THE ROLE OF THE VENTRAL HIPPOCAMPUS ON CONTEXTUAL LEARNING AND ACTIVE AVOIDANCE: IMPLICATIONS FOR PTSD

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ABSTRACT

The Role of the Ventral Hippocampus on Contextual Learning and Active Avoidance: Implications for PTSD

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A hallmark coping mechanism of post-traumatic stress disorder (PTSD) is avoidance, which is a behavior that decreases the likelihood of encountering a perceived threatening stimulus. Seventy-five Sprague Dawley rats were obtained for two way signaled active avoidance (SAA) in which the rat must completely cross to the other side of the conditioning box during a tone CS to prevent a footshock US and terminate the CS. In the first experiment, the rats were trained in one of two contexts for either 4 or 8 days and then tested under extinction conditions in both contexts. Rats tested in a different context from the one they were trained in showed significantly reduced levels of avoidance responding and increased freezing compared to their responding in the same context as training. In a second experiment, rats were trained for 4 days in the two-way SAA. To test if ventral hippocampus is responsible for the behavioral effect, the ventral hippocampus was inactivated with muscimol or injected with vehicle as a control. Rats given vehicle injections showed the same context shift deficit when tested in a novel context. However, rats given muscimol injections had similar levels of avoidance responses in

both the novel and original contexts for testing. These results exemplify that ventral hippocampus is important for constraining avoidance to the training context and improper functioning of this brain area could lead to context dysregulation of avoidance.

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Contributors

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All other work conducted for the thesis was completed by the student independently.

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1. INTRODUCTION

Being able to learn and respond adaptively to frightening or stressful events is essential for functioning in all living organisms (Cavdaroglu et al., 2020; Maren, 2008; Moscarello & Hartley, 2017). Threatening stimuli evoke a particularly salient emotional and autonomic reaction out of the animal for a quick response; this process may depend on past experiences associated with the environment or context it occurs in (Maren et al., 2013; Szekely et al., 2017). One part of an innate learning system that animals may utilize according to environmental demands and type of threat perceived is avoidance (Cain & LeDoux, 2008). Avoidance occurs when the animal partakes in some type of response (including no response) in order to decrease the likelihood of encountering threatening stimuli that are expected to occur under certain conditions (LeDoux et al., 2017; Mowrer, 1956). It is a behavior utilized by the organism when considered as the most advantageous result and only becomes negative when used excessively, or not enough, by the organism (Cain & LeDoux, 2008). Too little avoidance may costly as it can leave the animal vulnerable to the threat; this is exemplified in the conditioning box by rats known as poor avoiders that never escape the footshock due to excessive freezing (Choi et al., 2010; Lazaro-Munoz, 2010). Excessive avoidance can also lead to negative results due to maintenance of high fear levels and prevention of extinction to the fear stimulus when it no longer conveys a threat (Cain & LeDoux, 2008; Lovibond et al., 2009). Impaired use of avoidance is often a problem in psychological disorders.

A hallmark symptom for many fear- or- trauma based disorders is avoidance, and animal models of avoidance can be used in the lab to better understand etiology and treatment for such disorders (American Psychological Association, 2013). In post-traumatic stress disorder (PTSD),

the traumatic event serves as a strong emotional memory, which often creates complex associations with the aspects of the environment detected by each of the senses (American Psychological Association, 2013). As these reminders or cues are associated with the initial trauma, they are extremely anxiogenic and reducing exposure through avoidance helps prevent re-experiencing the trauma, though it is often brought into one's mind involuntarily though flashbacks and nightmares (American Psychological Association, 2013). This can be quite debilitating to the person as he or she might not be able to participate in a wide range of activities due to the potential of encountering the anxiogenic cues (Glogan et al., 2020). While avoidance can serve as a powerful reinforcer by reducing interaction with potentially anxiogenic stimuli, one's fear is not able to be contradicted as excessive to the situation and is maintained by the individual (Lovibond et al., 2009). As evidenced in a human instrumental avoidance study, participants who were able to perform "safety behaviors," or avoidance of a shock through button pressing, during extinction training experienced impaired extinction of fear compared to those who could not avoid the shock (Lovibond et al., 2009). In an effort to better understand these setbacks, we use animal models in the lab to understand how avoidance is operating when it is adaptive and what brain structures may be impaired when avoidance becomes maladaptive.

Conceptually, avoidance is an umbrella term that can encapsulate many behaviors and may change depending on the species (LeDoux et al., 2017). It can be broken down into two main subtypes- passive and active. Passive avoidance occurs when the animal does not partake in action to avoid the threat, while active avoidance requires some type of action in order for the threat to be neutralized (Kemle & Tapp, 1968). The focus of this study is two-way signaled active avoidance (SAA) in which the rat is placed into a conditioning chamber and must cross through a divider to the other side of the chamber when the tone CS is presented to prevent the

oncoming footshock US and terminate the anxiogenic warning CS. This task can be used to model avoidance in humans because people with PTSD symptoms have exhibited higher acquisition rates and expression of avoidance in a SAA computer simulated video game when compared to controls (Sheynin et al., 2017). An important modulator of avoidance memories is context, or the physical and internal representation of one's environment during the encoding of information (Maren et al., 2013). One's context serves as a powerful retrieval cue for fear associated stimuli formed during a traumatic event and dysregulation of feared stimuli can spread to novel, but similar contexts in a process known as fear generalization (Pittig et al., 2020). Importantly, our experiment first focuses on how context impacts avoidance in healthy rats so that we can eventually look into how rats with PTSD symptoms differ.

Avoidance is a complex, multi-stage learning mechanism that may be more dependent on context and resistant to extinction than Pavlovian fear conditioning (Lovibond et al., 2009). Pavlovian fear conditioning creates a direct association between the CS and US so the freezing response is very robust and can generalize in a context independent manner. In contrast, avoidance creates two memories: the initial tone shock association and then the avoidance behavior that eliminates the threat. Since the initial defensive memory is still wired in the brain, the animal must choose the best behavior (i.e., freezing or shuttling) for the optimal response and may rely on retrieval cues found in the context to remember similar past behaviors and environmental outcomes (Szekley et al., 2016). This requires inhibiting the initial conditioned response to the stimulus, such as freezing, so that the active avoidance behavior may take place, and the animal can avoid the threatening stimulus (Pittig et al., 2020). Second things learned like extinction, which creates an inhibitory CS-no US association to the original CS-US association, have been shown to be context-dependent (Bouton et al., 2004). Extinction being restricted to the

original context may be detrimental for extinction-based exposure therapies for conditions like PTSD, as life does not occur in one context. Avoidance may be similarly context-dependent due to its two-stage learning, which could be preventative measure so that it does not interfere with approach behaviors necessary for resource seeking in a safe context (Elliot, 2006). In pathological disorders, this compartmentalization of avoidance may be impaired, leading to dysregulation of avoidance across contexts. Both contextual learning and extinction, which is a highly context-dependent learning mechanism, rely on activation of the hippocampus (Ji & Maren, 2007). An interesting question posed is if avoidance is also dependent on context and therefore affected by hippocampus.

Contextual learning is primarily acquired in the hippocampus and consists of representations of stimuli in the environment, including how those stimuli interact with each other to form a comprehensive understanding of one's space (Fanselow & Dong, 2010; McDonald & White, 1993; Maren et al., 2013). When considering context, a key part of this memory is found in the spatial configuring of one's specific environment. On a neural level, the hippocampus contains neurons called place cells that fire for specific portions of the environment and respond uniquely to different locations (Moser & Moser, 1998). Rats with lesions to the hippocampus have been shown to be impaired in tasks that require spatial memory, such as the win-shift task, in which the rat uses relationships among stimuli to remember where the rewards are located in the testing environment (McDonald & White, 1993). In an aversive double dissociation study, hippocampal lesions reduced freezing to context, but not to the cue (tone), indicating its specific role in context for fear learning (Philips & LeDoux, 1992). Hippocampal memories of avoidance seem to be highly context specific in regulation of behavior, as lesioning hippocampal projections prevented proper context discrimination when the same CS was used

for avoidance in one context and approach in a different context (Smith et al., 2004). Thus, the hippocampus is a likely target for any potential contextual regulation in the two-way SAA.

The hippocampus has dorsal and ventral subregions that have distinct functions in contextual memory (Bannerman et al., 2004; Fanselow & Dong, 2010). The dorsal hippocampus (DH) has a primary role in processing the broader aspects of spatial memory while the ventral hippocampus (VH) responds more specifically to innately anxiogenic environments (Bannerman et al., 2004). Some neuroanatomical evidence to support this theory include a higher proportion of place cells in the DH, and the observation that it sends projections to areas involved with spatial processing or locomotion like the mammillary nuclei, anterior cingulate cortex, lateral septal nucleus, entorhinal cortex, and nucleus accumbens (Fanselow & Dong, 2010). Comparatively, the VH sends projections to olfactory areas, amygdala, infralimbic cortex, medial prefrontal cortex, bed nuclei of the stria terminalis, and hypothalamus, which are all areas involved in emotional memory and regulation or the physical processing of emotions (Fanselow & Dong, 2010). As the VH is more involved with the emotional component of context, disruption to the ventral, but not dorsal, hippocampus results in decreased anxiety in a plus maze (Kjelstrup et. al, 2002; Jimenez et al., 2018). VH lesions seem to produce a broad anxiolytic effect in a variety of anxiety producing contexts, such as the light portion of the light dark box or in the open field test (Bannerman et al., 2004). This literature supports the idea that the VH may code for anxiety producing contexts, with inactivation leading to anxiolytic reactions to previously threatening environments.

Although exact functions of VH are not known, another key hypothesis is that this structure plays an important role in regulating behavior to novel, threatening contexts (Cavdaroglu et al., 2020). In the Cavdaroglu et al. (2020) study, rats with lesions to VH were trained to extinguish

avoidance in the presence of a safety signal, and VH impairment served to facilitate extinction when compared to sham lesions. The VH may regulate how contextual stimuli, such as the safety signal, serve as cues to signal that the aversive event will or will not happen; no longer being able to access these cues leads to a weaker avoidance memory and stronger extinction (Cavdaroglu et al., 2020). Contexts can create key retrieval cues from past events to predict future responses in the same context, and inactivation of the VH prevents the brain from accessing such retrieval cues to have context specific behavior. The VH seems to have a primary role in specifically contextual aversive memories, as silencing of the VH is shown to disrupt contextual fear while leaving other kinds of fear intact, such as cued fear (Twining et al., 2020). Thus, we aimed to examine the importance of the VH in regulating avoidance across contexts. If such a role is identified, the ventral hippocampus may be a key area of interest for people with trauma and anxiety disorders, such as PTSD.

The research question of the current study is to determine if two-way SAA is context dependent. In order to test this question, rats were trained using the two-way SAA paradigm for four or eight days in one of two contexts (Context A or Context B) and tested afterwards for two days, one day for each context presented in a counterbalanced order. We found that two-way SAA is context dependent as rats performed significantly fewer avoidance responses in a different context from training when compared to responses in the same context. To study whether the VH is important for this effect, we implanted cannula into a second group of rats for injecting muscimol (a $GABA_A$ agonist) to inactive the VH. For the second experiment, we trained rats in the two-way SAA paradigm for four days and injected them with muscimol, or vehicle as a control, immediately before testing them in both contexts (one context per day in a counterbalanced order). There was an observed context shift deficit in the control group, with

higher avoidance responses on the testing day for the same context than the different context, which was similar to the results found in the first experiment. However, the VH inactivation performed a similar number of avoidance responses in the same and different contexts during testing, which demonstrates that the VH is responsible for the context dependence of two-way SAA.

2. METHODS

2.1 Subjects

We obtained 75 experimentally naïve, adult, male Sprague Dawley rats (200-300g) from the Envigo supplier for experiments one and two. The rats were housed in individual cages with ad libitum access to food and water inside a temperature/humidity-controlled room for the experimental process. They were maintained on a 14/10 light/dark cycle that started at 7 AM, and all of the experiments were run during the light phase of the light/dark cycle. Five days before the experiment, each rat was handled for about two minutes per day to habituate them to the experimenters. These experiments were done with approval from the Animal Care and Use Committee at Texas A&M University.

2.2 Surgical procedure

A week before training for experiment two, the rats underwent a surgical procedure. The rats were anesthetized with isoflurane (starting at 5% and gradually decreasing during the surgery) and were fixed to a stereotaxic apparatus (from Kopf Instruments). After an incision on the scalp was cut, the bregma was found, and holes were drilled for 3-4 jewelers screws, as well as two more holes for cannula placement. The two cannulae (which were 11mm, 26 gauge, Plastics 1), were placed into the ventral hippocampus (A/P: -5.25, M/L: +/- 5, D/V: -7.3 from bregma) and were fixed to the skull using dental cement. Cannula dummies (12 mm, 30 gauge, Plastics 1) were twisted onto the cannula so that there would not be blockage. After the surgery, the rats were allowed seven days to recover before behavior sessions. The cannula dummies were switched out twice so that the rats would become habituated for the infusion day.

2.3 Drug infusions

For the infusion procedure, the rats were transported to a different room in the laboratory via 5-gallon white buckets with a small layer of bedding inside. The dummies were removed, and the experimenter placed stainless steel injectors (11mm, 33 gauge) into the cannulae. The stainless-steel injectors were attached to polyethylene tubing. This tubing was joined to 10 µl Hamilton syringes, which were mounted in an infusion pump (Kd Scientific). First the tubing was filled with distilled water and an air bubble was created to separate water from the drug or vehicle that was inserted until the right amount was reached. The drug, muscimol (0.1 µg/µl with sterile saline dilution), was infused into the VH at a pace of 0.1 ul/min for 5 minutes (0.5 µl, .05µg total). Afterwards, the injectors were left for three minutes, taken out, and then new dummies were inserted into the cannula. The movement of the air bubbles ensured proper infusion. The rats were transferred back to the avoidance room via the transport boxes described below.

2.4 Behavioral apparatus

Four uniform shuttle boxes made of plexiglass and metal (50.8 X 25.4 X 30.5 cm, L-X-W-X-H; Coulborn Instruments) were utilized for the behavior sessions. Each shuttle box was comprised of two chambers split by a metal divider with a small opening for the rats to cross over to the other side (8 X 9 cm, W-X-H). The floor was made of stainless-steel bars. For the conditioned stimulus (CS), there were speakers on top of each chamber at the furthest side for administering a 5 kHz, 80 db tone for 15 seconds at a time. There was also a 0.7 mA foot shock (unconditioned stimulus (US)) from a scrambled shocker at the end of the tone transmitted via the steel bars. Each of the shuttle boxes were put into larger chambers to control for outside light and sound. Shuttling (moving from one side of the chamber to the other side via the door opening) was

recorded by two infrared rays, which were made of five emitter-detector pairs. This system was placed in the center of the wall for each shuttle box compartment. Different cues were used to establish two different contexts. In Context A, there was a house light and compartment lights (0.5 W light bulb), the doors of the larger chamber were closed to lessen sound, black and white stripped papers were placed on the back walls of the shuttle box, and a 3 % acetic odor was wiped on the walls. The rats were transported to and from the training room using white transport boxes with a layer of bedding. The other context, Context B, did not have houselights or compartment lights turned on, the doors of the larger chamber were open (with lights off in the room), black paper with glow in the dark stars was placed on the walls of the shuttle box, and a 1% ammonia solution was wiped onto the walls. The rats were transferred using black transport boxes with no bedding. When the rat was placed into a different context than it was previously trained in, a black plexiglass floor was placed over the metal bars.

2.5 Two-way signaled active avoidance and testing

On the first day of avoidance training, all of the rats were exposed to one CS-US pairing of the tone and footshock to establish Pavlovian conditioning of the association. Next, the rats were given thirty CS trials in which the rats were able to cross to the other side during the 15 second presentation of the tone (CS) to prevent the shock and terminate the tone. The CSs were separated by ISIs of an average of 120 seconds. Successful crossing required all four feet to cross to the other side of the shuttle box, which was detected by the infrared array and automatically entered into the Graphic State program as an avoidance response. An avoidance response would stop the tone and the deliverance of the shock. Following the first day, only the 30 avoidance trials were used in the training paradigm for the remaining 3 or 7 days. Fewer than 6 avoidances

for the last 3 days of training resulted in the rat being considered a poor avoider. There were two testing sessions given to the rats, one in each context (one testing session per day for a total of two days), and the contexts were presented in a counterbalanced order. For the test, there were ten CS (tone) deliveries without the shock. While the rats were able to cross to the other side of the chamber, successful avoidance did not turn off the CS. Though the animals could cross multiple times during the CS presentations, the experimenter capped each CS presentation at one avoidance response (Fig. 1).

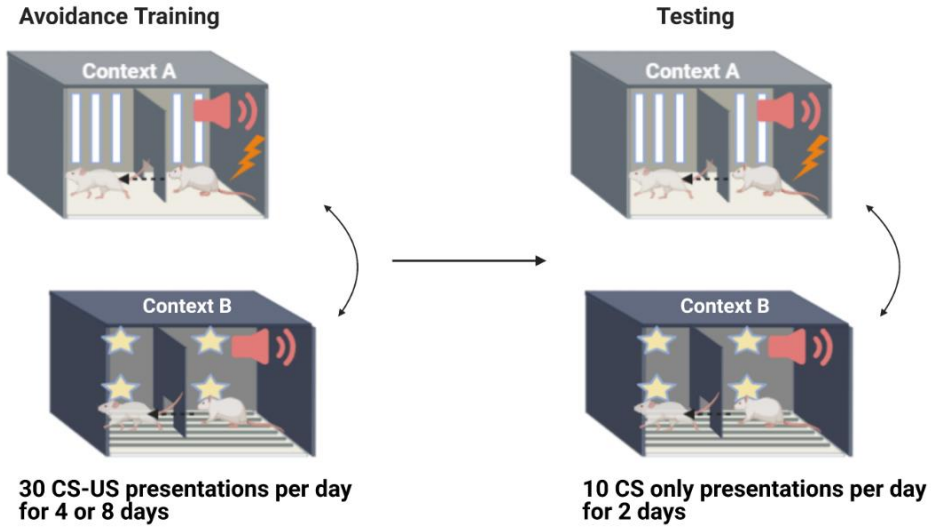

Figure 1: Experiment 1 Design. Rats were trained in two-way signaled active avoidance in either Context A or Context B with 30 CS-US presentations per day for days 1 through 4 and 7 through 10. On days 5 through 6 and 11 through 12, rats were tested under extinction conditions with 10 CS only presentations per day. Avoidance training and testing were both counterbalanced.

2.6 Experiment 1: context dependence of avoidance after 4 and 8 days of training

The goal of experiment one was to assess if two-way SAA was context- dependent. In this paradigm, the rats were trained for eight days with tests after the fourth and eighth days. On the fifth and sixth days (and 11th and 12th days), the rats were tested using the paradigm established above in which the rats were tested in both contexts, with one context per day.

2.7 Experiment 2: impact of ventral hippocampus on context dependence

For this experiment, all of the rats were trained in solely Context B since there were context shift effects in both training environments to lessen the number of rats needed (Fig. 2). Training was also limited to four days since a similar effect was seen at both four and eight days. For the two counterbalanced tests, the rats were given either muscimol or vehicle infusions in the VH immediately before each avoidance testing session with each rat receiving the same infusion for both context tests depending on drug group. Drug assignments were determined after the last day of training so that performance in both groups would be similar.

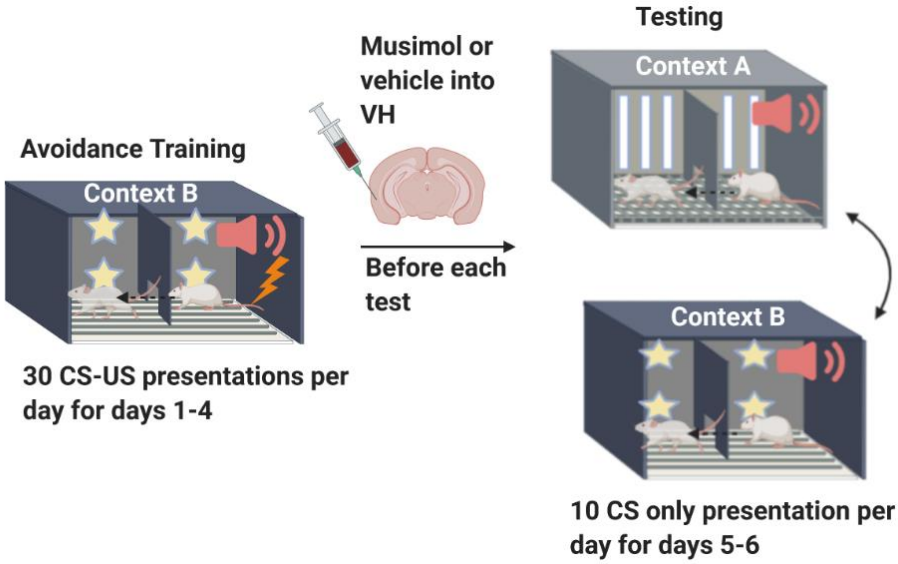

Figure 2: Experiment 2 Design. Rats were trained in two-way signaled active avoidance with 30 CS-US presentations per day for days 1 through 4. Rats were tested under extinction conditions with 10 CS only presentations per trial for days 5 and 6. Immediately before each testing session rats were injected with muscimol or saline into the VH. Avoidance training was done in Context B and testing used both contexts in a counterbalanced order.

2.8 Histological analysis

Rats were euthanized using sodium pentobarbital (Fatal Plus; 100 mg/ml, 0.75 ml) and were perfused with saline and 10% formalin. The brains were taken out and stored in 10% formalin for no longer than 24 hours before being transferred to a 30% sucrose solution at 4 degrees C for

at least 72 hours. Fixed brains were placed in a cryostat (-20 degrees C) and coronal brain sections (40 µm) were made starting at the ventral hippocampus. The slices were mounted on subbed microscope slides and stained using a 0.25% thionin solution to see cannula positions. Cannula placements for both saline and muscimol group animals in experiment 2, which guided the placement of the drug into the ventral hippocampus, are represented in figure 3 below. Experiment two started with 43 rats, but 7 dislodged their headcaps, 7 had cannula that missed the ventral hippocampus, and 4 were poor avoiders, so the total was 13 rats in the muscimol group (n=13) and 12 rats in the vehicle group (n=12).

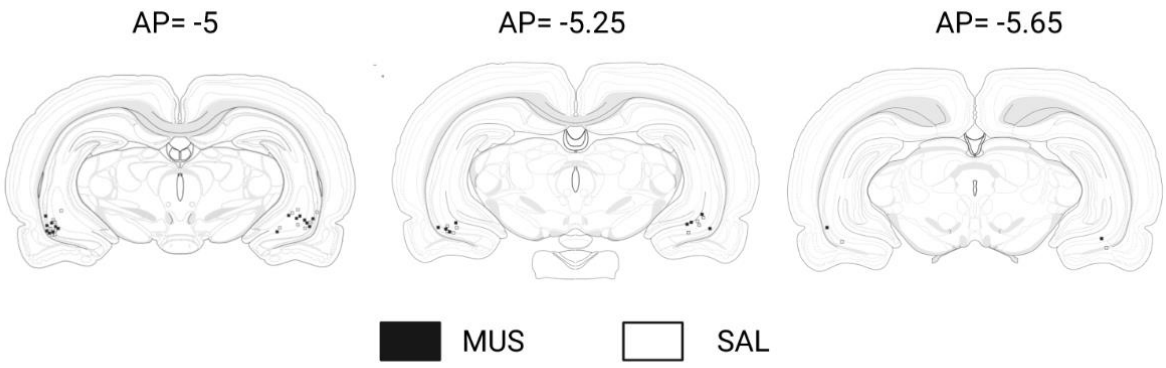

Figure 3: Cannula placements in the ventral hippocampus for all subjects included in the analysis (n=25), with muscimol (n=13) and saline (n=12) groups (atlas images from: Swanson, 1998).

2.9 Data analysis

Scoring of the freezing behavior was done by hand by an experimenter blind to the experimental condition with stopwatches from videos recorded by a digital camera. Freezing was measured during the 15 second presentation of each CS and well as a baseline 15 seconds before the initial CS. Freezing percentages were calculated as time spent freezing/ 15 seconds x 100. The active avoidance training was analyzed using a two-way repeated measures ANOVA while the active avoidance test data was conducted with a three factor ANOVA of mixed design. All of the

ventral hippocampus impairment data used only a two-way ANOVA. A paired t-test was conducted between the muscimol and vehicle conditions in experiment 2. These analyses were done using Statview Version 5.0.1 (SAS Institute) through a MacOS open- source emulator.

3. RESULTS

3.1 Experiment 1: context dependence of avoidance at 4 and 8 days of training

3.1.1 Avoidance responses for 4 and 8 day avoidance acquisition

To examine whether active avoidance is mediated by context in normal subjects, and if this process is affected by training length, 32 rats were trained for 8 days total in a signaled two-way active avoidance training paradigm, with tests after both 4 and 8 days (Fig. 1). One rat was excluded for being a poor avoider, and 3 were not included in the freezing analysis due to technological issues. Using a two way repeated measures ANOVA with the within-subjects factor of Day and between-subjects factor of Training Context, we found a main effect of Day as both groups increased in avoidance responses in the training sessions over the days [$F(7,203)= 41.945$, $p< 0.0001$]. There was a Day by Training Context interaction in which the rats in Context A had less overall avoidance responses compared to those trained in Context B [$F(7,203)= 2.597$, $p= 0.0138$]. This data shows that the rats improved with avoidance responses over the days, but there was some discrepancy with training contexts as the rats seemed to perform better in Context B, which was the dark context (Fig. 4A).

3.1.2 Avoidance and freezing responses for 4 and 8 days avoidance testing

For 2 days after the training sessions, the rats were tested for avoidance in both the same and different contexts in a counterbalanced manner. Utilizing a three factor ANOVA of mixed design with two within subjects factors of Test Context (same or different) and Day (four or eight days) and a between subjects factor of Training Context (A or B), the results showed a main effect of Day for the avoidance responses, indicating an increase in avoidance responses over time

[F(1,29)= 7.019, p= 0.0129]. However, there was not an interaction of Test Context by Day, meaning that the context the rats were tested in did not impact avoidance responses over the days [F(1,29)= 0.032, p= 0.8600]. There was a main effect of Test Context on avoidance responses, with higher levels of avoidance responses in the same context as training compared to those in the different context [F(1,29)= 44.493, p< 0.0001]. We found a Test Context by Training Context interaction [F(1,29)= 15.809, p= 0.0004] in which rats tested in Context A did not show as large as a context shift deficit as rats trained in context B (Fig. 4B). Freezing responses to the CS were also analyzed using a three factor ANOVA of mixed design with the same factors. There was a main effect of Test Context on freezing [F(1,26)= 34.088, p<0.0001], indicating a statistically significant higher level of freezing in the different context compared to the same context as the training (Fig. 4C). We did not observe a Test Context by Training Context interaction for freezing [F(1,26)= 2.257, p= 0.1450]. Overall, this data shows higher levels of avoidance and lower levels of freezing in the same context as training than in the different context.

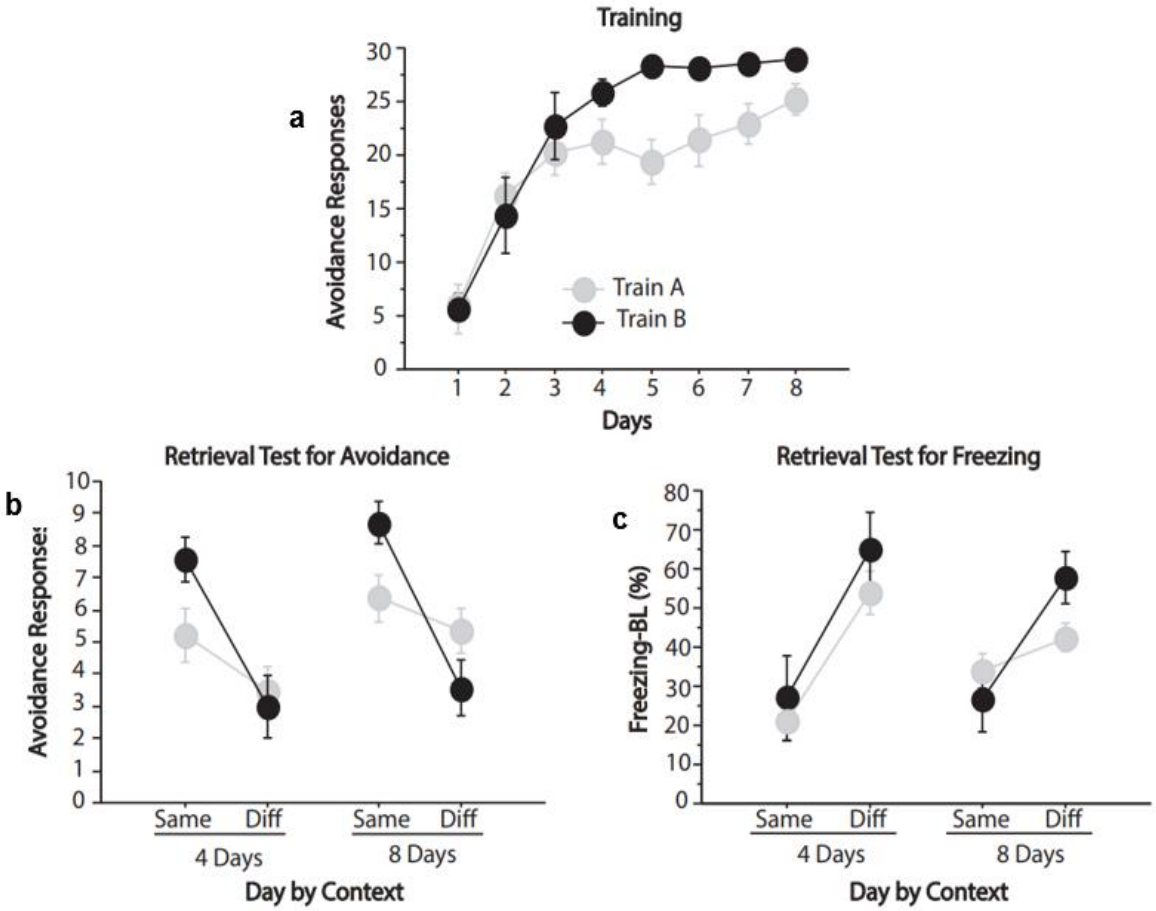

Figure 4: Two-way SAA is diminished by a change in context. (a) Avoidance responses per training day in the light context (train A) or dark context (train B) for 8 days. Those trained in B (n=7) performed significantly more avoidance responses than those trained in A (n=24). (b) Retrieval avoidance responses capped at 1 response/ CS for testing in the same and different contexts for 4 or 8 days. (c) Freezing percentages (with baseline subtracted) for testing in the same and different context for 4 or 8 days. Rats performed significantly more avoidance responses and spent significantly less time freezing in the same context compared to the different context though this effect was muted in animals trained in A. All data are presented as mean ± SEM.

3.2 Experiment 2: ventral hippocampus mediates context dependence of avoidance

3.2.1 Avoidance responses for ventral hippocampus inactivation avoidance acquisition

A separate cohort of rats was trained in two-way signaled active avoidance for four days in Context B and were given muscimol or vehicle injections into the ventral hippocampus before each testing session (Fig. 2). All data analysis was conducted using a two factor ANOVA. There

was a main effect of Day in which the rats increased their avoidance responses from day one compared to day four [F(3,69)= 49.403, p< 0.0001]. There was no Day by Drug interaction [F(3,69)= 0.859, p= 0.466], which indicated the rats in both the muscimol and vehicle groups were matched for performance (Fig. 5A).

3.2.2 Avoidance and freezing responses for ventral hippocampus inactivation avoidance testing

Immediately after ventral hippocampus infusions, the rats were tested under extinction conditions in the two-way SAA paradigm on days 5 and 6. Avoidance and freezing responses were analyzed using a two factor ANOVA. There was a Test Context by Drug interaction, which displayed that rats in the muscimol group were statistically significantly different in their avoidance responses compared to the vehicle group [F(1,23)= 7.150, p= .0136]; rats in the saline group performed fewer responses in the different context while rats in the muscimol group had similar levels of avoidance in both contexts (Fig. 5B). A paired t test was also conducted to examine group differences in the vehicle vs muscimol conditions for the avoidance responses in the same vs different test context. There was a statistically significant different amount of avoidance responses for the same vs different contexts for the vehicle group [t(11) = 4.535, p = 0.0009], but not for the muscimol group [t(12) = 0.410, p = 0.688). There was a main effect of Test Context for freezing responses as the rats in both groups froze significantly more in the same context than in the different context [F(1,23)= 5.530, p= .0276]. There was no test context by drug interaction [F(1,23)= 3.087, p= .0922], meaning the rats in the muscimol group demonstrated the same increase in freezing that the vehicle animals showed in the different

context (Fig. 5C). Thus, the data shows that the context shift deficit found in the vehicle group was abolished in the muscimol group as there were similar avoidance responses in both contexts.

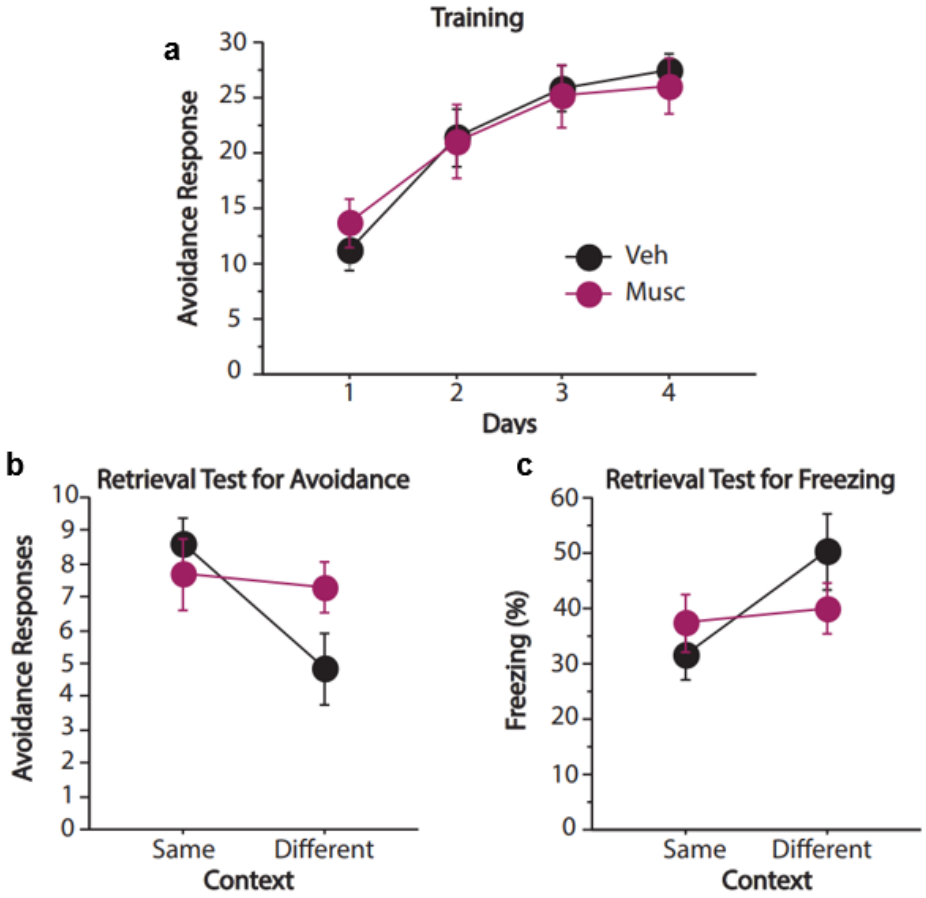

Figure 5: Ventral hippocampus inactivation abolishes the context shift deficit(a) Avoidance responses during 4 days of training for the muscimol and vehicle groups. (b) Retrieval avoidance responses during testing in the same or different context as training for the muscimol and vehicle groups. (c) Freezing responses during testing for the muscimol and vehicle groups in the same or different context as training. Rats injected with muscimol performed significantly more avoidance responses in the different context than those injected with vehicle. All data are presented as means ± SEM.

4. CONCLUSION

This study provides evidence that two-way signaled active avoidance is mediated by context, and this process requires the ventral hippocampus. Specifically, our experiments show animals will perform fewer avoidance responses and spend more time freezing when exposed to a different context than training. This difference in avoidance responding can be prevented through ventral hippocampus inactivation with muscimol during retrieval sessions, which demonstrates that the context dependence of avoidance can be localized to the ventral hippocampus. These results are aligned to a previous study which demonstrated that wheel-turning avoidance responses in rabbits are context-dependent, and lesions to the entorhinal cortex, which surrounds the hippocampus, abolished this effect (Freeman et al., 1997). It is a possibility that our results could be similar due to muscimol spread to entorhinal cortex or the ventral subiculum, a region receiving projections from entorhinal cortex that Burhans and Gabriel (2007) found to be important in their avoidance studies as part of the "ESA pathway". However, their work sometimes showed that the lesions extended to VH, and our cannula placements were not always in a proximal distance to entorhinal cortex, so it is likely that both regions are relevant to the context dependence of avoidance (Burhans & Gabriel, 2007; Freeman et al., 1997). In this case, our study focused on just the ventral hippocampus as a key structure for contextual memory.

There was some disparity of avoidance responses between the two contexts used in experiment one. Rats tended to perform more avoidance responses during training and had larger shifts in the different context for Context B compared to Context A. The reason this effect occurred may be due to differing baseline fear levels between the two contexts, as Context A has

the light turned on. Since rats prefer darkness, they will inherently feel higher levels of fear to Context A compared to the dark context, Context B. If the rats are unable to inhibit their natural fear responses to the light, this will lead to higher freezing and less avoidance responses during the avoidance training and testing especially since brighter lighting tends to decrease locomotion in rodents (Crawley, 1985). While anxiety clearly plays a role in the context shift deficit, it cannot fully explain our data since those trained in the A context still show a significant decrease in avoidance even when shifted to the dark context where they are more comfortable.

The first widely known explanation of the mechanisms behind avoidance originated from Mower's Two-Factory theory in which avoidance learning occurs in two steps based on separate learning systems (Mowrer, 1956). The animal first acquires classical fear conditioning when a tone becomes paired with an unescapable shock (US), becoming the conditioned stimulus (CS) (Mowrer, 1956). This is an unconscious learning mechanism in which the pairing of the CS and US creates a CS-US association, and the animals intrinsically attaches "fear" to the CS as the connection to the US has been strengthened in the brain's firing network (Cain & LeDoux, 2008; Maren, 2008). This learning primarily takes place in the amygdala, as the basolateral amygdala (BLA) creates the CS-US connection while the central amygdala (CeA) coordinates the appropriate biological response, such as freezing (Cain & LeDoux, 2008). A major input to the BLA is the ventral hippocampus, which may connect the contextual learning to the cued learning found in the amygdala for a retrieval cue when required (Fanselow & Dong, 2010). Next, the animal learns a behavior through instrumental conditioning, such as shuttling or lever pressing, so that it will avoid the aversive event (Mowrer, 1956). Instrumental conditioning has a behavior consciously conducted by the animal, with the likelihood of repeating said behavior determined by the environmental response, like absence of the shock (Maren, 2008; Mowrer, 1956). Both the

Pavlovian fear response and instrumental conditioning are remembered by the animal, which must choose the correct response according to the context and other relevant factors.

Active avoidance requires the initial fear response from the central amygdala to be inhibited by the medial prefrontal cortex so that the instrumental behavior may be performed (Moscarello & LeDoux, 2013). This competition between freezing and avoidance can be thought of as memory interference, in which information learned in one time period affects the information learned in another time (Bouton, 1993). When responses are learned in a second order, such as in extinction, they rely on contextual stimuli to help settle the correct action in response to the stimulus presented (Bouton, 2004). Since active avoidance requires two steps, it may operate in a similar manner. Moreover, the first learned behavior dominates response in new contexts, which may explain why there is lower avoidance in novel contexts and higher avoidance responses in the known test context, since the animal first learned to freeze (Bouton, 2004). In the case of avoidance, it may be biologically advantageous for the behavior to be restricted to the original fear-inducing context, so it does not interfere with proper extinction and prolong fear once the danger has passed (Lovibond et al.,2009; Pittig et al.,2020). When avoidance is not confined to the original context, it can promote context dysregulation of avoidance to other situations and unhealthy coping mechanisms. One important consideration for the persistence of avoidance is the reinforcement mechanism behind the behavior.

There is an important distinction behind the reinforcements of shorter vs prolonged active avoidance behavior. Our study shows that animals had similar behavior after both the four and eight days of training, however other studies have focused on even longer avoidance training, such as over 20 days (Cullen et al., 2015). In the shorter training trials, animals are motivated to avoid through negative reinforcement (removal of shock), which relies on dopaminergic

modulation of cortico-limbic-striatal structures (Stanley et al., 2021). Though, rats will continue to display avoidance even under extinction conditions when there is no shock (LeDoux et al., 2017). This contradiction occurs due to prolonged training switching the neural circuitry from primarily relying on negative reinforcement to a habit-based system that is independent of reward (Cain et al., 2019). Persistent avoidance training has been shown to increase dendritic spines in the dorsal medial striatum in correlation to the intensity of the US; this may represent the neural basis of memory retention needed for habit memory (Stanley et al., 2021). Habitual information stored in the striatum has previously been shown to be more resistant to extinction than goal- directed negative reinforcement (Wendler et al., 2014). Many human studies have confirmed that avoidance is resistant to extinction, perhaps because of this reliance on habit rather than more situationally relevant factors (Lovibond et al., 2009). If individuals with PTSD are impaired in learning this extinction process, then they will continue to avoid even when they are in safe situations and it is no longer adaptive to do so.

An important factor for many trauma and anxiety based mental disorders is the excessive use of avoidance stemming from dysregulation of feared stimuli across contexts. Transferring feared stimuli to novel, but similar, contexts leads to maintenance of one's fear and utilizing excessive avoidance as a coping mechanism for the individual (Glogan et al., 2020). If there is not a compartmentalization of fear to the original trauma, there may not be a safe space for the individual, and this can be detrimental to one's quality of life. In a study researching fearful cues from sexual assault survivors, there was a correlation found between severity of PTSD and avoidance of both sexual and non- sexual threatening images (Fleurkens et al., 2014). Those who scored higher in PTSD symptomology generalized fear from sexually threatening images to threatening images in general, such as a car wreck; this effect was not seen in those with little to

no PTSD symptoms (Fleukens et al., 2014). Fear may generalize quite broadly to other threatening stimuli, depending on one's internal state of anxiety and perception of the threat (Fleurkens et al., 2014). A similar theme of fear generalization, or extending threatened feelings from the original context the threat was experienced in, was evident to be at a heightened rate for those with anxiety or PTSD (Glogan et al., 2020; Sheynin et al., 2020; van Meurs et al., 2014). As noted with our study, the hippocampus has a broad role in connecting context to memory, so when this area is not working properly, it may lead to impaired cognition and behavior.

Other research has found a link between PTSD severity and hippocampal damage or abnormalities (Joshi et al., 2020). In the Gilbertson et al. (2002) study, a genetic predisposition was identified in monozygotic twins, as a combat exposed veteran twin with PTSD and his civilian twin with no- PTSD both had smaller hippocampal volumes than a non-related combat exposed veteran without PTSD. This provides evidence that an individual may be predisposed to disorders such as PTSD through hippocampal abnormalities and will be more likely to develop PTSD through stressful life events or other psycho-social factors. Debate still occurs if PTSD-predisposition is entirely genetic, as extreme stress has also been shown to create hippocampal atrophy in the CA3 region, leading to inefficient processing (Bremner et al., 2001). A vicious cycle may entail in which the stress from the original trauma is maintained through ineffective coping mechanisms, like excessive avoidance, leading to sustained stress and further damage to the hippocampus. Damage to this area, whether through genetic factors, stress, or injury, may impact the hippocampus' functions, including contextual memory regulation. In patients with PTSD, the hippocampal function of preventing the avoidance response in new contexts could be inhibited due some type of abnormality, resulting in maladaptive avoidance generalization.

An important consideration of this study may be the long-term role of the ventral hippocampus, as whether it mediates the context-dependence of avoidance in both short-term and long-term avoidance. This study found that prolonged training of avoidance (eight days) was still context dependent, though we did not confirm the ventral hippocampus was required for this effect. In the Cullen et al. (2015) study, the ventral hippocampus was required for recent contextual memory, but after 28 days the information had shifted to prefrontal cortex control. Shifting information away from the ventral hippocampus may cause a memory to lose its context specificity and create a more abstract memory that can easily be generalized across contexts (Cullen et al., 2015). PTSD often goes years without treatment and may worsen with time, so considering the long-term functions of the ventral hippocampus is functionally relevant. Much research still needs to be conducted in terms of how the ventral hippocampus' projections to cortico-limbic-striatal brain structures are involved in regulating fear and anxiety, and how brain circuitry may shift as a function of time. Understanding this circuity may bring researchers one step closer into understanding the etiology behind complex diseases such as PTSD and elucidate novel treatments.

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