Role of the Ventral CA1 to Primary Auditory Cortex Projection in Associative Learning

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ABSTRACT

As we understand more about individual brain regions, more studies begin to focus on how different regions communicate with each other. Having long been established as the center for learning and memory, the hippocampus has connections to most of neocortex as well as many subcortical regions. Some recent findings revealed that the hippocampus not only receive input from primary sensory cortices but also provide feedback projection to these areas. Do these projections play a role in sensory associative learning? Our project focused on the role of hippocampus to primary auditory cortex (A1) projections in auditory associative learning. Optogenetics was used to study the function of a direct projection from the ventral CA1 region of the hippocampus to primary auditory cortex (A1) in an auditory go/no-go task using head-fixed mice. Preliminary results show that activation of this projection does not affect to task acquisition or generalization. Our next step is to investigate the effect of inhibiting the CA1 to A1 projections.

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INTRODUCTION

1.1 Associative Learning

In the early 20th century, two behavioral paradigms—Pavlovian conditioning and instrumental conditioning, were founded by Pavlov and Thorndike respectively to understand animal learning. Both paradigms understand animal learning behavior as forming associations between stimuli, between stimuli and actions or between actions and outcomes. The formation of these associations is reinforced by behaviorally relevant reinforcers, such as food rewards. In Pavlovian conditioning, animals learn the association between a conditioned stimulus (CS, e.g. a tone) and an unconditioned stimulus (US, e.g. meat bones). The US has intrinsic behavioral significance to the animal and acts as a reinforcer of the learning process. The US also triggers an unconditioned response (UR, e.g. drooling) in naïve animals. After repeated presentation of the US after the CS, animals learn to exhibit a conditioned response (CR, e.g. drooling) after the presentation of the CS in anticipation of the US that will follow soon after. Thus, animals learn to predict the value of the US after seeing the CS.

In instrumental conditioning, animals learn to form an association between a stimulus (S) and a response (R), when the response is repeatedly followed by a behaviorally relevant reinforcer. Thus, in this case animals have to learn to predict the value of the expected reinforcer from the stimulus response pair. There are two types of instrumental conditioning: habitual learning and goal-directed learning. In habitual learning, action-selection is only driven by the stimulus, while in goal-directed learning, action-selection is also driven by the current value of the associated outcome. Outcome devaluation and contingency degradation are two tests commonly used to distinguish between habitual and goal-directed learning. The outcome devaluation test measures the action-outcome association by selectively decreasing the value of an outcome. If action-selection is driven by the value of the outcome, then action leading to the devalued outcome should be performed less often. Contingency degradation decreases the difference between the probability of earning a specific outcome given a response and the probability of earning that outcome given no response. Animals engaged in goal-directed learning should respond less often

after contingency degradation.

Reinforcement learning has been used as a powerful theoretical framework for understanding both Pavlovian and instrumental conditioning. In Pavlovian conditioning, agents learn V(s), which is the expected future reward of state s, regardless of the action chosen in state s. In instrumental conditioning, agents learn Q(s, a), which is the expected future reward of taking action a in state s. In this framework, both V(s) and Q(s, a) are updated by prediction errors, which measures the discrepancy between rewards expected and that actually obtained. Thus, according to the reinforcement learning theories, associative learning is driven by non-zero prediction errors.

Brain Regions Involved in Pavlovian Conditioning

Different Pavlovian conditioning paradigms employ largely non-overlapping brain regions (Fanselow and Poulos, 2005). The brain regions involved depend on the particular US and CS used in the conditioning paradigm. For cued fear conditioning, the US (e.g. pain) is represented by a variety of brain regions, such as the posterior thalamus and the anterior cingulate cortex. The CS (e.g. an auditory tone) is represented by the auditory cortex, the thalamus and the hippocampus. Information regarding the CS and the US converge in the amygdala (Figure 1.1). Learning is mediated by the strengthening of projections carrying CS or US information to the lateral amygdala (LA) via long term potentiation (LTP). For example, the projection from the thalamus to the LA is strengthened after conditioning, resulting in greater response to auditory stimuli in the LA. Another widely studied Pavlovian conditioning paradigm is eye-blink conditioning, which mainly engages the cerebellum (Fanselow and Poulos, 2005).

Brain Regions Involved in Instrumental Conditioning

The prefrontal cortex (PFC), striatum, and the amygdala are the main brain regions involved in instrumental conditioning. In particular, different regions of the striatum are involved in different associative processes in instrumental learning. The striatum can be divided into the dorsolateral striatum (DLS), the dorsomedial striatum (DMS) and the ventral striatum (VS). The DLS has been shown to be responsible for habitual learning, i.e. the learning of stimulus-response associations (Yin, Knowlton, and Bernard W. Balleine, 2004). Rats with DLS lesions reduced their response to a devalued outcome when the learning was habitual (Yin, Knowlton, and Bernard W. Balleine, 2004). The DMS and VS are proposed to be parts of two distinct

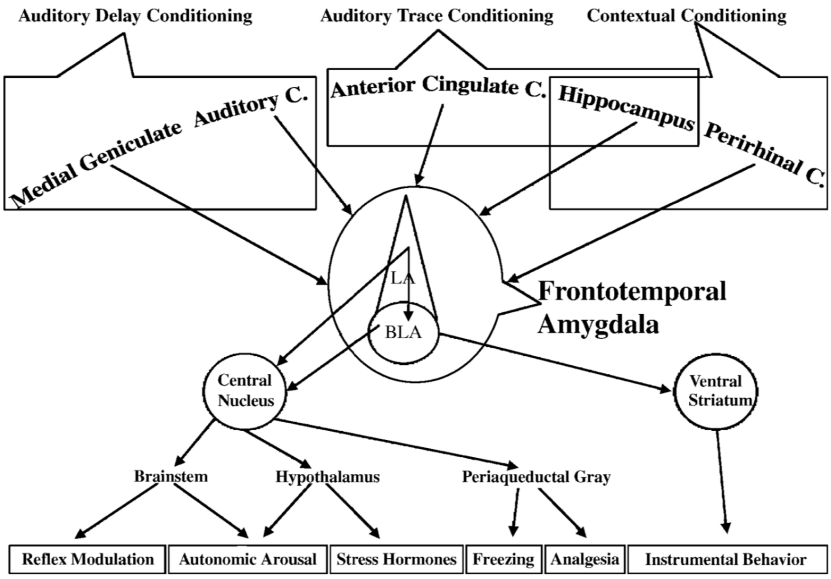

Figure 1.1: The basic for contextual fear conditioning. LA, lateral nucleus of the amygdala; BLA, basolateral nucleus of the amygdala. Adapted from (Fanselow and Poulos, 2005).

streams controlling goal-directed behavior (Figure 1.2) (Hart, Leung, and Bernard W. Balleine, 2014). The prelimbic area (PL) to posterior DMS (pDMS) pathway form the dorsal stream. Multiple parts of the PFC project to the ventral striatum, including the PL, the infralimbic cortex (IL) and the orbitalfrontal cortex (OFC) and these form the ventral stream. The PL and the pDMS are both necessary for acquisition of action-outcome associations (Laura H Corbit and Bernard W Balleine, 2003; Yin, Ostlund, et al., 2005). The pDMS but not the PL is also necessary for consolidation or storage of learnt action-outcome associations (Hart, Leung, and Bernard W. Balleine, 2014).

On the other hand, the ventral stream is involved in converting action-outcome associations to motor outputs (or goal-directed performance) (Hart, Leung, and Bernard W. Balleine, 2014). Two categories of factors can influence performance 1) experienced value of instrumental outcomes (outcome-guided action selection); 2) expected value of outcomes predicted by Pavlovian cues (stimulus-guided action selection) (Hart, Leung, and Bernard W. Balleine, 2014). Nucleus accumbens (NAc) is the main region of the ventral striatum and can be further divided into a core and a shell. The NAc core is necessary for outcome-guided action selection, while the NAc shell is necessary for stimulus-guided action selection. Information regarding Pavlovian and instrumental outcome values are hypothesized to be encoded in the

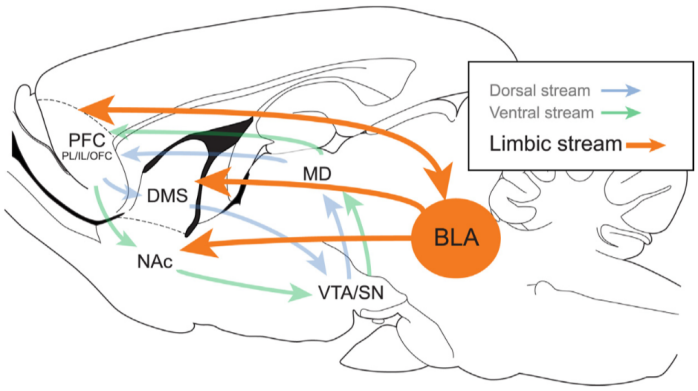

Figure 1.2: Simplified saggital representation of the corticostriatal loops that constitutes the dorsal learning stream (blue) and the ventral performance stream (green). PFC: prefrontal cortex; PL: prelimbia cortex; IL: infralimbic cortex; OFC: orbitalfrontal cortex; DMS: dorsomedial striatum; NAc: nucleus accumbens; MD: mediodorsal thalamus; VTA/SN: ventral tegmental area/substantia nigra. Adapted from (Hart, Leung, and Bernard W. Balleine, 2014).

amygdala and routed separately to the NAc shell and core respectively (Hart, Leung, and Bernard W. Balleine, 2014).

Another brain region that has extensive projections to prefrontal cortex, the striatum and the amygdala is the hippocampus. There are three pathways connecting the hippocampus and the prefrontal cortex (PFC): 1) a direct projection from ventral CA1/ventral subiculum to PFC, 2) a bidirectional pathway connecting dorsal CA1 and PFC via the thalamic nucleus reuniens (RE) and 3) a bidirectional pathway connecting dorsal CA1 and PFC via perirhinal cortex (PRC) and lateral entorhinal cortex (LEC) (Figure 1.3) (Howard Eichenbaum, 2017). Ventral striatum also receives heavy innervation from the hippocampus (Figure 1.4) (Pennartz et al., 2011). Ventral CA1/ventral subiculum mainly project to the NAc shell, while sparser projections from dorsal hippocampus innervate both NAc shell and core. In the next section, I will review the anatomical structure and function of the hippocampus, with a focus on its role in associative learning.

1.2 Hippocampus: Structure and Function

The hippocampus proper (referred to as the hippocampus from now on) is composed of four subregions: CA1, CA2, CA3 and the dentate gyrus (Langston et al., 2010). The hippocampal formation includes the hippocampus proper and the retrohippocampal region, which includes the subiculum, presubiculum/postsubiculum,

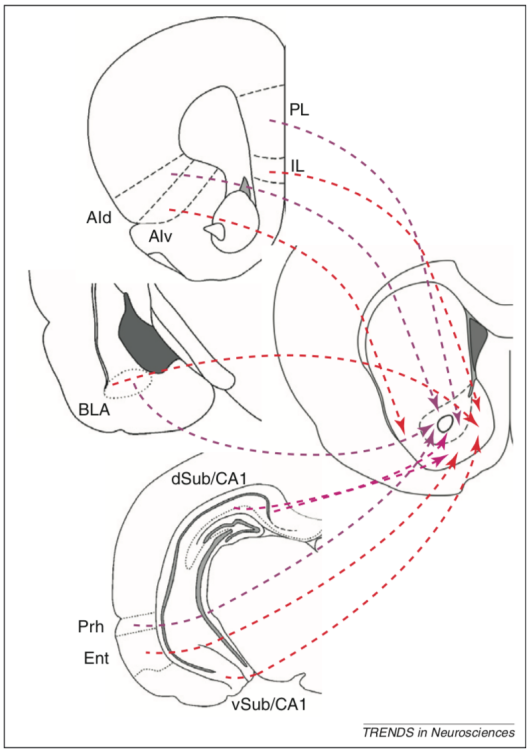

Figure 1.3: Main hippocampal inputs to the ventral striatum. Purple and red arrows indicate projections predominantly to the VS core and shell respectively. Prh: perirhinal cortex; Ent: entorhinal cortex, vSub: ventral subiculum; dSub: dorsal subiculum. Adapted from (Pennartz et al., 2011).

parasubiculum, and the entorhinal area. Input to the hippocampus comes from the entorhinal cortex and information flow from the layer II of the entorhinal cortex to the dentate gyrus, then to CA3 and then to CA1 in a mostly feedforward manner (Figure 1.5). There is also a direct projection from layer III of the entorhinal cortex to CA1 (Figure 1.5). While the CA1 region of the hippocampus has very dense projections to the subiculum and the entorhinal cortex, there are also direct innervations to many areas outside of the hippocampal formation (Cenquizca and Swanson, 2007). Moreover, the projection patterns differ along the longitudinal extent of the hippocampus (Figure 1.6). The dorsal CA1 pathway has a moderate innervation to the retrosplenial cortex. The intermediate CA1 projects rostrally through the fimbria-fornix to innervate moderately the infralimbic area and lightly the prelimbic and anterior cingulate areas. The ventral two-thirds of the CA1 region has the most diverse projections. It innervates virtually all sensory areas including primary and higher associative regions as well as basolateral amygdala and orbital areas (Cenquizca and Swanson, 2007).

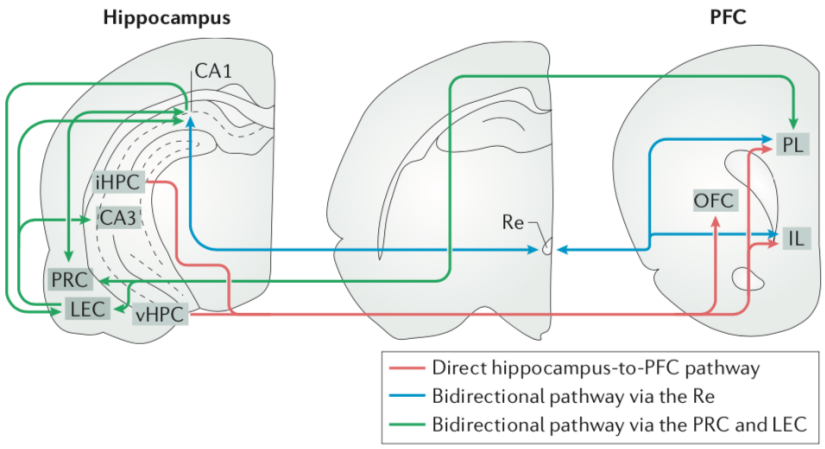

Figure 1.4: Indirect and direct prefrontal-hippocampal pathways. PFC: prefrontal cortex; PL: prelimbia cortex; IL: infralimbic cortex; OFC: orbitalfrontal cortex; Re: thalamic nucleus reuniens; iHPC: intermediate hippocampus; vHPC: ventral hippocampus; PRC: perirhinal cortex; LEC: lateral entorhinal cortex. Adapted from (Howard Eichenbaum, 2017).

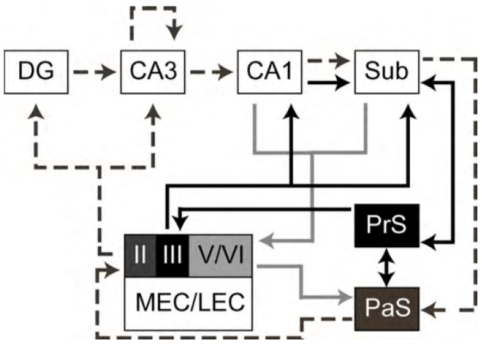

Figure 1.5: Simplified schematic of intrahippocampal connectivity. DG: dentate gyrus, Sub: subiculum, MEC: medial entorhinal cortex, LEC: lateral entorhinal cortex, PrS: presubiculum, PaS: parasubiculum. Solid black indicates interactions with layer III of EC, dashed line indicates interactions predominantly with layer II of EC, grey lines indicates interactions predominantly with layer V/VI of EC. Adapted from Langston et al. (2010).

The different regions within the hippocampus have been demonstrated to play different roles in learning and memory formation. The CA3 is characterized by its recurrent connections between pyramidal neurons and is hypothesized to be involved in pattern completion (Langston et al., 2010). Modeling of the recurrent network suggests that CA3 stores previously formed memories as attractor states (Hopfield and Tank, 1986). Inputs from the entorhinal cortex initiates the CA3 network in a

state representing incomplete memory, which then evolves towards the closest attractor state, resulting in correct memory recall. The dentate gyrus is characterized by a large number of projection neurons and sparse firing, and is hypothesized to be involved in pattern separation (Langston et al., 2010). Disruption of NMDA receptors in the dentate gyrus impairs the ability of mice to discriminate between similar contexts (McHugh et al., 2007). Since our study focuses on ventral CA1 and associative learning, the function of hippocampus and in particular, CA1, in associative learning paradigms will be reviewed in detail in the next section.

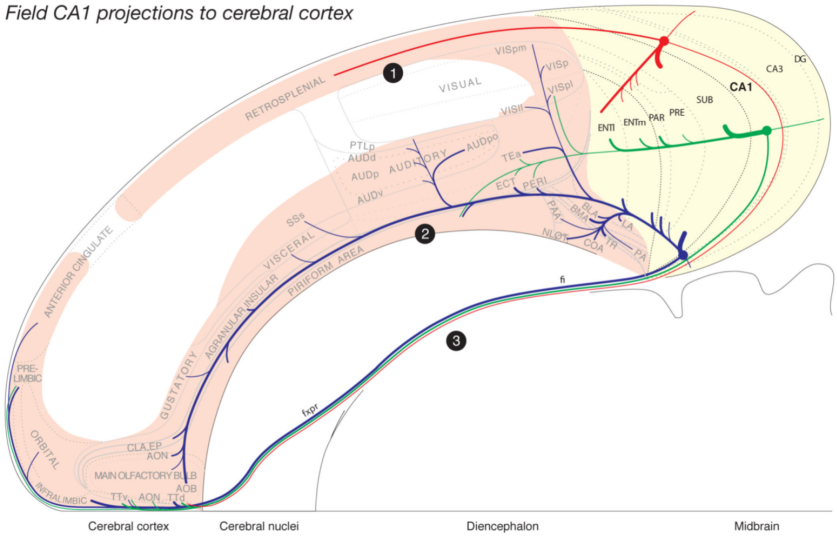

Figure 1.6: Projection from dorsal (red), intermediate (green), and ventral (blue) levels of CA1 to the rest of hippocampal formation (shaded yellow), and then the rest of the cortical mantle (shaded light red). Adapted from Cenquizca & Swanson (2007).

1.3 The Hippocampus and Associative Learning

Growing evidence indicate that the hippocampus is not only involved in spatial navigation but also in declarative memory in general. Associative memory is one kind of declarative memory. The hippocampus and especially the CA1 region has been implicated in many different aspects of associative memory.

Classical Conditioning

Eye blink conditioning is a classical conditioning paradigm where rabbits are often used to learn to associate an auditory tone (CS) with an aversive air-puff to the eye (US). Naïve animals blink in response to the US but gradually learns to preemptively blink in response to the CS during training. In delay eye blink conditioning, the onset

of the CS is before that of the US but both stimuli terminate at the same time. While in trace eye blink conditioning, there is a temporal delay between the offset of the CS and onset of the US such that the CS and US never overlap. Lesion studies show that the hippocampus is necessary for acquiring trace eye blink conditioning task but not delay eye blink conditioning (McEchron and Disterhoft, 1999). Moreover, electrophysiology recordings in the CA1 region during training revealed learning-related changes in the pyramidal cell activities (McEchron and Disterhoft, 1999; Berger, Alger, and R. F. Thompson, 1976). During both delay and trace eye blink conditioning, CA1 pyramidal cells developed a response to the US after a few trials of training. In delay eye blink conditioning, as behavioral conditioning develops, the CA1 pyramidal cell response also shifts forward in time, temporally preceding and paralleling the amplitude time course of the eye blink response (Berger, Alger, and R. F. Thompson, 1976). During trace eye blink conditioning, CA1 response to the CS developed a few trials prior to initial CR increase. However, in contrast to the uniform response profile reported for delay eye blink conditioning, CA1 pyramidal cells had a variety of different response profiles during trace eye blink conditioning that often do not parallel the amplitude time course of nictitating membrane response. In aged rabbits that weren't able to learn the task, CA1 response to the US was delayed. After asymptotic performance level is reached, CA1 responses to the CS become indistinguishable from those of pseudoconditioned animals and CA1 responses to the US actually become inhibited (McEchron and Disterhoft, 1999). These results suggest in order to form an association between the CS and the aversive US, the hippocampus needs to first encode the behaviorally-relevant US and then associate it with the CS. Moreover, the hippocampus is only required during the acquisition of the task but not during its long-term retention, as suggested by both the decrease in CA1 activity in response to the CS and US during asymptotic performance (McEchron and Disterhoft, 1999) and other lesion studies (J. J. Kim, Clark, and Richard F. Thompson, 1995).

Similarly, the hippocampus is necessary for trace cued fear conditioning but not for the delayed version (McEchron and Disterhoft, 1999). In rodents, the CS used in cued fear conditioning is often an auditory cue and the US a foot-shock. The trace intervals used in cued fear conditioning is often tens of second instead of less than a second, as often seen for eye blink conditioning. Similar to trace eyeblink conditioning, single neuron recordings done in CA1 during cued fear conditioning showed diverse response profiles (McEchron, Tseng, and Disterhoft, 2003). Neurons can be inhibited or excited by the CS and/or US. As a result,

average activity of CA1 pyramidal cells is modulated by neither the CS nor the US during conditioning. However, a significant percentage of CA1 pyramidal cells showed maximal firing during CS-only retention trials timed at the expected onset of the US. Moreover, this percentage of neurons decreased as the fear response decreased during extinction trials (McEchron, Tseng, and Disterhoft, 2003). This result suggest that the hippocampus support trace conditioning by encoding the trace interval. Population analysis of CA1 activity during cued fear conditioning shows that population activity during CS presentation, US presentation and rest can be clearly separated using dimensionality reduction analysis. Population activity travels from the US state to the CS state after US presentation during conditioning, supporting the hippocampus plays a role during the association between the CS and the US (Chen, Wang, and Tsien, 2009).

The hippocampus is also necessary for the acquisition and recall of contextual fear memory (McEchron and Disterhoft, 1999). Rabbits with hippocampal lesions showed more movement in the conditioned context than control animals 24 hours after contextual fear memory encoding (McEchron and Disterhoft, 1999). Moreover, optogenetically stimulating dentate gyrus neurons that were active during fear memory encoding can elicit freezing response in mice in a context different from that used for fear memory encoding (Ramirez et al., 2013).

These studies of classical conditioning paradigms suggest that the hippocampus is not required for forming associations between discrete CSs and USs. However, it is required when there is a temporal discontiguity between the CS and the US and the trace interval is encoded in the CA1 region. The hippocampus is also required when the CS is not discrete (e.g. a contextual stimulus). Moreover, the activities of CA1 pyramidal cells are modulated by learning. Single neurons likely exhibit diverse profiles during conditioning, and information regarding the CS and the US and association between the two might have to be decoded from population activity.

Arbitrary Stimulus-Stimulus Associations

Classical conditioning paradigms are special cases of arbitrary stimulus-stimulus associations. Apart from being necessary for classical conditioning when there is a temporal discontiguity between a CS and a US, the CA1 region of the hippocampus is also necessary when associations need to be formed between arbitrary non-spatial stimuli with temporal discontiguity. Rats with complete hippocampus lesions were not impaired in learning an object-odor paired associate task (Gilbert and Kesner,

2002). However, when a trace interval is added in between the object and odor stimuli, rats with dorsal CA1 lesions were significantly impaired in the acquisition of the task (Kesner, Hunsaker, and Gilbert, 2005). Since these two studies differ only in the trace interval, it is safe to conclude that the dorsal CA1 is necessary for bridging the temporal gap between stimuli.

Although the hippocampus is likely not involved in forming associations between arbitrary non-spatial stimuli, a few studies demonstrated that the hippocampus is necessary for the generalization of associations (Bunsey and H. Eichenbaum, 1996; Tse, Langston, et al., 2007). In one study (Bunsey and H. Eichenbaum, 1996), rats were trained to perform an odor-odor paired associate task, in which rats are presented with a sample odor and have to dig for food in the correct choice odor well. Rats were also tested for their ability to make inferences. In the transitivity test, rats need to infer odor A maps to odor C after being trained on AB and BC. In the symmetry test, rats need to infer CB after learning BC. Results show that rats with complete neurotoxic hippocampus lesions were not impaired in the initial acquisition of this task but were significantly impaired in both the transitivity and symmetry tests. This study agrees with the others that the hippocampus is not necessary for learning of arbitrary associations between non-spatial stimuli. It also extends the picture that the hippocampus is necessary when flexible representable of such associations is needed to make inferences.

In a separate study, the hippocampus was shown to be necessary for generalization in a flavor-place paired associate task (Tse, Langston, et al., 2007). In this task, rats learnt six flavor-place associations during the training period. After training, control rats were able to learn two new paired associations within a single session and retain the memory for 24 hours, while hippocampus-lesioned rats were not able to rapidly acquire new paired associates. The authors proposed that the new knowledge are learnt quickly and retained for a long time because the rats formed a mental schema of the task from the first six learnt paired associations.

Aside from behavioral evidence, a recent study discovered “event cells” in the hippocampus of mice, which might represent transferable and abstract representation of task states (Sun et al., 2020). In the task used in this study, mice were trained to run laps on a square maze and were only rewarded every four laps. The authors discovered place cells whose firing rates are modulated by lap number. Moreover, when mice are exposed to a new circular maze environment, the place fields of these place/event cells remapped but the preferred lap number remained very similar to

that in the square. This study provides neural evidence that the brain represents abstract task states in addition to action and state values.

Taken all these studies together, there is increasing amount of evidence suggesting the hippocampus is necessary for applying knowledge of previously learnt associations to efficiently solve new tasks.

Instrumental Conditioning

Classical conditioning requires learning the values of states, while instrumental learning requires learning the values of actions. Many studies agree that the hippocampus is not involved in forming stimulus-response associations involving discrete cues. Lesions to the dorsal hippocampus did not impair the ability of rats to acquire an instrumental task involving pressing a lever to obtain food (Laura H. Corbit and Bernard W. Balleine, 2000). Rats with hippocampal lesions were also not impaired in the win-stay task on a standard 8-arm radial maze, which requires learning the association between lit maze arms and food reward (White and McDonald, 2002).

The involvement of the hippocampus in goal-directed behavior is more controversial. Early lesion studies show that rats with dorsal hippocampal lesions behave comparably to control rats in food devaluation tests and contingency degradation tests (Laura H. Corbit and Bernard W. Balleine, 2000; Laura H. Corbit, Ostlund, and Bernard W. Balleine, 2002). On the other hand, more recent studies employing multi-step reinforcement learning paradigms show that the hippocampus play a positive role in goal-directed behavior. In one study, rats with hippocampus lesions were less likely to employ goal-direct strategies when performing a two-step decision task (Miller, Botvinick, and Brody, 2017). In this task, rats first need to pick one of two nose ports, each of which leads to one of two reward port becoming available with probability 80% and the other reward port becoming available with probability 20%. In the second step, rats are instructed to enter the available reward port and receives reward with a probability associated to the reward port. Rats can either perform this task using a purely model-free strategy by repeating rewarded actions, or they can adopt a model-based strategy by learning the state transition probabilities. Rats with reversible dorsal hippocampal inactivation show a decrease in their tendency to rely on a model-based strategy. Another study using human epileptic patients with unilateral anterior temporal lobectomy and a similar two-step decision task corroborated this finding (Vikbladh et al., 2019). Although a caveat

of this study is that the reliance on model-free strategies could also be caused by temporal lobe lesions outside of the hippocampus or the epileptic history of the patients.

Some studies have implicated a role for the ventral hippocampus in goal-directed behavior. Normal regulation of synaptic strengths and plasticity in the ventral hippocampus is necessary for goal-directed behavior. Stress-sensitive tyrosine kinase receptor B (trkB) is a regulator for synaptic strengths and plasticity. Overexpression of a truncated form of trkB in the ventral hippocampus was able induce an overreliance on habits, as evidenced by an insensitivity to contingency degradation (Barfield et al., 2017). The same manipulation did not affect acquisition of the operant task or performance in a outcome devaluation task. Chemogenetically silencing the ventral hippocampus makes the animal more sensitive to degradation of action-outcome contingencies (Barker, Bryant, and Chandler, 2019). Moreover, optogenetically silencing the ventral hippocampus enhances the ability of the animal to maintain ongoing goal-directed behavior and also increases motivation (Yoshida et al., 2019).

The studies that suggest the hippocampus is not necessary for goal-directed behavior used tasks that involve learning a single action-outcome association and lesions in the dorsal hippocampus. Thus, it is possible that the hippocampus is necessary for more complicated tasks that require multiple action-outcome associations and have stochastic outcomes. Moreover, the ventral hippocampus could be necessary specifically for encoding action-outcome contingencies.

Go/No-Go Tasks

The go/no-go task is a specific example of instrumental conditioning and one study has suggested that the hippocampus is not necessary for this task. In (Gilbert and Kesner, 2002), a discriminatory go/no-go task was used to test whether learning an object-odor association is hippocampus-dependent. Rats were instructed to only dig for food reward when the correct pairing of object and odor stimuli is presented. Rats with complete hippocampus lesions and control rats learnt the task with comparable speed across six training blocks, each consisting of 60 trials (Figure 1.7). Moreover, rats with hippocampus lesions were also not impaired in their ability to discriminate odors or visual stimuli, which were also assessed with the same discriminatory go/no-go task. Since these rats have complete electrolytic lesion of the hippocampus, the hippocampus and the fibers passing through the

hippocampus are both likely not necessary for the acquisition of go/no-go tasks.

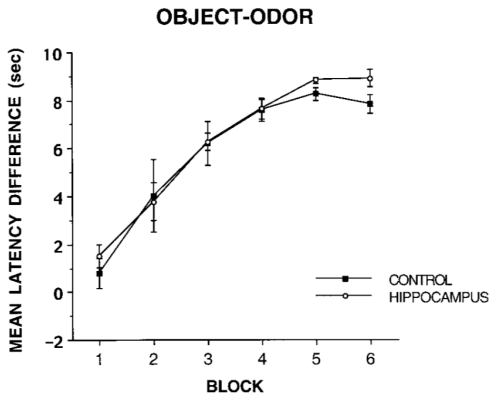

Figure 1.7: Mean (± SEM) latency differences (latency on mispaired trials - latency on paired trials) as a function of blocking of control and hippocampus-lesioned rats on acquisition of the object-odor paired associate task. Adapted from Gilbert and Kesner (2002).

Taken all the studies together, we can see that the hippocampus and in particular CA1 is mainly involved in forming stimulus-stimulus associations involving spatial stimuli. During tasks when non-spatial stimuli are involved, CA1 is only necessary when the stimuli needing to be associated are separated by a trace interval. However, there is a potential role for the hippocampus in making inferences using previously learnt associations. Furthermore, the hippocampus is likely not necessary for forming stimulus-response associations needed during acquisition of instrumental conditioning. The hippocampus is also likely not involved learning a single action-outcome association, but might be necessary with multiple action-outcome associations need to be learnt. Results from more recent studies are starting demonstrate a role of the ventral hippocampus in representing the contingency between an action and its outcome and in maintaining goal-directed behavior.

Caveat of Lesion Studies

In order to understand the functional of the hippocampus, most of the studies reviewed above used lesions of the whole hippocampus or subregions of the hippocampus. However, the function of each hippocampus region might be heterogeneous and the different functions might be carried out by neurons projecting to different brain regions. Moreover, lesion studies have low temporal and spatial resolution and cannot help us understand the real-time contributions of the hippocampus in as-

sociative learning. Some recent studies using optogenetics to manipulate neuronal activity showed disparate findings compared to previous lesion studies (Goshen, 2014). This could be because the role of the hippocampus can be compensated by other cortical areas when the brain is given some time to recover after lesion (Goshen, 2014). Another limitation of lesion studies is that it only investigates the role of loss of function in the hippocampus in associative learning. Whereas we now have chemogenetic and optogenetic tools to investigate the effect of gain of function.

In the next section, I will review our current understanding of the function carried out by CA1 pyramidal cells projecting to the prefrontal cortex, the striatum, the amygdala and primary sensory cortices. These brain regions are selected because of their established roles in associative learning.

1.4 Information Routed by Ventral Hippocampal Outputs

In the overview of hippocampus anatomy, we showed that direct projection from the hippocampus to prefrontal cortex, striatum, amygdala and primary sensory cortices mostly arise from ventral CA1. By recording single neuron activity in the ventral CA1 (vCA1) during three different behavioral tasks, a study showed that different types of information are routed from the vCA1 to prefrontal cortex, nucleus accumbens and the amygdala (Ciocchi et al., 2015). Using an elevated plus maze task, the study showed that ventral CA1 pyramidal neurons projecting to the prefrontal cortex are more likely to signal anxiety. An open field exploration task was used to show that spatial information is routed equally to all three brain areas. Moreover, vCA1 uses largely non-overlapping pyramidal cell populations to signal anxiety versus spatial information. A third goal-directed spatial navigation task was used to assess the representation of goal (reward) information in the vCA1. vCA1 pyramidal exhibited two distinct reward response profiles, with the excited neurons increasing their firing rate prior to reward and inhibited neurons decreasing their firing rate prior to reward. The excited neurons are more enriched in the population double projecting to mPFC and nucleus accumbens while the inhibited neurons are enriched in the population projecting to only nucleus accumbens.

Routing of Spatial/Contextual Information

Consistent with the study described above, causal manipulations applied to the projections from the hippocampus to other brain areas have been used to disrupt task performances requiring spatial or contextual information. Disconnection between

the hippocampus and the NAc shell region using unilateral asymmetric lesions of each structure impaired place conditioning and context dependent retrieval of CS-US associations (Ito et al., 2008). In this study, rats first learnt Pavlovian CS-US associations in three distinct spatial environments with discrete CS cues (light flashes). Disconnection between the hippocampus and the NAc shell did not impair learning with discrete cues. Then rats were only rewarded after CS presentation in a specific spatial environment. Disconnecting the hippocampus and the NAc shell slowed the extinction of CR response in irrelevant spatial environments and prevented rats from forming a preference for the rewarded spatial environments. The authors proposed that the functional circuit involving the hippocampus and the nucleus accumbens shell mediates spatial contextual control over associative learning. This paper also confirmed previous results that the hippocampus is not involved in discrete cue conditioning.

Another study showed that the direct projection from vCA1 to the prefrontal cortex is necessary during the encoding phase of a delayed non-match to place task (DNMTP) (Spellman et al., 2015). The DNMTP task uses a T-maze and has an encoding phase and a retrieval phase. During the encoding phase, only one arm of the T-maze is open. During the retrieval phase, mice have to go to the arm that was closed during the encoding phase in order to receive a reward. The study showed that inhibition of vCA1 terminals in the mPFC during the encoding phase but not the retrieval phase impaired task performance. Moreover, inhibition vCA1 to mPFC input impaired goal-location-selective firing of mPFC neurons during the encoding phase but not the retrieval phase. Encoding of non-spatial information such as task phase was not impaired in the mPFC. This study provides another example where vCA1 routes goal-relevant spatial information to other brain regions.

The hippocampus also routes spatiotemporal information to sensory areas. One study showed that inhibiting the direction projection from ventral hippocampus (vHPC) to medial anterior olfactory nucleus (mAON) and that from intermediate hippocampus to lateral AON impaired spatial odor memory, while only inhibiting the vHPC to mAON projection impaired temporal odor memory (Aqrabawi and J. C. Kim, 2018). Inhibition of the HPC terminals in AON was done using optogenetics and during the retrieval phase of the task. Taken all three studies together, we see that outputs from the ventral hippocampus routes spatial and/or temporal information to nucleus accumbens, prefrontal cortex and anterior olfactory nucleus. The routing of this information can be necessary for task performance during the encoding phase

and/or during the retrieval phase of the task.

Routing of Non-Spatiotemporal Information

The hippocampus also routes non-spatial information to other brain areas. One well-established one is the routing of anxiety-related information to the prefrontal cortex. One study showed that inhibition of vCA1 terminals in the mPFC in mice decreased open-arm avoidance in an elevated plus-maze task and decreased center-avoidance in an open field exploration task (Padilla-Coreano et al., 2016). Both of these behavior measures indicate a decrease in anxiety level. Inhibition of the vCA1 terminal in the mPFC also decreased theta synchrony between vCA1 and mPFC. Activation of the vCA1 terminal in the mPFC with an 8 Hz (in the theta range) sinusoidal light stimulation increased mice anxiety level but stimulation at 20 Hz had no behavioral effect (Padilla-Coreano, 2016, PhD Thesis). These results also indicate at the hippocampus communicate with the prefrontal cortex with specific frequencies.

Regarding sensory perception, the ventral hippocampus (vHPC) can also exert top-down control on odor sensitivity via its direct projection to AON (Aqrabawi, Browne, et al., 2016). Inhibition of vHPC terminals in AON using DREADDS decreased latency to uncover buried food and increased sociability and social recognition of previously encountered conspecifics in mice. On the other hand, activating the same terminals using optogenetics increased latency to uncover buried food and decreased social recognition.

Other studies have shown that hippocampus and the prefrontal cortex are both necessary for generalization of learnt associations (also referred to knowledge transfer or schema assimilation), but it is unclear whether the communication between the two brain structures is necessary and if so, what specific circuitry is involved. Knowledge transfer refers the ability of an agent to use knowledge learnt from past successful experiences to efficiently solve a new task. This process involves recognizing the common elements shared across tasks or making logical inferences based on past knowledge. Using a flavor-place paired-associate task, a study found that after rats reached plateau performance for six flavor-place pairs over more than one week of training, they were able to acquire two new flavor-place associations over a single trial and maintain the memory for over 24 hours (Tse, Langston, et al., 2007). Acquisition of the new pairs is dependent on the hippocampus but not the retrieval 48 hours. Acquisition of the new pairs also requires both the AMPA and NMDA

receptors in the prelimbic area and retrieval only requires the AMPA receptors 24 hours after acquisition (Tse, Takeuchi, et al., 2011). However, it is unclear from this study whether the hippocampus and the prelimbic area function independently or whether communication between the two areas is needed.

A separate study demonstrated distinct roles for the hippocampus and the PFC in a transitive inference task in humans (Zeithamova and Preston, 2010). The transitive inference requires subjects to infer the association between stimulus A and C after learning the association between A and B and that between B and C. The authors hypothesized that binding of the two associations (AB and BC) could either happen during the encoding of BC or the presentation of AC could engage an active inference process that requires retrieval of AB and BC associations. The authors showed the hippocampus is engaged in the encoding of BC and retrieval of learnt AB and BC associations while the PFC is more likely to be engaged in the active inference process during the presentation of AC. The study found strong but indirect functional connection between the hippocampus and the inferior frontal cortex through the parahippocampal cortex.

Taken all of these studies together, there is strong evidence supporting the hippocampus routes spatiotemporal information to many brain areas including olfactory areas and routes anxiety-related information selectively to medial prefrontal cortex. There is little evidence supporting the role of the hippocampus in associative tasks involving discrete cues. However, there is strong evidence supporting the role of the hippocampus in the generalization of learnt associations to new discrete or continuous stimuli. It is unclear which hippocampal output mediate this function, but the connection to the prefrontal cortex seems to be a likely candidate. Moreover, very few studies have tried to unveil the function of the hippocampal projections to primary or higher sensory areas. The interactions between the hippocampus and olfactory areas are the most studied.

1.5 Hypotheses

Given the lack of studies on projections from vCA1 to sensory areas, we decided to investigate the function of the projection from vCA1 to primary auditory cortex. Specifically, we propose this projection has a role in auditory associative learning. Given the established role of the hippocampus in the acquisition of many associative learning paradigms such as trace conditioning and spatial/contextual learning, we hypothesized that the CA1 to A1 projection plays a positive role in auditory asso-

ciative learning. The requirement of the hippocampus in knowledge transfer and model-based planning also led to our hypothesis that the hippocampus facilitates generalization of learnt associations. We first investigated the role of this projection during initial learning using an auditory go/no-go task and optogenetics. Then, we investigated its role during learning generalization of the same task. We expected to see enhanced learning speed during both initial learning and generalization when the CA1-A1 projection is stimulated and impairment in learning when the projection is inhibited. Our results will help elucidate the role of the hippocampus to sensory cortex projections in sensory associative learning.

Chapter 2

METHODS

2.1 Animals and Training Environment.

All mice used for this study belonged to the C57BL/6E strain and were male. Mice were kept on a water restricted schedule from Monday to Friday and were given 1-1.2 ml of water per day. Free access to water was given between 7 pm on Friday and 11 am on Sunday. All experiments were performed in an acoustically and electromagnetically shielded room. All mice were head fixed on spherical treadmill during behavioral conditioning and were allowed to run and walk freely. A water port was placed right in front of each mouse and within reach of the tongue. Licking was detected using an IR beam within the water port.

2.2 Behavior Conditioning

The basic behavioral task used for this thesis was an auditory go/no-go task. Mice were trained for session per day, 100-150 trials per session. In each trial a pure tone of one of two possible frequencies, assigned to the valence CS+ or CS-, were displayed. For the rest of the paper, tone pairs will be written as CS+/CS- (e.g. 14k/17k, 14k is the CS+ tone with frequency 14 kHz). Mice decided whether to lick the water port based on the tone frequency. Licking within 3s of CS+ tone presentation (hit) triggered the delivery of 6.5 ul of water. Licking within 3s of CS- presentation (false positive) resulted in increasing the inter-trial interval to 30s. Correct rejection of CS- led to no inter-trial interval, while all CS+ trials had a inter-trial interval of 5 to 20s. Mice learnt to lick the water port (go response) after the presentation of tone CS+ and to avoid licking after the presentation tone CS- (no-go response). All auditory stimuli used were between 5 kHz and 36 kHz. Mice were not asked to discriminate auditory frequencies less than 0.9 octaves apart. This frequency separation was much larger than their physical limit of frequency discrimination (Hoz and Nelken, 2014).

Shaping

All animals went through a shaping period to facilitate training. After one day of water restriction, naive animals were first put into cage of the same kind as their home cage. A water port is placed at one end of the cage and would deliver whenever

licks are detected. Animals were left in the cage to lick for reward in order to get familiar with the water port. Immediately after that, animals were habituated to the spherical treadmill for 20 minutes in the head-fixed position. On the next day, animals were trained to lick for reward on the ball without any auditory stimuli. After animals learnt to lick the water port, they were exposed to only the CS+ tone and learnt to associate water rewards with the tone. After performance exceeded chance level, animals were assigned to training on one of the tasks described below.

Four-Tone Task

Two variants of this basic auditory task were used in this study. The four-tone task was used to assess the role of CA1 to A1 projection in initial learning. Mice were trained on two different tone pairs on alternating days. For one of the two tone pairs, mice received channelrhodopsin stimulation paired to both CS+ and CS- during tone presentation in each trial. Mice did not receive optogenetic stimulation on the first training session of either tone pair in order to gauge their baseline performance. This experiment let us explore the effect of activating hippocampus to primary auditory cortex (A1) projection on initial learning by comparing the performance of each animal on the control tone pair versus the optogenetic stimulation paired tone pair.

Generalization Task

This task was used to assess learning generalization. Mice were trained with the following tone pair sequence: 8k/15k, 10k/18.7k,12.5k/23.4k, 6.4/12k, 5.1k/9.6k, 10k/18.7k, 19k/35.5k. The CS+ tone and CS- tone in all tone pairs had a 0.9 octave separation to ensure that the difficulty of all tone pairs were the same. Mice moved on to a new tone pair after reaching asymptotic performance on the current tone pair. Learning speed was measured by the number of days taken to reach a certain performance level.

2.3 Behavioral Analysis.

Timestamps of tone presentation, licks and reward delivery were acquired with a custom LabVIEW program. Performance was assessed using signal detection measure d', which was calculated with the following formula,

$$d' = z(H) - z(FA)$$

where z is the z-score of the left-tailed p-value. H is the hit rate (percentage of go response in response to the CS+ stimulus). FA is the false alarm rate (percentage of go response in response to the CS- stimulus).

2.4 Optogenetics.

An adeno-associated viral vector carrying channelrhodopsin under a CamKIIa promoter was injected into the hippocampus. Axonal fibers and terminals of infected cells were optically stimulated in neocortex through an optic fiber in head-fixed mice. The effectiveness of optogenetic stimulation was verified by whole-cell patch clamp recordings: brief pulses (1 to 3 milliseconds duration) of blue light (470nm) evoked EPSPs postsynaptic to the channelrhodopsin expressing cells.

Channelrhopdopsin stimulation parameters

During the behavior experiments, the optical stimulation (470 nm) consisted of 3 pulses (each lasting 3 milliseconds). These light pulses were delivered during the auditory stimulation, starting 50 milliseconds after tone onset with 35 millisecond inter-pulse intervals. Light pulses were paired to both CS+ and CS- tone frequencies.

Halorhodopsin stimulation parameters

During the behavior experiments, the optical stimulation (590 nm) starts at 5% maximum amplitude and is continuous on for one second. Then the light intensity ramps down over 100 ms in 5 ms steps. The ramping was designed to avoid rebound firing after activity inhibition in the CA1 axon terminals (Mahn et al., 2016). The optical stimulation starts at 50 milliseconds after tone onset. Light pulses were paired to both CS+ and CS- tone frequencies.

Chapter 3

RESULTS

3.1 Inconclusive effect of optogenetic activation of CA1 terminals in A1 on task acquisition

We first tested out hypothesis that activating vCA1 to A1 projection improves acquisition of an auditory go/no-go task using one control and two experimental mice. All three animals alternated between two different tone-pairs (11k/20k, 14k/17k) on consecutive days. Optogenetic stimulation was paired to both the CS+ and CS- tone in the harder tone pair (14k/17k has a smaller frequency separation compared to 11k/20k). As expected, the control animal showed clear improvement in performance over the course of six weeks for both tone pairs and acquired the harder tone pair (14k/17k) slower than the easier tone pair (11k/20k) (Figure 3.1). However, optogenetic stimulation failed to increase the learning speed for the harder tone pair. One experimental animal showed clear learning for 11k/20k but neither experimental animal showed significant improvement in performance for 14k/17k (Figure 3.1). Performance was averaged for each week because the experimental animals showed a lot of behavioral fluctuations on each day. A closer look at these fluctuations revealed that the experimental animals typically showed a decrease in their performance on the second day of the week and only for the optogenetically stimulated tone pair (Figure 3.2, exp 1: $p < 0.01$; exp 2: $p = 0.16$, one-tailed t-test). The result for the second experimental animal is insignificant. This is probably due to small sample size, since performance comparisons could only be made for three out of five weeks for this animal. These results seem to suggest that contrary to our expectations, activation of CA1 to A1 projection might impair learning.

We recruited one more mouse to explicitly test this alternative hypothesis. Two tone pairs with equal difficulty (5k/12k, 10k/24k) were used and optogenetic stimulation was paired to the tone pair with better initial performance (10k/24k). The animal continued to perform better to the optogenetically paired tone pair compared to the control tone pair (Figure 3.3), indicating that optogenetic activation of the CA1 to A1 projection has no obvious effect on task acquisition. However, this mouse also received a lower concentration of viral vector injection compared to the first two experimental animals and thus could be expressing insufficient amount of channel-

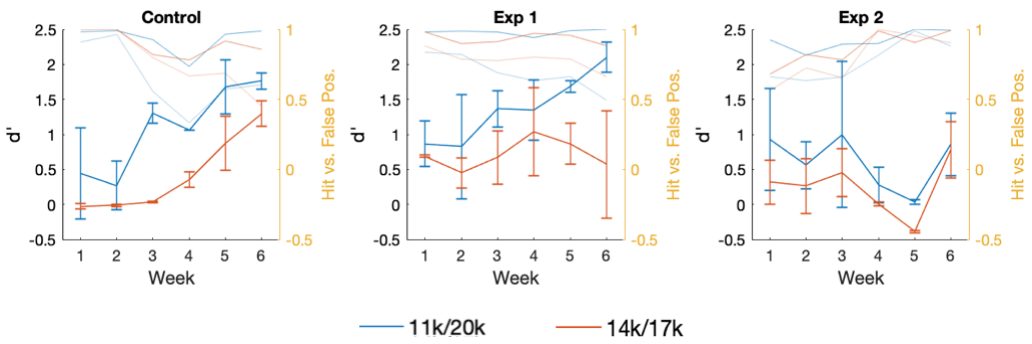

Figure 3.1: Optogenetic activation of vCA1 to A1 projection does not improve initial learning. Performance measured with d' are presented one control (left) and two experimental animals (middle and right) for both the control tone pair (blue with error bar) and the optogenetic stimulation paired tone pair (red with error bar). Performance is averaged for each week and presented for six weeks in total. For reference, hit (semi transparent trace) and false positive (most transparent trace) rates are also presented for each tone pair in their respective colors. Error bars are standard deviations of session performances during the week.

rhodopsin in vCA1. Therefore, the results for task acquisition are inconclusive.

3.2 Optogenetic activation of CA1 terminals in A1 does not impair task generalization

Since task generalization has not been demonstrated for auditory go/no-go tasks using pure tones in mice, we first explored whether mice can generalize on this task. One mouse was trained on the following sequence of tone pairs: 8k/15k, 10k/18.7k,12.5k/23.4k, 6.4/12k, 5.1k/9.6k, 10k/18.7k, 19k/35.5k. Throughout training, the lower frequency in each tone pair is always rewarded. The first three tone pairs and the fifth tone pair were designed such that the CS+ tone in the new tone pair is close in frequency to the CS+ in the previous tone pair and the same goes for the CS- tone frequency. While the other tone pairs were designed such that the new CS+ tone is close in frequency to the old CS- tone or vice versa. With the exception of 19k/35.5k, learning speed for all subsequent tone pairs was faster than that for the first tone pair (Figure 4). Specifically, the mouse has difficulty generalizing when the new CS+ tone (19k/35.5k) was close in frequency to the old CS- tone (10k/18.7k), but not when the new CS- (6.4k/12k) was close in frequency to the old CS+ (12.5k/23.4k) (Figure 3.4, 3.5). Our results show that mice can generalize across the meaning of specific frequencies but they do not understand that low frequency sound always maps to reward while high frequency always maps

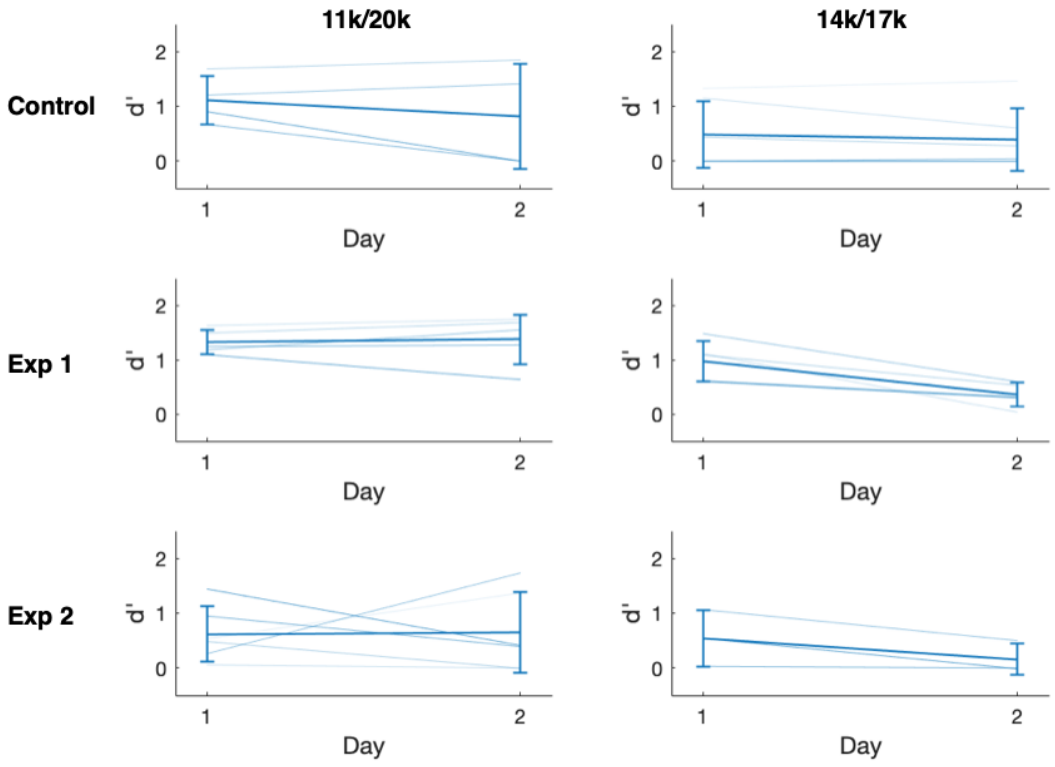

Figure 3.2: Performance for the optogenetic stimulation paired tone pair decrease on the second day of the week. Performance for 14k/17k is significantly lower for experimental animal 1 on the second day of each week compared to on the first day of each week ($p < 0.01$, one-tailed t-test). The same result is not significant for experimental animal 2, probably due to the lack of data for 2 out of 5 weeks. The faded blue lines in each plot are performance from each week with less faded lines representing the earlier training stages. The control animal is lacking data for 11k/20k for weeks 3 and 4. Experimental animal 2 is lacking data for 14k/17k for weeks 2-4. Error bars are standard errors of d' estimated using bootstrapping.

to no reward. To test the effect of CA1 to A1 projection in task generalization, we paired the fifth tone pair (5.1k/9.6k) to optogenetic stimulation. The learning speed for this tone pair was comparable to that for the third tone pair (12.5k/23.4k), indicating that generalization was not impaired.

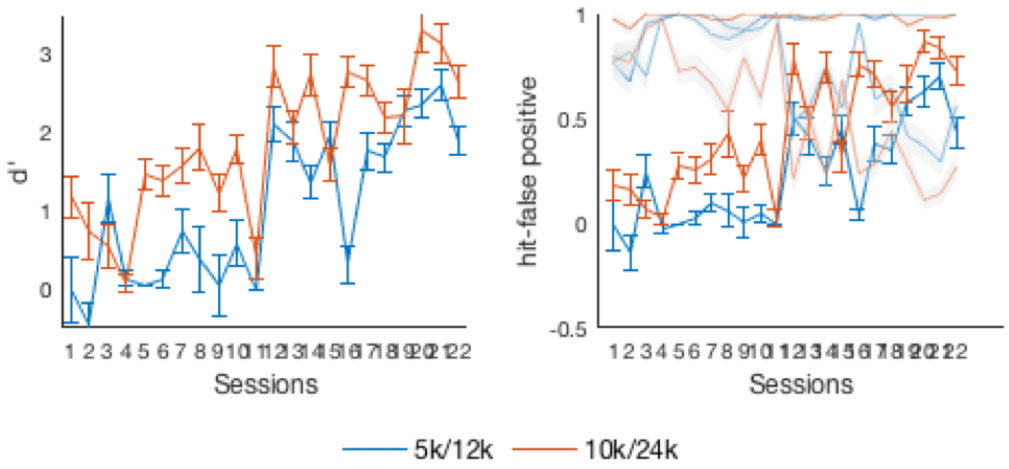

Figure 3.3: Optogenetic activation of vCA1 to A1 projection does not impair initial learning. Left, performance measured with d' for the control tone pair (5k/12k, blue) and optogenetic stimulation paired tone pair (10k/24k, red) are presented across 22 sessions. The first 10k/24k session were done without optogenetic stimulation. Error bars are bootstrapped standard errors of d' within each session. Right, same as on the left, except performance is measured by hit rate - false positive rate. For reference (semi transparent trace) and false positive (most transparent trace) rates are also presented for each tone pair in their respective colors.

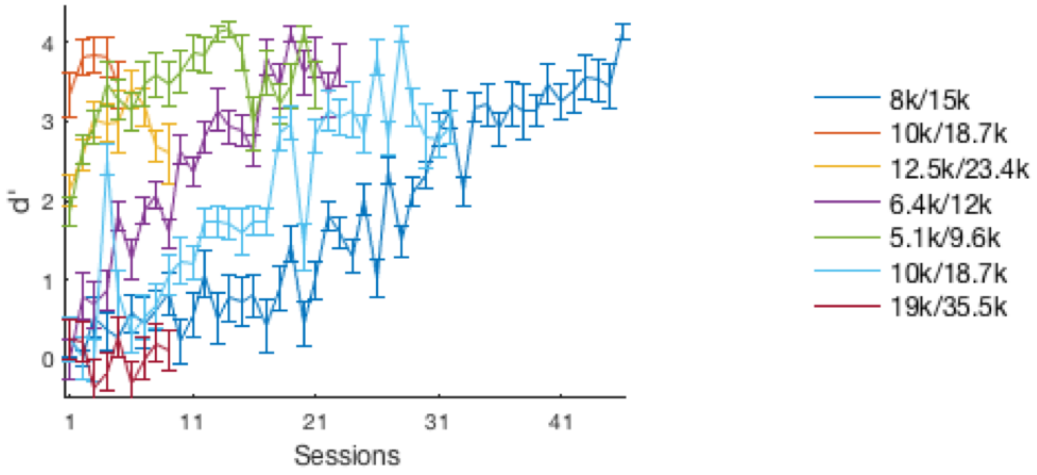

Figure 3.4: Learning generalization across seven tone pairs. The legend from top to bottom lists the tone pairs to which the animal was trained on in that sequence. Learning of the tone pair 5.1k/9.6k is paired with optogenetic activation of hippocampus to primary auditory cortex projection (green). Error bars are standard errors of d' estimated using bootstrapping.

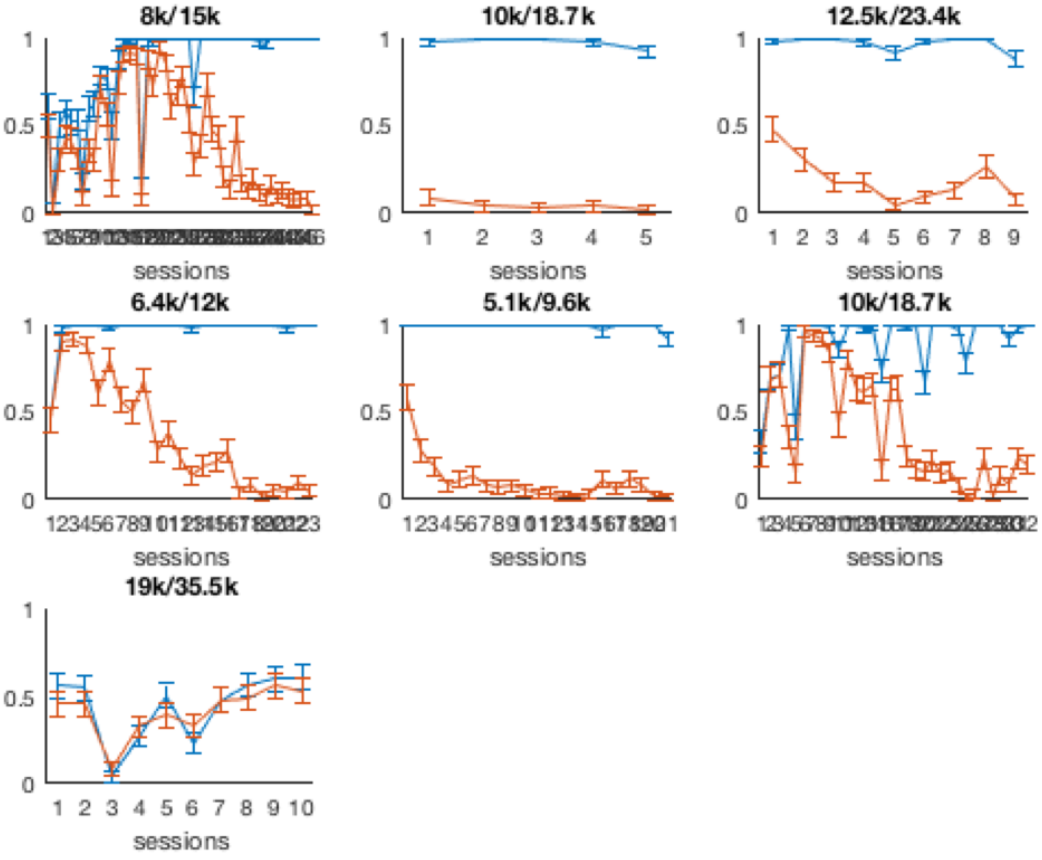

Figure 3.5: Hit rate and false positive rate for each of the seven tone pairs shown in Figure 3.4 across sessions. Error bars are SEM calculated from trial outcomes within each session.

Chapter 4

DISCUSSION AND FUTURE DIRECTIONS

Our preliminary results were inconclusive for the role of CA1 to A1 projection in auditory task acquisition but show that the activation of this projection does not impair learning generalization. Moreover, we show that mice can generalize over the meanings of specific frequencies but not the mapping from high vs. low frequencies to reward vs. no reward. However, there are many places for improvement to make our results more conclusive.

One possible explanation for why we do not see obvious effects with optogenetic stimulation of CA1 to A1 projection is that task information is transmitted from CA1 to A1 through a specific subset of neurons. Previous studies have shown that activation of a specific subset of hippocampal neurons that were involved in memory encoding can cause artificial recall of contextual fear memory (Josselyn and Tonegawa, 2020; Kitamura et al., 2017; Ramirez et al., 2013). The optogenetic stimulation used in our study does not select for neurons involved in task acquisition and thus might be too unspecific to the task to improve learning speed.

Another possibility is that the hippocampus does not participate in the acquisition of go/no-go tasks. A previous study used a discriminatory go/no-go task to test whether learning an object-odor association is hippocampus-dependent (Gilbert and Kesner, 2002). Rats were instructed to only dig for food reward when the correct pairing of object and odor stimuli is presented. Rats with complete electrolytic hippocampus lesions learnt the task as quickly as control mice across six training blocks. Hippocampus-lesioned rats were also not impaired in their ability to discriminate between odors or visual stimuli, assessed using the same go/no-go paradigm. This study differs from ours in the sensory stimuli used (odor vs. pure tone), the action required and the type of reward, but the overall task structure is identical. However, since this is only a loss of function study, it doesn't eliminate the possibility that gain of function in the hippocampus has an effect on task acquisition. Moreover, lesion studies suffer from lack of temporal and spatial resolution and does not investigate the real-time contribution of the hippocampus in task acquisition (Goshen, 2014). Some rats in this study also had incomplete lesions in the ventral hippocampus, which could be necessary for task acquisition. Taken these possibilities together,

investigating the effect of optogenetic inhibition of the vCA1 to A1 projection on task acquisition would make our results more conclusive.

Regarding task generalization, our results generate three different observations. The simplest observation is that mice can transfer their knowledge when the frequency of the new CS+/CS- tone is close to that of the previous CS+/CS- tone. Furthermore, this generalization is not affected by vCA1-A1 projection activation. For example, after a mouse reached good performance level with 8k/15k (d' > 3), it started the first session of 10k/18.7k with good performance (d' > 3). In this case, the CS+ and CS- tones are about 0.32 octave higher than the previous CS+ and CS- tones respectively. This result is consistent with existing literature and is likely to be an intrinsic property of the mouse auditory system (Hoz and Nelken, 2014). Using a latent inhibition paradigm, de Hoz & Nelken, 2014 showed that mice learn to associate a tone with an aversive air-puff slower if they have been previously exposed the same or similar tones in a safe context. The generalization gradient spans about half an octave and is steeper towards higher frequencies. The authors propose that this might be a result of the tuning curve widths of the mouse auditory nerve fibers, which is around half an octave in the 5-16 kHz range. If this is case, then our optogenetics result is also expected, since the hippocampus shouldn't affect a physical property that's intrinsic to the mouse auditory system.

The second observation is that mouse has difficulty generalizing when the new CS+ tone is close in frequency to the previous CS- tone, but not when the new CS- is close in frequency to the previous CS+. Since mice can generalize across frequencies, it is reasonable to assume here mice simply assumed the new tones to have the same behavioral meaning as the familiar tones that are close in frequency. In the case where CS+ switches to CS-, mice initially lick in response to the new CS- and then gradually learn to decrease its licking response since the behavior is no longer reinforced by water. In the case where CS- switches to CS+, mice initially refrain from licking in response to the new CS+ and therefore learns slower because its behavior is not reinforced by reward. However, mice seem to learn slower in this case compared to initial learning, despite having comparable hit rates in the first few sessions (Figure 3.5), indicating their previous experience has an inhibitory effect on current learning. This phenomenon is similar to latent inhibition, where pre-exposure to a neutral stimulus prevents subsequent association between this stimulus and an aversive outcome. Rabbits with bilateral dorsal hippocampus lesions did not exhibit latent inhibition in an eye blink paradigm (Solomon and Moore, 1975).

Rats with postnatal bilateral lesions of the ventral hippocampus lesions did not exhibit latent inhibition in a two-way shuttle box cued fear conditioning paradigm (Grecksch et al., 1999). We should try inhibiting the vCA1 to primary auditory cortex projection and see whether we can facilitate learning when a previous CS- tone becomes the new CS+ tone. If so, then the vCA1 to A1 projection could mediate latent inhibition.

A third observation is that during learning of any tone pair mice always go through a period when they lick in response to both the CS+ and the CS- tone. This behavior indicates that mice do not understand the higher-order relationship between the two tones. Studies demonstrating the ability of animal to perform model-based tasks have been done in rats (Miller, Botvinick, and Brody, 2017). Rats with hippocampus lesions have been shown to reduce their reliance on model-based strategy in a two-step decision task (Miller, Botvinick, and Brody, 2017). Another study showed that human test subjects can understand the anti-correlation between two stimuli in a two-armed bandit task and this understanding of higher-order task structures is likely mediated by the vmPFC (Hampton, Bossaerts, and O'Doherty, 2006). Thus, it is possible that the vCA1 to A1 projection facilitates learning when the learning process relies on understanding the relationship between different auditory cues.

No definite conclusions can be made based on our preliminary results since all of our experiments suffer from small sample size. In particular, the animal used to test the whether stimulating vCA1 to A1 projection impairs initial learning received a lower dose of viral vector injection compared to other mice used in this study. Moreover, all of our results regarding task generalization is based on one mouse. For our next step, we will expand our sample size and carry out optogenetic inhibition of the vCA1 to A1 projection to help draw a more definitive conclusion regarding the role of this projection in initial learning and generalization. Electrophysiology recording and calcium imaging of the CA1 terminals in A1 in a variety of other behavioral paradigms can help us identify a behavior that engages this projection. Some behaviors that are known to involve the ventral hippocampus include trace conditioning, spatial navigation and anxiety paradigms (Ciocchi et al., 2015; McEchron and Disterhoft, 1999). Another possible for the vCA1-A1 projection is facilitating transfer of information from the hippocampus to the auditory cortex during long-term memory consolidation. A previous study showed that the activity in dorsal CA1 predicts the activity in the auditory cortex during sharp wave ripples (Rothschild, Eban, and Frank, 2017).

The hippocampus plays many diverse roles in associative learning, spatial navigation, model based-planning, memory consolidation and anxiety. With the invention of optogenetics, we are beginning to understand the specific projections from the hippocampus mediating its roles in these functions. Understanding the interaction between the hippocampus and sensory cortices will help us move one step closer to understanding the hippocampus in relation to the rest of the brain.

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Appendix A

UNFINISHED EXPERIMENTS

A.1 Optogenetic Inactivation of vCA1 to A1 Projection

We are currently training two animals injected with halorhodopsin on the four tone experiment. One animal has started the four tone experiment and is being trained with 6.2k/11.5k and 17.4k/32.4k on alternating days. Both tone pairs are separated by 0.9 octaves. Halorhodopsin stimulation was paired to 17.4k/32.4k, the tone pair with slightly better performance on the control session. So far the performance for the two tone pairs are similar (Figure 4). The animal is responding to 32.4k with short lick latency and comparable number of licks relative to the other tone frequencies, indicating it has no trouble hearing this high frequency tone (Figure 5). The experiment would need to be carried out longer to see whether halorhodopsin inhibition of CA1 to A1 projections impairs learning.

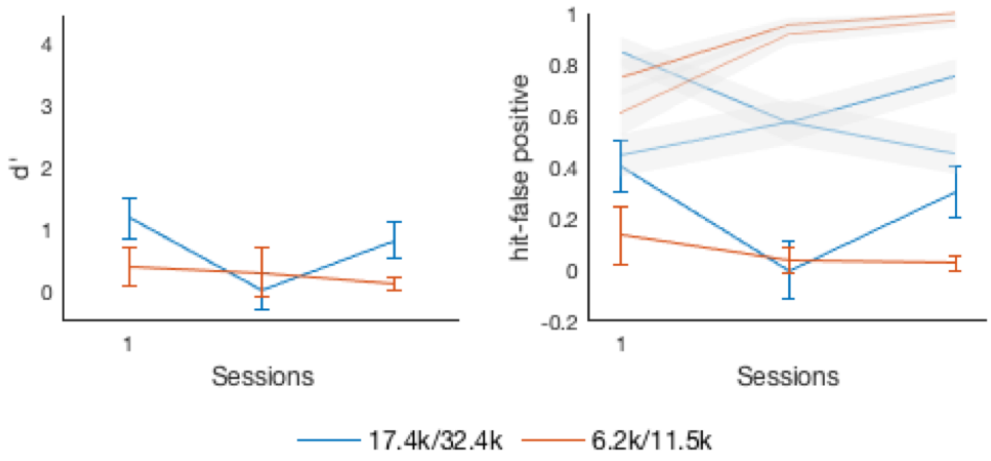

Figure A.1: Effect of optogenetic inhibition of hippocampus to A1 projection on learning within animal comparison. The first red session were done without optogenetic stimulation. Details are the same as Figure 2.

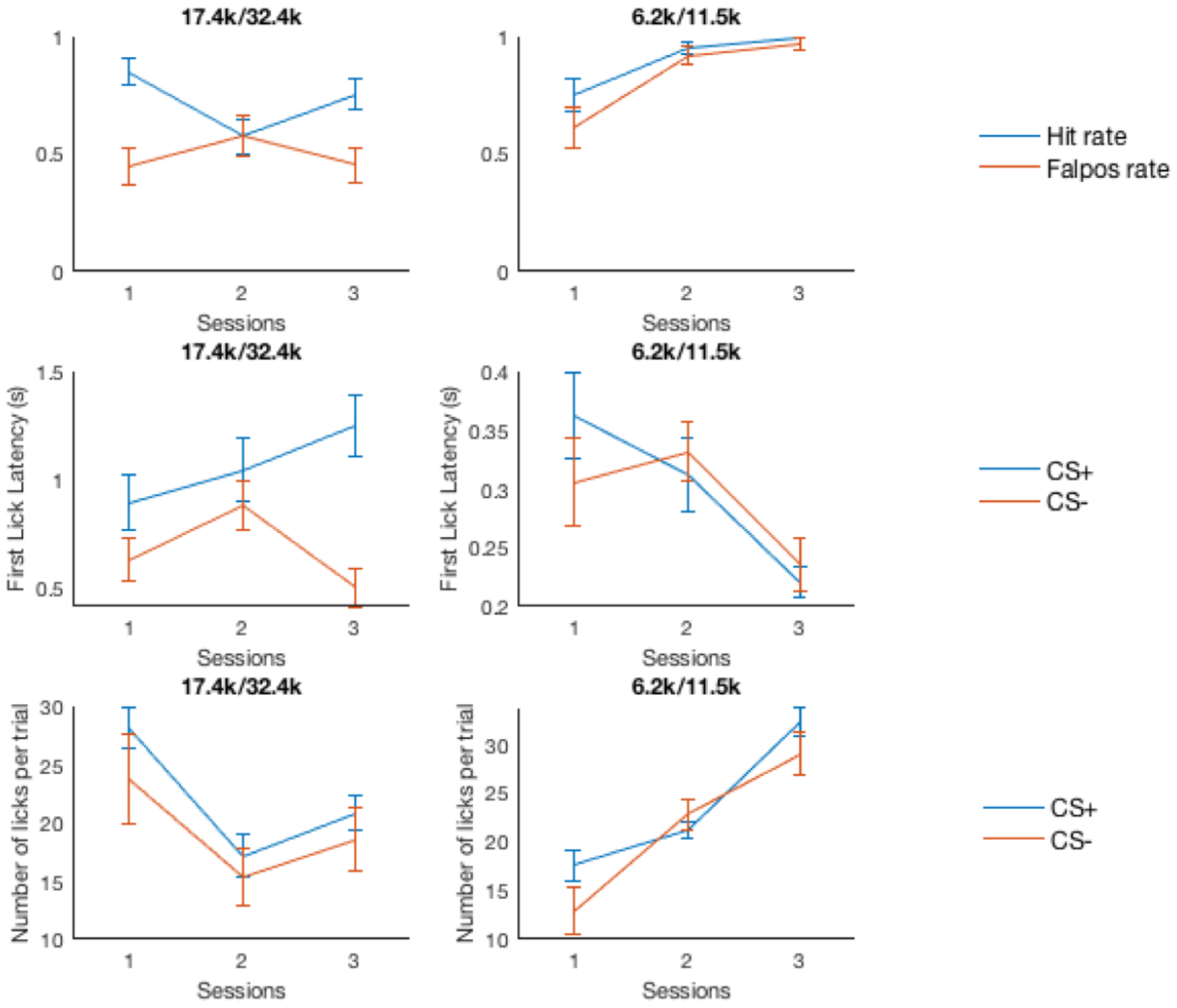

Figure A.2: Quantification of hit rate false-positive rate, first lick latency and number of licks per session for the same animal as in Figure 4.

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