–501. https://doi.org/10.1016/j.conb.2011.01.003

Bast, T., Wilson, L. A., Witter, M. P., & Morris, R. G. M. (2009). From rapid place learning to behavioral performance: A key role for the intermediate hippocampus. PLoS Biology, 7(4), 0730–0746. https://doi.org/10.1371/journal.pbio.1000089

Bearden, C. E., Thompson, P. M., Avedissian, C., Klunder, A. D., Nicoletti, M., Dierschke, N., … Soares, J. C. (2009). Altered hippocampal morphology in unmedicated patients with major depressive illness. ASN Neuro, 1(4), 265–273. https://doi.org/10.1042/AN20090026

Beasley, C. L., & Reynolds, G. P. (1997). Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. Schizophrenia Research, 24(3), 349–355. https://doi.org/10.1016/S0920-9964(96)00122-3

Beckstead, R. M., Domesick, V. B., & Nauta, W. J. H. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Research, 175(2), 191–217. https://doi.org/10.1016/0006-8993(79)91001-1

Benchenane, K., Peyrache, A., Khamassi, M., Tierney, P. L., Gioanni, Y., Battaglia, F. P., & Wiener, S. I. (2010). Coherent Theta Oscillations and Reorganization of Spike Timing in the Hippocampal- Prefrontal Network upon Learning. Neuron, 66(6), 921–936. https://doi.org/10.1016/j.neuron.2010.05.013

Berendse, H. W., & Groenewegen, H. J. (1990). Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. Journal of Comparative Neurology, 299(2), 187–228. https://doi.org/10.1002/cne.902990206

Bezaire, M. J., & Soltesz, I. (2013). Quantitative assessment of CA1 local circuits: Knowledge base for interneuron-pyramidal cell connectivity. Hippocampus, 23(9), 751–785. https://doi.org/10.1002/hipo.22141

Bian, X.-L., Qin, C., Cai, C.-Y., Zhou, Y., Tao, Y., Lin, Y.-H., … Zhu, D.-Y. (2019). Anterior Cingulate Cortex to Ventral Hippocampus Circuit Mediates Contextual Fear Generalization. The Journal of Neuroscience, 39(29), 5728–5739. https://doi.org/10.1523/jneurosci.2739-18.2019

Birrell, J. M., & Brown, V. J. (2000). Medial Frontal Cortex Mediates Perceptual Attentional Set Shifting in the Rat. The Journal of Neuroscience, 20(11), 4320–4324. https://doi.org/10.1523/JNEUROSCI.20-11-04320.2000

Bissonette, G. B., Martins, G. J., Franz, T. M., Harper, E. S., Schoenbaum, G., & Powell, E. M. (2008). Double Dissociation of the Effects of Medial and Orbital Prefrontal Cortical Lesions on Attentional and Affective Shifts in Mice. Journal of Neuroscience, 28(44), 11124–11130. https://doi.org/10.1523/JNEUROSCI.2820-08.2008

Block, A. E., Dhanji, H., Thompson-Tardif, S. F., & Floresco, S. B. (2007). Thalamic-prefrontal cortical-ventral striatal circuitry mediates dissociable components of strategy set shifting. Cerebral Cortex, 17(7), 1625–1636. https://doi.org/10.1093/cercor/bhl073

Bokor, H., Csáki, Á., Kocsis, K., & Kiss, J. (2002). Cellular architecture of the nucleus reuniens thalami and its putative aspartatergic/glutamatergic projection to the hippocampus and medial septum in the rat. European Journal of Neuroscience, 16(7), 1227–1239. https://doi.org/10.1046/j.1460-9568.2002.02189.x

Boulougouris, V., Dalley, J. W., & Robbins, T. W. (2007). Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. Behavioural Brain Research, 179(2), 219–228. https://doi.org/10.1016/j.bbr.2007.02.005

Bowie, C. R., & Harvey, P. D. (2006). Cognitive deficits and functional outcome in schizophrenia. Neuropsychiatric Disease and Treatment, 2(4), 531–536. https://doi.org/10.2147/nedt.2006.2.4.531

Brady, A. M. (2009). Neonatal ventral hippocampal lesions disrupt set-shifting ability in adult rats. Behavioural Brain Research, 205(1), 294–298. https://doi.org/10.1016/j.bbr.2009.07.025

Brady, A. M., Saul, R. D., & Wiest, M. K. (2010). Selective deficits in spatial working memory in the neonatal ventral hippocampal lesion rat model of schizophrenia. Neuropharmacology, 59(7–8), 605–611. https://doi.org/10.1016/j.neuropharm.2010.08.012

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. Proceedings of the National Academy of Sciences of the United States of America, 101(40), 14515–14520. https://doi.org/10.1073/pnas.0406344101

Brooks, J. M., Pershing, M. L., Thomsen, M. S., Mikkelsen, J. D., Sarter, M., & Bruno, J. P. (2012). Transient inactivation of the neonatal ventral hippocampus impairs attentional set-shifting behavior: Reversal with an α7 nicotinic agonist. Neuropsychopharmacology, Vol. 37, pp. 2476–2486. https://doi.org/10.1038/npp.2012.106

Brown, V. J., & Tait, D. S. (2016). Attentional Set-Shifting Across Species. In Current Topics in Behavioral Neurosciences (pp. 363–395). https://doi.org/10.1007/7854_2015_5002

Bubenikova-Valesova, V., Stuchlik, A., Svoboda, J., Bures, J., & Vales, K. (2008). Risperidone and ritanserin but not haloperidol block effect of dizocilpine on the active allothetic place avoidance task. Proceedings of the National Academy of Sciences, 105(3), 1061–1066. https://doi.org/10.1073/pnas.0711273105

Bueno-Junior, L. S., & Leite, J. P. (2018). Input Convergence, Synaptic Plasticity and Functional Coupling Across Hippocampal-Prefrontal-Thalamic Circuits. Frontiers in Neural Circuits, 12(40), 1–17. https://doi.org/10.3389/fncir.2018.00040

Bures, J., Fenton, A. A., Kaminsky, Y., & Zinyuk, L. (1997). Place cells and place navigation. Proceedings of the National Academy of Sciences of the United States of America, 94(1), 343–350. https://doi.org/10.1073/pnas.94.1.343

Burke, K. A., Takahashi, Y. K., Correll, J., Leon Brown, P., & Schoenbaum, G. (2009). Orbitofrontal inactivation impairs reversal of Pavlovian learning by interfering with “disinhibition” of responding for previously unrewarded cues. European Journal of Neuroscience, 30(10), 1941–1946. https://doi.org/10.1111/j.1460-9568.2009.06992.x

Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1997a). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel pavlovian autoshaping procedure for the rat: Implications for the neurobiology of emotion. Behavioral Neuroscience, 111(5), 908–919. https://doi.org/10.1037/0735-7044.111.5.908

Bussey, T. J., Muir, J. L., Everitt, B. J., & Robbins, T. W. (1997b). Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. Behavioral Neuroscience, 111(5), 920–936. https://doi.org/10.1037/0735-7044.111.5.920

Cannon, T. D., Thompson, P. M., van Erp, T. G. M., Toga, A. W., Poutanen, V.-P., Huttunen, M., … Kaprio, J. (2002). Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. Proceedings of the National Academy of Sciences, 99(5), 3228–3233. https://doi.org/10.1073/pnas.052023499

Carr, D B, & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 20(10), 3864–3873. https://doi.org/http://www.jneurosci.org/content/20/10/3864

Carr, David B., & Sesack, S. R. (1996). Hippocampal afferents to the rat prefrontal cortex: Synaptic targets and relation to dopamine terminals. Journal of Comparative Neurology, 369(1), 1–15. https://doi.org/10.1002/(SICI)1096-9861(19960520)369:1<1::AID-CNE1>3.0.CO;2-7

Carter, C. S., Braver, T. S., Barch, D. M., Botvinick, M. M., Noll, D., & Cohen, J. D. (1998). Anterior cingulate cortex, error detection, and the online monitoring of performance. Science, 280(5364), 747–749. https://doi.org/10.1126/science.280.5364.747

Castañé Anna, A., Theobald, D. E. H., & Robbins, T. W. (2010). Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats. Behavioural Brain Research, 210(1), 74–83. https://doi.org/10.1016/j.bbr.2010.02.017

Chambers, R. A., Moore, J., McEvoy, J. P., & Levin, E. D. (1996). Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia. Neuropsychopharmacology, 15(6), 587–594. https://doi.org/10.1016/s0893-133x(96)00132-7

Chan, R. C. K., Di, X., McAlonan, G. M., & Gong, Q. -y. (2011). Brain Anatomical Abnormalities in High-Risk Individuals, First-Episode, and Chronic Schizophrenia: An Activation Likelihood Estimation Meta-analysis of Illness Progression. Schizophrenia Bulletin, 37(1), 177–188. https://doi.org/10.1093/schbul/sbp073

Chrapusta, S. J., Egan, M. F., Wyatt, R. J., Weinberger, D. R., & Lipska, B. K. (2003). Neonatal ventral hippocampal damage modifies serum corticosterone and dopamine release responses to acute footshock in adult Sprague-Dawley rats. Synapse, 47(4), 270–277. https://doi.org/10.1002/syn.10179

Christopher, G., & MacDonald, J. (2005). The impact of clinical depression on working memory. Cognitive Neuropsychiatry, 10(5), 379–399. https://doi.org/10.1080/13546800444000128

Chudasama, Y., Passetti, F., Rhodes, S. E. V., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: Differential effects on selectivity, impulsivity and compulsivity. Behavioural Brain Research, 146(1–2), 105–119. https://doi.org/10.1016/j.bbr.2003.09.020

Cimadevilla, J. M., Wesierska, M., Fenton, A. A., & Bures, J. (2001). Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. Proceedings of the National Academy of Sciences of the United States of America, 98(6), 3531–3536. https://doi.org/10.1073/pnas.051628398

Cimadevilla, Jose M, Fenton, A. A., & Bures, J. (2001). New spatial cognition tests for mice: Passive place avoidance on stable and active place avoidance on rotating arenas. Brain Research Bulletin, 54(5), 559–563. https://doi.org/10.1016/S0361-9230(01)00448-8

Cimadevilla, Jose M, Kaminsky, Y., Fenton, A., & Bures, J. (2000). Passive and active place avoidance as a tool of spatial memory research in rats. Journal of Neuroscience Methods, 102, 155–164.

Clarke, H. F., Robbins, T. W., & Roberts, A. C. (2008). Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. Journal of Neuroscience, 28(43), 10972–10982. https://doi.org/10.1523/JNEUROSCI.1521-08.2008

Condé, F., Maire-Lepoivre, E., Audinat, E., & Crépel, F. (1995). Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. The Journal of Comparative Neurology, 352(4), 567–593. https://doi.org/10.1002/cne.903520407

Corcoran, K. A., & Quirk, G. J. (2007). Activity in Prelimbic Cortex Is Necessary for the Expression of Learned, But Not Innate, Fears. Journal of Neuroscience, 27(4), 840–844. https://doi.org/10.1523/JNEUROSCI.5327-06.2007

Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A., & Buzsáki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. Journal of Neuroscience, 19(1), 274–287. https://doi.org/10.1523/jneurosci.19-01-00274.1999

Cummings, J. L. (1995). Anatomic and Behavioral Aspects of Frontal-Subcortical Circuits. Annals of the New York Academy of Sciences, 769(1), 1–14. https://doi.org/10.1111/j.1749-6632.1995.tb38127.x

Davoodi, F. G., Motamedi, F., Naghdi, N., & Akbari, E. (2009). Effect of reversible inactivation of the reuniens nucleus on spatial learning and memory in rats using Morris water maze task. Behavioural Brain Research, 198(1), 130–135. https://doi.org/10.1016/j.bbr.2008.10.037

de Bruin, J. P. C., Sànchez-Santed, F., Heinsbroek, R. P. W., Donker, A., & Postmes, P. (1994). A behavioural analysis of rats with damage to the medial prefrontal cortex using the morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. Brain Research, 652(2), 323–333. https://doi.org/10.1016/0006-8993(94)90243-7

de Hoz, L., Knox, J., & Morris, R. G. M. (2003). Longitudinal axis of the hippocampus: Both septal and temporal poles of the hippocampus support water maze spatial learning depending on the training protocol. Hippocampus, 13(5), 587–603. https://doi.org/10.1002/hipo.10079

Delatour, B., & Gisquet-Verrier, P. (2000). Functional role of rat prelimbic-infralimbic cortices in spatial memory: Evidence for their involvement in attention and behavioural flexibility. Behavioural Brain Research, 109(1), 113–128. https://doi.org/10.1016/S0166-4328(99)00168-0

Dockery, C. A., & Wesierska, M. J. (2010). A spatial paradigm, the allothetic place avoidance alternation task, for testing visuospatial working memory and skill learning in rats. Journal of Neuroscience Methods, 191(2), 215–221. https://doi.org/10.1016/j.jneumeth.2010.06.029

Dominguez, G., Faucher, P., Henkous, N., Krazem, A., Piérard, C., & Béracochéa, D. (2014). Stress induced a shift from dorsal hippocampus to prefrontal cortex dependent memory retrieval: Role of regional corticosterone. Frontiers in Behavioral Neuroscience, 8, 1–11. https://doi.org/10.3389/fnbeh.2014.00166

Drewe, E. A. (1974). The Effect of Type and Area of Brain Lesion on Wisconsin Card Sorting Test Performance. Cortex, 10(2), 159–170. https://doi.org/10.1016/S0010-9452(74)80006-7

Everett, J., Lavoie, K., Gagnon, J. F., & Gosselin, N. (2001). Performance of patients with schizophrenia on the Wisconsin Card Sorting Test (WCST). Journal of Psychiatry and Neuroscience, 26(2), 123–130.

Fanselow, M. S., & Dong, H. W. (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? Neuron, 65(1), 7–19. https://doi.org/10.1016/j.neuron.2009.11.031

Farinelli, M., Deschaux, O., Hugues, S., Thevenet, A., & Garcia, R. (2006). Hippocampal train stimulation modulates recall of fear extinction independently of prefrontal cortex synaptic plasticity and lesions. Learning and Memory, 13(3), 329–334. https://doi.org/10.1101/lm.204806

Fellows, L. K., & Farah, M. J. (2003). Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. Brain, 126(8), 1830–1837. https://doi.org/10.1093/brain/awg180

Ferbinteanu, J., Ray, C., & McDonald, R. J. (2003). Both dorsal and ventral hippocampus contribute to spatial learning in Long-Evans rats. Neuroscience Letters, 345(2), 131–135. https://doi.org/10.1016/S0304-3940(03)00473-7

Flores, G., Alquicer, G., Silva-Gómez, A. B., Zaldivar, G., Stewart, J., Quirion, R., & Srivastava, L. K. (2005). Alterations in dendritic morphology of prefrontal cortical and nucleus accumbens neurons in post-pubertal rats after neonatal excitotoxic lesions of the ventral hippocampus. Neuroscience, 133(2), 463–470. https://doi.org/10.1016/j.neuroscience.2005.02.021

Floresco, S B, Seamans, J. K., & Phillips, a G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 17(5), 1880–1890. https://doi.org/10.1016/0306-4522(87)90179-5

Floresco, Stan B., Block, A. E., & Tse, M. T. L. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behavioural Brain Research, 190(1), 85–96. https://doi.org/10.1016/j.bbr.2008.02.008

Floresco, Stan B., Ghods-Sharifi, S., Vexelman, C., & Magyar, O. (2006). Dissociable roles for the nucleus accumbens core and shell in regulating set shifting. Journal of Neuroscience, 26(9), 2449–2457. https://doi.org/10.1523/JNEUROSCI.4431-05.2006

Floresco, Stan B., Todd, C. L., & Grace, A. A. (2001). Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. Journal of Neuroscience, 21(13), 4915–4922. https://doi.org/10.1523/jneurosci.21-13-04915.2001

Floresco, Stan B., Zhang, Y., & Enomoto, T. (2009). Neural circuits subserving behavioral flexibility and their relevance to schizophrenia. Behavioural Brain Research, 204(2), 396–409. https://doi.org/10.1016/j.bbr.2008.12.001

Fusar-Poli, P., Deste, G., Smieskova, R., Barlati, S., Yung, A. R., Howes, O., … Borgwardt, S. (2012). Cognitive Functioning in Prodromal Psychosis. Archives of General Psychiatry, 69(6), 562–571. https://doi.org/10.1001/archgenpsychiatry.2011.1592

Fuxe, K., Hökfelt, T., Johansson, O., Jonsson, G., Lidbrink, P., & Ljundahl, Å. (1974). The origin of the dopamine nerve terminals in limbic and frontal cortex. Evidence for meso-cortico dopamine neurons. Brain Research, 82(2), 349–355. https://doi.org/10.1016/0006-8993(74)90618-0

Gabbott, P., Headlam, A., & Busby, S. (2002). Morphological evidence that CA1 hippocampal afferents monosynaptically innervate PV-containing neurons and NADPH-diaphorase reactive cells in the medial prefrontal cortex (Areas 25/32) of the rat. Brain Research, 946(2), 314–322. https://doi.org/10.1016/S0006-8993(02)02487-3

Garey, L. J., Ong, W. Y., Patel, T. S., Kanani, M., Davis, A., Mortimer, A. M., … Hirsch, S. R. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. Journal of Neurology, Neurosurgery & Psychiatry, 65(4), 446–453. https://doi.org/10.1136/jnnp.65.4.446

Genzel, L., Dresler, M., Cornu, M., Jäger, E., Konrad, B., Adamczyk, M., … Goya-Maldonado, R. (2015). Medial prefrontal-hippocampal connectivity and motor memory consolidation in depression and schizophrenia. Biological Psychiatry, 77(2), 177–186. https://doi.org/10.1016/j.biopsych.2014.06.004

Ghahremani, D. G., Monterosso, J., Jentsch, J. D., Bilder, R. M., & Poldrack, R. A. (2010). Neural Components Underlying Behavioral Flexibility in Human Reversal Learning. Cerebral Cortex, 20(8), 1843–1852. https://doi.org/10.1093/cercor/bhp247

Ghanbarian, E., & Motamedi, F. (2013). Ventral Tegmental Area Inactivation Suppresses the Expression of CA1 Long Term Potentiation in Anesthetized Rat. PLoS ONE, 8(3), e58844. https://doi.org/10.1371/journal.pone.0058844

Ghods-Sharifi, S., Haluk, D. M., & Floresco, S. B. (2008). Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. Neurobiology of Learning and Memory, 89(4), 567–573. https://doi.org/10.1016/j.nlm.2007.10.007

Gigg, J., Tan, A. M., & Finch, D. M. (1994). Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. Hippocampus, 4(2), 189–198. https://doi.org/10.1002/hipo.450040209

Granon, S., & Poucet, B. (2000). Involvement of the rat prefrontal cortex in cognitive functions : A central role for the prelimbic area. Psychobiology, 28(2), 229–237. https://doi.org/https://doi.org/10.3758/BF03331981

Groenewegen, H. J. (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience, 24(2), 379–431. https://doi.org/10.1016/0306-4522(88)90339-9

Groenewegen, H. J., Vermeulen-Van der Zee, E., Te Kortschot, A., & Witter, M. P. (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. Neuroscience, 23(1), 103–120. https://doi.org/10.1016/0306-4522(87)90275-2

Haluk, D. M., & Floresco, S. B. (2009). Ventral striatal dopamine modulation of different forms of behavioral flexibility. Neuropsychopharmacology, 34(8), 2041–2052. https://doi.org/10.1038/npp.2009.21

Hamilton, D. A., & Brigman, J. L. (2015). Behavioral flexibility in rats and mice: Contributions of distinct frontocortical regions. Genes, Brain and Behavior, 14(1), 4–21. https://doi.org/10.1111/gbb.12191

Hauer, B. E., Pagliardini, S., & Dickson, C. T. (2019). The Reuniens Nucleus of the Thalamus Has an Essential Role in Coordinating Slow-Wave Activity between Neocortex and Hippocampus. ENeuro, 6(5), ENEURO.0365-19.2019. https://doi.org/10.1523/ENEURO.0365-19.2019

Haut, M. W., Cahill, J., Cutlip, W. D., Stevenson, J. M., Makela, E. H., & Bloomfield, S. M. (1996). On the nature of Wisconsin Card Sorting Test performance in schizophrenia. Psychiatry Research, 65(1), 15–22. https://doi.org/10.1016/0165-1781(96)02940-X

Heckers, S., & Konradi, C. (2015). GABAergic mechanisms of hippocampal hyperactivity in schizophrenia. Schizophrenia Research, 167(1–3), 4–11. https://doi.org/10.1016/j.schres.2014.09.041

Heinrichs, R. W., & Zakzanis, K. K. (1998). Neurocognitive deficit in schizophrenia: A quantitative review of the evidence. Neuropsychology, 12(3), 426–445. https://doi.org/10.1037/0894-4105.12.3.426

Henke, P. G. (1990). Hippocampal Pathway to the Amygdala and Stress Ulcer Development. Brain Research Bulletin, 25, 691–695. https://doi.org/10.1016/0361-9230(90)90044-Z

Herman, J. P., Cullinan, W. E., Morano, M. I., Akil, H., & Watson, S. J. (1995). Contribution of the Ventral Subiculum to Inhibitory Regulation of the Hypothalamo-Pituitary-Adrenocortical Axis. Journal of Neuroendocrinology, 7(6), 475–482. https://doi.org/10.1111/j.1365-2826.1995.tb00784.x

Herman, J. P., Cullinan, W. E., Young, E. A., Akil, H., & Watson, S. J. (1992). Selective forebrain fiber tract lesions implicate ventral hippocampal structures in tonic regulation of paraventricular nucleus corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) mRNA expression. Brain Research, 592(1–2), 228–238. https://doi.org/10.1016/0006-8993(92)91680-D

Hobin, J. A., Ji, J., & Maren, S. (2006). Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. Hippocampus, 16(2), 174–182. https://doi.org/10.1002/hipo.20144

Honzel, N., Justus, T., & Swick, D. (2014). Posttraumatic stress disorder is associated with limited executive resources in a working memory task. Cognitive, Affective and Behavioral Neuroscience, 14(2), 792–804. https://doi.org/10.3758/s13415-013-0219-x

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Structure and Function, 212(2), 149–179. https://doi.org/10.1007/s00429-007-0150-4

Hunt, P. R., & Aggleton, J. P. (1998). Neurotoxic lesions of the dorsomedial thalamus impair the acquisition but not the performance of delayed matching to place by rats: A deficit in shifting response rules. Journal of Neuroscience, 18(23), 10045–10052. https://doi.org/10.1523/jneurosci.18-23-10045.1998

Insausti, R. (1993). Comparative anatomy of the entorhinal cortex and hippocampus in mammals. Hippocampus, 3(1 S), 19–26. https://doi.org/10.1002/hipo.1993.4500030705

Ishikawa, A., & Nakamura, S. (2006). Ventral Hippocampal Neurons Project Axons Simultaneously to the Medial Prefrontal Cortex and Amygdala in the Rat. Journal of Neurophysiology, 96(4), 2134–2138. https://doi.org/10.1152/jn.00069.2006

Izaki, Y., Hori, K., & Nomura, M. (2000). Disturbance of rat lever-press learning by hippocampo-prefrontal disconnection. Brain Research, 860(1–2), 199–202. https://doi.org/10.1016/S0006-8993(00)02039-4

Jay, T. M., Glowinski, J., & Thierry, A. M. (1989). Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. Brain Research, 505(2), 337–340. https://doi.org/10.1016/0006-8993(89)91464-9

Jay, T. M., Thierry, A. -M, Wiklund, L., & Glowinski, J. (1992). Excitatory Amino Acid Pathway from the Hippocampus to the Prefrontal Cortex. Contribution of AMPA Receptors in Hippocampo-prefrontal Cortex Transmission. European Journal of Neuroscience, 4(12), 1285–1295. https://doi.org/10.1111/j.1460-9568.1992.tb00154.x

Jay, T. M., & Witter, M. P. (1991). Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. Journal of Comparative Neurology, 313(4), 574–586. https://doi.org/10.1002/cne.903130404

Johnstone, B., Hogg, J. R., Schopp, L. H., Kapila, C., & Edwards, S. (2002). Neuropsychological deficit profiles in senile dementia of the Alzheimer's type. Archives of Clinical Neuropsychology, 17(3), 273–281. https://doi.org/10.1016/S0887-6177(01)00112-3

Jung, M. W., Wiener, S. I., & McNaughton, B. L. (1994). Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. Journal of Neuroscience, 14(12), 7347–7356. https://doi.org/10.1523/jneurosci.14-12-07347.1994

Karl, A., Schaefer, M., Malta, L. S., Dörfel, D., Rohleder, N., & Werner, A. (2006). A meta-analysis of structural brain abnormalities in PTSD. Neuroscience and Biobehavioral Reviews, 30(7), 1004–1031. https://doi.org/10.1016/j.neubiorev.2006.03.004

Keefe, R. S. E., Eesley, C. E., & Poe, M. P. (2005). Defining a cognitive function decrement in schizophrenia. Biological Psychiatry, 57(6), 688–691. https://doi.org/10.1016/j.biopsych.2005.01.003

Kelemen, E., & Fenton, A. A. (2010). Dynamic Grouping of Hippocampal Neural Activity During Cognitive Control of Two Spatial Frames. PLoS Biology, 8(6), e1000403. https://doi.org/10.1371/journal.pbio.1000403

Kenney, J., & Manahan-Vaughan, D. (2013). Learning-facilitated synaptic plasticity occurs in the intermediate hippocampus in association with spatial learning. Frontiers in Synaptic Neuroscience, 5(10), 1–7. https://doi.org/10.3389/fnsyn.2013.00010

Khani, A. (2014). Partially dissociable roles of OFC and ACC in stimulus-guided and action-guided decision making. Journal of Neurophysiology, 111(9), 1717–1720. https://doi.org/10.1152/jn.00323.2013

Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H.-A., Murison, R., Moser, E. I., & Moser, M.-B. (2002). Reduced fear expression after lesions of the ventral hippocampus. Proceedings of the National Academy of Sciences, 99(16), 10825–10830. https://doi.org/10.1073/pnas.152112399

Klausberger, T., Magill, P. J., Márton, L. F., Roberts, J. D. B., Cobden, P. M., Buzsáki, G., & Somogyi, P. (2003). Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature, 421(6925), 844–848. https://doi.org/10.1038/nature01374

Knight, R. T., & Nakada, T. (1998). Cortico-limbic circuits and novelty: A review of EEG and blood flow data. Reviews in the Neurosciences, 9(1), 57–70. https://doi.org/10.1515/REVNEURO.1998.9.1.57

Kolb, B., & Whishaw, I. Q. (1983). Performance of schizophrenic patients on tests sensitive to left or right frontal, temporal, or parietal function in neurological patients. Journal of Nervous and Mental Disease, Vol. 171, pp. 435–443. https://doi.org/10.1097/00005053-198307000-00008

Kosaki, Y., & Watanabe, S. (2012). Dissociable roles of the medial prefrontal cortex, the anterior cingulate cortex, and the hippocampus in behavioural flexibility revealed by serial reversal of three-choice discrimination in rats. Behavioural Brain Research, 227(1), 81–90. https://doi.org/10.1016/j.bbr.2011.10.039

Laroche, S., Jay, T. M., & Thierry, A. M. (1990). Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. Neuroscience Letters, 114(2), 184–190. https://doi.org/10.1016/0304-3940(90)90069-L

Lee, H., Dvorak, D., Kao, H. Y., Duffy, Á. M., Scharfman, H. E., & Fenton, A. A. (2012). Early Cognitive Experience Prevents Adult Deficits in a Neurodevelopmental Schizophrenia Model. Neuron, 75(4), 714–724. https://doi.org/10.1016/j.neuron.2012.06.016

Lee, S. L., Lew, D., Wickenheisser, V., & Markus, E. J. (2019). Interdependence between dorsal and ventral hippocampus during spatial navigation. Brain and Behavior, 9(10), 1–14. https://doi.org/10.1002/brb3.1410

Li, L., & Shao, J. (1998). Restricted lesions to ventral prefrontal subareas block reversal learning but not visual discrimination learning in rats. Physiology and Behavior, 65(2), 371–379. https://doi.org/10.1016/S0031-9384(98)00216-9

Linley, S. B., Gallo, M. M., & Vertes, R. P. (2016). Lesions of the ventral midline thalamus produce deficits in reversal learning and attention on an odor texture set shifting task. Brain Research, 1649, 110–122. https://doi.org/10.1016/j.brainres.2016.08.022

Lipska, B. K. (2002). Neonatal disconnection of the rat hippocampus: A neurodevelopmental model of schizophrenia. Dialogues in Clinical Neuroscience, 4(4), 361–367.

Liu, X., & Carter, A. G. (2018). Ventral hippocampal inputs preferentially drive corticocortical neurons in the infralimbic prefrontal cortex. Journal of Neuroscience, 38(33), 7351–7363. https://doi.org/10.1523/JNEUROSCI.0378-18.2018

Loureiro, M., Lecourtier, L., Engeln, M., Lopez, J., Cosquer, B., Geiger, K., … Pereira De Vasconcelos, A. (2012). The ventral hippocampus is necessary for expressing a spatial memory. Brain Structure and Function, 217(1), 93–106. https://doi.org/10.1007/s00429-011-0332-y

Malá, H., Andersen, L. G., Christensen, R. F., Felbinger, A., Hagstrøm, J., Meder, D., … Mogensen, J. (2015). Prefrontal cortex and hippocampus in behavioural flexibility and posttraumatic functional recovery: Reversal learning and set-shifting in rats. Brain Research Bulletin, 116, 34–44. https://doi.org/10.1016/j.brainresbull.2015.05.006

Marek, R., Jin, J., Goode, T. D., Giustino, T. F., Wang, Q., Acca, G. M., … Sah, P. (2018). Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. Nature Neuroscience, 21(3), 384–392. https://doi.org/10.1038/s41593-018-0073-9

Maren, S. (1999). Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats. Behavioral Neuroscience, 113(2), 283–290. https://doi.org/10.1037/0735-7044.113.2.283

Maren, S., & Holt, W. G. (2004). Hippocampus and Pavlovian Fear Conditioning in Rats: Muscimol Infusions Into the Ventral, but Not Dorsal, Hippocampus Impair the Acquisition of Conditional Freezing to an Auditory Conditional Stimulus. Behavioral Neuroscience, 118(1), 97–110. https://doi.org/10.1037/0735-7044.118.1.97

Marquis, J. P., Goulet, S., & Doré, F. Y. (2008). Neonatal ventral hippocampus lesions disrupt extra-dimensional shift and alter dendritic spine density in the medial prefrontal cortex of juvenile rats. Neurobiology of Learning and Memory, 90(2), 339–346. https://doi.org/10.1016/j.nlm.2008.04.005

Martig, A. K., Jones, G. L., Smith, K. E., & Mizumori, S. J. Y. (2009). Context dependent effects of ventral tegmental area inactivation on spatial working memory. Behavioural Brain Research, 203(2), 316–320. https://doi.org/10.1016/j.bbr.2009.05.008

Martig, A. K., & Mizumori, S. J. Y. (2011). Ventral tegmental area and substantia nigra neural correlates of spatial learning. Learning & Memory, 18(4), 260–271. https://doi.org/10.1101/lm.1895211

Martin, J. H. (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neuroscience Letters, 127(2), 160–164. https://doi.org/10.1016/0304-3940(91)90784-Q

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behavioural Brain Research, 146(1–2), 97–103. https://doi.org/10.1016/j.bbr.2003.09.019

McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P., & Bannerman, D. M. (2004). Amygdala and Ventral Hippocampus Contribute Differentially to Mechanisms of Fear and Anxiety. Behavioral Neuroscience, 118(1), 63–78. https://doi.org/10.1037/0735-7044.118.1.63

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., … Rauch, S. L. (2009). Neurobiological Basis of Failure to Recall Extinction Memory in Posttraumatic Stress Disorder. Biological Psychiatry, 66(12), 1075–1082. https://doi.org/10.1016/j.biopsych.2009.06.026

Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. Nature, 420(6911), 70–74. https://doi.org/10.1038/nature01138

Monchi, O., Ko, J. H., & Strafella, A. P. (2006). Striatal dopamine release during performance of executive functions: A [11C] raclopride PET study. NeuroImage, 33(3), 907–912. https://doi.org/10.1016/j.neuroimage.2006.06.058

Monchi, O., Petrides, M., Petre, V., Worsley, K., & Dagher, A. (2001). Wisconsin card sorting revisited: Distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. Journal of Neuroscience, 21(19), 7733–7741. https://doi.org/10.1523/jneurosci.21-19-07733.2001

Morris, L. S., Kundu, P., Dowell, N., Mechelmans, D. J., Favre, P., Irvine, M. A., … Voon, V. (2016). Fronto-striatal organization: Defining functional and microstructural substrates of behavioural flexibility. Cortex, 74, 118–133. https://doi.org/10.1016/j.cortex.2015.11.004

Moser, E., Moser, M. B., & Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. Journal of Neuroscience, 13(9), 3916–3925. https://doi.org/10.1523/jneurosci.13-09-03916.1993

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. Proceedings of the National Academy of Sciences, 92(21), 9697–9701. https://doi.org/10.1073/pnas.92.21.9697

Naber, P. A., Lopes da Silva, F. H., & Witter, M. P. (2001). Reciprocal connections between the entorhinal cortex and hippocampal fields CA1 and the subiculum are in register with the projections from CA1 to the subiculum. Hippocampus, 11, 99–104. https://doi.org/10.1002/hipo.1028

Nagano-Saito, A., Leyton, M., Monchi, O., Goldberg, Y. K., He, Y., & Dagher, A. (2008). Dopamine depletion impairs frontostriatal functional connectivity during a set-shifting task. Journal of Neuroscience, 28(14), 3697–3706. https://doi.org/10.1523/JNEUROSCI.3921-07.2008

Ng, C., Noblejas, M. I., Rodefer, J. S., Smith, C. B., & Poremba, A. (2007). Double Dissociation of Attentional Resources : Prefrontal Versus Cingulate Cortices. 27(45), 12123–12131. https://doi.org/10.1523/JNEUROSCI.2745-07.2007

O'Doherty, J., Kringelbach, M. L., Rolls, E. T., Hornak, J., & Andrews, C. (2001). Abstract reward and punishment representations in the human orbitofrontal cortex. Nature Neuroscience, 4(1), 95–102. https://doi.org/10.1038/82959

O'Donnell, P. (2012). Cortical disinhibition in the neonatal ventral hippocampal lesion model of schizophrenia: New vistas on possible therapeutic approaches. Pharmacology and Therapeutics, 133(1), 19–25. https://doi.org/10.1016/j.pharmthera.2011.07.005

Orsini, C. A., Kim, J. H., Knapska, E., & Maren, S. (2011). Hippocampal and Prefrontal Projections to the Basal Amygdala Mediate Contextual Regulation of Fear after Extinction. Journal of Neuroscience, 31(47), 17269–17277. https://doi.org/10.1523/JNEUROSCI.4095-11.2011

Padilla-Coreano, N., Bolkan, S. S., Pierce, G. M., Blackman, D. R., Hardin, W. D., Garcia-Garcia, A. L., … Gordon, J. A. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron, 89(4), 857–866. https://doi.org/10.1016/j.neuron.2016.01.011

Pantelis, C., Barber, F. Z., Barnes, T. R. E., Nelson, H. E., Owen, A. M., & Robbins, T. W. (1999). Comparison of set-shifting ability in patients with chronic schizophrenia and frontal lobe damage. Schizophrenia Research, 37(3), 251–270. https://doi.org/10.1016/S0920-9964(98)00156-X

Paolo, A. M., Axelrod, B. N., Tröster, A. I., Blackwell, K. T., & Koller, W. C. (1996). Utility of a Wisconsin Card Sorting Test short form in persons with Alzheimer's and Parkinson's disease. Journal of Clinical and Experimental Neuropsychology, 18(6), 892–897. https://doi.org/10.1080/01688639608408310

Park, E. H., Reilly, K. C. O., Taborga, D., Nicholas, K., & Ahmed, A. S. (2019). Is the rat prefrontal cortex crucial for cognitive control during spatial cognition ?

Parron, C., Poucet, B., & Save, E. (2001). Re-evaluation of the spatial memory deficits induced by hippocampal short lasting inactivation reveals the need for cortical co-operation. Behavioural Brain Research, 127(1–2), 71–79. https://doi.org/10.1016/S0166-4328(01)00357-6

Pearson-Leary, J., Eacret, D., Chen, R., Takano, H., Nicholas, B., & Bhatnagar, S. (2017). Inflammation and vascular remodeling in the ventral hippocampus contributes to vulnerability to stress. Translational Psychiatry, 7, e1160. https://doi.org/10.1038/tp.2017.122

Phillips, J. M., & Brown, V. J. (2000). Anticipatory errors after unilateral lesions of the subthalamic nucleus in the rat: Evidence for a failure of response inhibition. Behavioral Neuroscience, 114(1), 150–157. https://doi.org/10.1037/0735-7044.114.1.150

Pikkarainen, M., Rönkkö, S., Savander, V., Insausti, R., & Pitkänen, A. (1999). Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. The Journal of Comparative Neurology, 403(2), 229–260. https://doi.org/10.1002/(SICI)1096-9861(19990111)403:2<229::AID-CNE7>3.0.CO;2-P

Place, R., Farovik, A., Brockmann, M., & Eichenbaum, H. (2016). Bidirectional prefrontal-hippocampal interactions support context-guided memory. Nature Neuroscience, 19(8), 992–994. https://doi.org/10.1038/nn.4327

Placek, K., Dippel, W. C., Jones, S., & Brady, A. M. (2013). Impairments in set-shifting but not reversal learning in the neonatal ventral hippocampal lesion model of schizophrenia: Further evidence for medial prefrontal deficits. Behavioural Brain Research, 256, 405–413. https://doi.org/10.1016/j.bbr.2013.08.034

Popik, P., & Nikiforuk, A. (2015). Attentional set-shifting paradigm in the rat. Current Protocols in Neuroscience, 72, 9.51.1-9.51.13. https://doi.org/10.1002/0471142301.ns0951s72

Pothuizen, H. H. J., Zhang, W. N., Jongen-Rêlo, A. L., Feldon, J., & Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: A within-subject, within-task comparison of reference and working spatial memory. European Journal of Neuroscience, 19(3), 705–712. https://doi.org/10.1111/j.0953-816X.2004.03170.x

Potvin, O., Allen, K., Thibaudeau, G., Doré, F. Y., & Goulet, S. (2006). Performance on spatial working memory tasks after dorsal or ventral hippocampal lesions and adjacent damage to the subiculum. Behavioral Neuroscience, 120(2), 413–422. https://doi.org/10.1037/0735-7044.120.2.413

Poucet, B., Herrmann, T., & Buhot, M. C. (1991). Effects of short-lasting inactivations of the ventral hippocampus and medial septum on long-term and short-term acquisition of spatial information in rats. Behavioural Brain Research, 44(1), 53–65. https://doi.org/10.1016/S0166-4328(05)80239-6

Poucet, B., Thinus-Blanc, C., & Muller, R. U. (1994). Place cells in the ventral hippocampus of rats. NeuroReport, 5(16), 2045–2048. https://doi.org/10.1097/00001756-199410270-00014

Prior, M., & Hoffmann, W. (1990). Brief report: Neuropsychological testing of autistic children through an exploration with frontal lobe tests. Journal of Autism and Developmental Disorders, 20(4), 581–590. https://doi.org/10.1007/BF02216063

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology, 33(1), 56–72. https://doi.org/10.1038/sj.npp.1301555

Ragozzino, M. E. (2002). The Effects of Dopamine D1 Receptor Blockade in the Prelimbic-Infralimbic Areas on Behavioral Flexibility. Learning & Memory, 9(1), 18–28. https://doi.org/10.1101/lm.45802

Ragozzino, M. E. (2007). The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. Annals of the New York Academy of Sciences, 1121, 355–375. https://doi.org/10.1196/annals.1401.013

Ragozzino, M. E., Detrick, S., & Kesner, R. P. (1999). Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. Journal of Neuroscience, 19(11), 4585–4594.

Ragozzino, M. E., Ragozzino, K. E., Mizumori, S. J. Y., & Kesner, R. P. (2002). Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. Behavioral Neuroscience, 116(1), 105–115. https://doi.org/10.1037/0735-7044.116.1.105

Rajasethupathy, P., Sankaran, S., Marshel, J. H., Kim, C. K., Ferenczi, E., Lee, S. Y., … Deisseroth, K. (2015). Projections from neocortex mediate top-down control of memory retrieval. Nature, 526(7575), 653–659. https://doi.org/10.1038/nature15389

Rich, E. L., & Shapiro, M. (2009). Rat prefrontal cortical neurons selectively code strategy switches. Journal of Neuroscience, 29(22), 7208–7219. https://doi.org/10.1523/JNEUROSCI.6068-08.2009

Rich, E. L., & Shapiro, M. L. (2007). Prelimbic/infralimbic inactivation impairs memory for multiple task switches, but not flexible selection of familiar tasks. Journal of Neuroscience, 27(17), 4747–4755. https://doi.org/10.1523/JNEUROSCI.0369-07.2007

Richmond, M. A., Pouzet, B., Veenman, L., Feldon, J., Yee, B. K., Rawlins, J. N. P., & Bannerman, D. M. (1999). Dissociating context and space within the hippocampus: Effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. Behavioral Neuroscience, 113(6), 1189–1203. https://doi.org/10.1037/0735-7044.113.6.1189

Rocher, C., Spedding, M., Munoz, C., & Jay, T. M. (2004). Acute Stress-induced Changes in Hippocampal/Prefrontal Circuits in Rats: Effects of Antidepressants. Cerebral Cortex, 14(2), 224–229. https://doi.org/10.1093/cercor/bhg122

Rogers, R. D., Andrews, T. C., Grasby, P. M., Brooks, D. J., & Robbins, T. W. (2000). Contrasting cortical and subcortical activations produced by attentional-set shifting and reversal learning in humans. Journal of Cognitive Neuroscience, 12(1), 142–162. https://doi.org/10.1162/089892900561931

Rolls, E. T. (1994). Neurophysiology and cognitive functions of the striatum. Revue Neurologique, Vol. 150, pp. 648–660.

Romanides, A. ., Duffy, P., & Kalivas, P. . (1999). Glutamatergic and dopaminergic afferents to the prefrontal cortex regulate spatial working memory in rats. Neuroscience, 92(1), 97–106. https://doi.org/10.1016/S0306-4522(98)00747-7

Rushworth, M. F. S., Hadland, K. A., Gaffan, D., & Passingham, R. E. (2003). The Effect of Cingulate Cortex Lesions on Task Switching and Working Memory. Journal of Cognitive Neuroscience, 15(3), 338–353. https://doi.org/10.1162/089892903321593072

Scatton, B., Simon, H., Le Moal, M., & Bischoff, S. (1980). Origin of dopaminergic innervation of the rat hippocampal formation. Neuroscience Letters, 18(2), 125–131. https://doi.org/10.1016/0304-3940(80)90314-6

Schobel, S. A., Chaudhury, N. H., Khan, U. A., Paniagua, B., Styner, M. A., Asllani, I., … Small, S. A. (2013). Imaging Patients with Psychosis and a Mouse Model Establishes a Spreading Pattern of Hippocampal Dysfunction and Implicates Glutamate as a Driver. Neuron, 78(1), 81–93. https://doi.org/10.1016/j.neuron.2013.02.011

Schobel, S. A., Kelly, M. A., Corcoran, C. M., Van Heertum, K., Seckinger, R., Goetz, R., … Malaspina, D. (2009). Anterior hippocampal and orbitofrontal cortical structural brain abnormalities in association with cognitive deficits in schizophrenia. Schizophrenia Research, 114(1–3), 110–118. https://doi.org/10.1016/j.schres.2009.07.016

Seamans, J K, Floresco, S. B., & Phillips, a G. (1998). D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 18(4), 1613–1621.

Seamans, Jeremy K., Lapish, C. C., & Durstewitz, D. (2008). Comparing the prefrontal cortex of rats and primates: Insights from electrophysiology. Neurotoxicity Research, 14(2–3), 249–262. https://doi.org/10.1007/BF03033814

Shafritz, K. M., Kartheiser, P., & Belger, A. (2005). Dissociation of neural systems mediating shifts in behavioral response and cognitive set. NeuroImage, 25(2), 600–606. https://doi.org/10.1016/j.neuroimage.2004.12.054

Shi, F., Liu, B., Zhou, Y., Yu, C., & Jiang, T. (2009). Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: Meta-analyses of MRI studies. Hippocampus, 19(11), 1055–1064. https://doi.org/10.1002/hipo.20573

Svoboda, J., Stankova, A., Entlerova, M., & Stuchlik, A. (2015). Corrigendum: Acute administration of MK-801 in an animal model of psychosis in rats interferes with cognitively demanding forms of behavioral flexibility on a rotating arena. Frontiers in Behavioral Neuroscience, 9. https://doi.org/10.3389/fnbeh.2015.00348

Swanson, L. W. (1981). A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Research, 217(1), 150–154. https://doi.org/10.1016/0006-8993(81)90192-X

Swick, D., Ashley, V., & Turken, A. U. (2008). Left inferior frontal gyrus is critical for response inhibition. BMC Neuroscience, 9, 1–11. https://doi.org/10.1186/1471-2202-9-102

Szatkowska, I., Szymańska, O., Bojarski, P., & Grabowska, A. (2007). Cognitive inhibition in patients with medial orbitofrontal damage. Experimental Brain Research, 181(1), 109–115. https://doi.org/10.1007/s00221-007-0906-3

Szczepanski, S. M., & Knight, R. T. (2014). Insights into Human Behavior from Lesions to the Prefrontal Cortex. Neuron, 83(5), 1002–1018. https://doi.org/10.1016/j.neuron.2014.08.011

Tait, D. S., Bowman, E. M., Neuwirth, L. S., & Brown, V. J. (2018). Assessment of intradimensional/extradimensional attentional set-shifting in rats. Neuroscience and Biobehavioral Reviews, 89(February), 72–84. https://doi.org/10.1016/j.neubiorev.2018.02.013

Takahashi, Y. K., Roesch, M. R., Stalnaker, T. A., Haney, R. Z., Calu, D. J., Taylor, A. R., … Schoenbaum, G. (2009). The Orbitofrontal Cortex and Ventral Tegmental Area Are Necessary for Learning from Unexpected Outcomes. Neuron, 62(2), 269–280. https://doi.org/10.1016/j.neuron.2009.03.005

Takita, M., Izaki, Y., Jay, T. M., Kaneko, H., & Suzuki, S. S. (1999). Induction of stable long-term depression in vivo in the hippocampal-prefrontal cortex pathway. European Journal of Neuroscience, 11(11), 4145–4148. https://doi.org/10.1046/j.1460-9568.1999.00870.x

Tekin, S., & Cummings, J. L. (2002). Frontal-subcortical neuronal circuits and clinical neuropsychiatry: An update. Journal of Psychosomatic Research, 53(2), 647–654. https://doi.org/10.1016/S0022-3999(02)00428-2

Tierney, P. L., Dégenètais, E., Thierry, A.-M., Glowinski, J., & Gioanni, Y. (2004). Influence of the hippocampus on interneurons of the rat prefrontal cortex. European Journal of Neuroscience, 20(2), 514–524. https://doi.org/10.1111/j.1460-9568.2004.03501.x

Torres-Berrío, A., Vargas-López, V., & López-Canul, M. (2019). The ventral hippocampus is required for behavioral flexibility but not for allocentric/egocentric learning. Brain Research Bulletin, 146, 40–50. https://doi.org/10.1016/j.brainresbull.2018.12.011

Trivedi, M. A., & Coover, G. D. (2004). Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. Neurobiology of Learning and Memory, 81(3), 172–184. https://doi.org/10.1016/j.nlm.2004.02.005

Vertes, R. P. (2002). Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens. The Journal of Comparative Neurology, 442(2), 163–187. https://doi.org/10.1002/cne.10083

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S. L., & Quirk, G. J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learning & Memory, 13(6), 728–733. https://doi.org/10.1101/lm.306106

Viena, T. D., Linley, S. B., & Vertes, R. P. (2018). Inactivation of nucleus reuniens impairs spatial working memory and behavioral flexibility in the rat. Hippocampus, 28(4), 297–311. https://doi.org/10.1002/hipo.22831

Volk, D. W., Austin, M. C., Pierri, J. N., Sampson, A. R., & Lewis, D. A. (2001). GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: Decreased expression in a subset of neurons. American Journal of Psychiatry, 158(2), 256–265. https://doi.org/10.1176/appi.ajp.158.2.256

Vorhees, C. V, & Williams, M. T. (2006). Morris water maze : procedures for assessing spatial and related forms of learning and memory. Nature Protocols, 1(2), 848–858. https://doi.org/10.1038/nprot.2006.116

Wang, G. W., & Cai, J. X. (2006). Disconnection of the hippocampal-prefrontal cortical circuits impairs spatial working memory performance in rats. Behavioural Brain Research, 175(2), 329–336. https://doi.org/10.1016/j.bbr.2006.09.002

Weinberger, D. R., Berman, K. F., Suddath, R., & Torrey, E. F. (1992). Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. American Journal of Psychiatry, 149(7), 890–897. https://doi.org/10.1176/ajp.149.7.890

Wesierska, M., Dockery, C., & Fenton, A. A. (2005). Beyond memory, navigation, and inhibition: Behavioral evidence for hippocampus-dependent cognitive coordination in the rat. Journal of Neuroscience, 25(9), 2413–2419. https://doi.org/10.1523/JNEUROSCI.3962-04.2005

Witter, M. P., & Amaral, D. G. (1991). Entorhinal Cortex of the Monkey .5. Projections to the Dentate Gyrus, Hippocampus, and Subicular Complex. The Journal of Comparative Neurology, 307, 437–459.

Wolf, R. C., Vasic, N., Sambataro, F., Höse, A., Frasch, K., Schmid, M., & Walter, H. (2009). Temporally anticorrelated brain networks during working memory performance reveal aberrant prefrontal and hippocampal connectivity in patients with schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33(8), 1464–1473. https://doi.org/10.1016/j.pnpbp.2009.07.032

Woodson, W., Nitecka, L., & Ben-Ari, Y. (1989). Organization of the GABAergic system in the rat hippocampal formation: A quantitative immunocytochemical study. Journal of Comparative Neurology, 280(2), 254–271. https://doi.org/10.1002/cne.902800207

Wouterlood, F. G., Saldana, E., & Witter, M. P. (1990). Projection from the nucleus reuniens thalami to the hippocampal region: Light and electron microscopic tracing study in the rat with the anterograde tracerPhaseolus vulgaris-leucoagglutinin. The Journal of Comparative Neurology, 296(2), 179–203. https://doi.org/10.1002/cne.902960202

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. Journal of Neuroscience, 31(21), 7811–7816. https://doi.org/10.1523/JNEUROSCI.1504-11.2011

Ye, X., Kapeller-Libermann, D., Travaglia, A., Inda, M. C., & Alberini, C. M. (2017). Direct dorsal hippocampal-prelimbic cortex connections strengthen fear memories. Nature Neuroscience, 20(1), 52–61. https://doi.org/10.1038/nn.4443

Zhang, Z. J., & Reynolds, G. P. (2002). A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophrenia Research, 55(1–2), 1–10. https://doi.org/10.1016/S0920-9964(01)00188-8

Zheng, C., & Zhang, T. (2015). Synaptic plasticity-related neural oscillations on hippocampus-prefrontal cortex pathway in depression. Neuroscience. https://doi.org/10.1016/j.neuroscience.2015.01.071