EFFECTS OF CUE TYPE AND OVERLAP IN CONTEXT CONDITIONING: THE ROLE OF THE HIPPOCAMPUS

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ABSTRACT

An environmental context can be represented as a collection of individual features, or as a conjunction of elements combined by relational associations formed within the hippocampus. Separating out different experiences and then retrieving the correct representation becomes more challenging when cue elements start to overlap or become degraded. I designed a series of highly ambiguous context discrimination tasks based on sensory features including olfaction, vision, and geometric shape. Normal rats were able to discriminate highly ambiguous contexts defined by olfactory cues, visual cues, and certain geometric shapes. Rats with hippocampal lesions were impaired in the olfactory and visual task, and showed enhanced performance on the geometric shape task. In a final experiment, I demonstrated that rats learned a novel pattern separation/completion task that utilized olfactory information as the retrieval cue. These studies suggest that in context discriminations, some features are more important than others and this influences hippocampal involvement.

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> "Never trust the storytelling. Only trust the story."
>
> Neil Gaiman, The Sandman #38: "The Hunt" (Adopted from D.H. Lawrence)

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LIST OF ABBREVIATIONS

DFCTC – discriminative fear conditioning to context
HPC – hippocampus
RA – retrograde amnesia
AA – anterograde amnesia
DHPC – dorsal hippocampus
VHPC – ventral hippocampus
EC – entorhinal cortex
DG – dentate gyrus
CA3 – Cornu Ammonis 3
CA1 – Cornu Ammonis 1

Chapter 1

General Introduction

An animal's survival is dependent on learning from their experiences to guide future behaviour. One critical component includes learning about the relationships among stimuli, environmental contingencies and ultimately producing actions or responses directed towards desired goals or away from potential hazards (Thorndike, 1911; Pavlov, 1927). Examining these kinds of learning and memory processes will provide an improved understanding of the organization of mammalian learning and memory processes and ultimately normal and abnormal behaviour (Hirsh, 1974; White & McDonald, 2002; Gruber & McDonald, 2012). The important role of environmental contexts in learning is well documented and its influence is multifaceted with external sensory stimuli interacting with intrinsic motivations to form complex representations that can be utilized to guide adaptive behavioural patterns (Fanselow & Tighe, 1988; Holland & Bouton., 1999). Context influences not only how an animal respond to positive or negative experiences but more generally how an animal's patterns of behaviour are expressed (Good & Honey, 1991; Bouton, 1993). Manipulating contextual details has allowed researchers a means to study animal behaviour and makes inferences on various different aspects of learning and memory processes (Thorndike, 1911; Tolman, 1932; O'Keefe & Nadel, 1978; Sutherland, et al., 1989; Antoniadis & McDonald, 1999).

An environmental context can be represented as a collection of individual features or cues, or as a conjunction of elements making up a learning experience (Nadel and Willner, 1980; O'Reilly and Rudy, 2001; Rudy and O'Reilly, 2001; Rudy and Sutherland, 1989). Animals experiencing context conditioning in learning experiments are exposed to either a positive or negative event associated specifically to the context itself or specific context features. There can be many different sources of information available in a context; the physical layout and position of visible objects, scent cues, ambient sound, and even the lighting to name a few. It is difficult to determine which elements of an environment contribute to the diverse representations formed in learning processes. Context conditioning paradigms can be designed to feature elements which dictate whether an event will be positive or negative, and only through associative processes, can these environments be approached or avoided, respectively. Thus, following training, subjects with no previous preference for either context display active approach or avoidance (appetitive/aversive) of the context paired with the positive or negative stimulus, indicating that a significant preference has been acquired following the conditioning process.

Fear conditioning paradigms require animals to associate stimuli or features of a situation or context with an aversive event. Through discriminative conditioning, neutral stimuli such as context or contextual cues are associated with a specific outcome, which, if acquired and retained by the subject, enables the capacity of specific stimuli to elicit an appropriate behavioural response (Kim & Fanselow, 1992; Maren, et al., 1997; Antoniadis & McDonald, 1999). Normally, fear conditioning tasks use a non-discriminative procedure constituted by using a single context chamber or limited pairing of the reinforcer stimulus (Kim & Fanselow, 1992). An early examination of fear conditioning paradigms provided the framework to explore the advantages in using discriminative vs non-discriminative paradigms, and reveal the limitations of unitary measures of fear (Antoniadis & McDonald, 1999; Antoniadis & McDonald, 2000). In the Antoniadis & McDonald research, they investigated hippocampal and amygdalar influences on different measures of fear in their discriminative fear conditioning to context task. It was found that the hippocampus and amygdala both participate in the conditioning of freezing, preference, locomotion and ultrasonic vocalizations; in addition to the amygdala mediating heart rate and the hippocampus mediating defecation and body temperature (Antoniadis & McDonald, 2000). More importantly, developing the discriminative paradigm alleviated certain confounds found in non-discriminative paradigms such as acquiring a fear response to unrelated static stimuli and sensitization (Antoniadis & McDonald, 1999). In our discriminative task all paired sessions are done in a separate room (shock room), while all unpaired sessions and testing sessions (freezing and preference) are done in a safe room. This is to show that any learned association demonstrated in testing is to the context itself and not the greater training room.

In fear conditioning, freezing or avoidance behaviour is indicative of discriminative conditioning as it shows that the animal learned an association between an event and an outcome. A problem persists though in experiments utilizing contextual conditioning tasks as little is known about what contextual features are guiding associative learning in these paradigms and what the nature of these representations might be. For example, certain experiments utilizing non-discriminative fear conditioning paradigms provide no information about which elements of the local and greater context influence conditioning (Antoniadis & McDonald, 1999). Discriminative fear conditioning paradigms can be explicitly designed to evaluate what types of cues and related representations may be influencing learned behaviour (Antoniadis & McDonald, 1999). Recent work from our lab has shown that altering the training procedure or presenting a less ambiguous discrimination was found to be HPC-independent (Unpublished data from our lab). Building from the initial research, I conceited to modify the paradigm into a high ambiguity model, increasing the difficulty of the discrimination (high feature overlap), and thereby possibly necessitating HPC involvement. To do this, we designed highly ambiguous discriminative fear conditioning to context tasks and examined performance in normal rats, and rats with lesions to the HPC.

Research Question

A fundamental component of cognition involves the ability to recall past experiences and make decisions based on previous outcomes. Complex relational representations that support episodic memories allow access to crucial information for the selection of appropriate behaviour in ambiguous situations. The hippocampus (HPC) is known for its participation in the rapid acquisition, storage, and retrieval of complex relational and contextual representations linked with events, cues, and actions experienced (Hirsh, 1974; O'Keefe & Nadel, 1978; Sutherland et al., 1989; Antoniadis & McDonald, 2000). Cues and contexts can hold individual meaning when associated with different outcomes such as a stimulus being reinforced in one context, but non-reinforced in another (Good & Honey, 1991). Contexts or situations defined by common elements make it difficult to separate the meaning of these similar experiences. For example, each day you park your vehicle in the same parking lot, there are consistent overlapping features as well as continually changing details which together create an ambiguous situation. Normally you would return to this lot around the same time each day offering an analogous contextual setting; a similar amount of cars, the same levels of illumination, vehicles with similar physical properties, shape or colours. In addition, the parking lot is constantly changing in terms of distribution of vehicles as a whole, as well as the individual vehicles located in the immediate vicinity of yours. Each day, when returning to the lot you must separate out the overlapping features while attending to the elements which are in flux. The task being more easily achieved on certain days with favorable conditions (i.e., a holiday with less vehicles present), or more challenging given numerous external or internal conditions (i.e., graduation day with more than the normal amount of vehicles, or the end of a hard work day with fatigue setting in).

Highly similar contexts with overlapping cues or features can result in the disruption of appropriate adaptive behaviour because of a misinterpretation of the context meaning. When experiencing highly ambiguous situations like these, detailed representations formed in the HPC can be used to discriminate between overlapping cues or contexts (O'Reilly & Rudy, 2001; Lee et al., 2015). Evidence suggests that the HPC is involved in this fundamental function of decreasing interference by separating events, cues, and contexts into distinct non-overlapping or orthogonalized representations (Leutgeb, et al., 2004; O'Reilly & Rudy, 2001). For my research described in this thesis, I developed various context paradigms for rats in which the cues defining the context are identical except for one. Specifically, multiple context paradigms were developed in which the unique cues included visual, olfactory, tactile, and geometric shape. Following development of these paradigms, we investigated the effects of neurotoxic lesions of the entire HPC completed before training (anterograde lesions). Anterograde lesions of the HPC were predicted to produce impairments in the discrimination of the ambiguous contexts with many overlapping features.

Learning and Memory

Background

The acquisition and storage of information based on experiences is a fundamental component to the guidance and execution of normal behaviour (Tolman, 1932; Tulving, 1972; McDonald & Hong, 2000). This is accomplished through the processing of a continuous flow of information from the external and internal environment of the individual to produce situation-appropriate responses. The ability to recall past experiences and make decisions based on previous outcomes is an essential part of survival, one aspect of memory often described as episodic memory (Tulving, 1972; 2002; O'Keefe & Nadel, 1978). Episodic memory is an adaptive process in which organisms encode unique environmental characteristics and features, including contextual cues, for later retrieval. This can occur even in instances when only subsets of cues or features are presented, referred to as pattern completion (O'Reilly & McClelland, 1994; Tulving; 2002; O'Reilly & Rudy, 2001). Episodic memory is composed of information tied to spatial, temporal, and contextual cues (Tulving, 1972; O'Keefe & Nadel, 1978), which is bound together through coincident representations of stimulus elements to be recalled from the presentation of full or partial input cues (O'Reilly & Rudy, 2001; Rudy, 2009). Evidence suggests that multimodal sensory elements of an episodic experience are connected through complex relational associations made by the HPC (O'Keefe & Nadel, 1978).

The HPC processes and encodes spatial contextual information (Moser & Moser, 1998; Pothuizen, et al., 2004; McDonald, et al., 2006), with increased activation in HPC neurons during exposure to unique or novel environments (McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995). Place cells within the HPC are responsible for the formation and storage of the representation of conjunctive relationships among cues in an environment (Gruber & McDonald, 2012; O'Keefe and Speakman, 1987; McNaughton et al., 1983). HPC damage or disruption produces impairments in learning specific spatial and contextual learning tasks for example, the Morris Water Task (MWT) or Radial Arm Maze (RAM) task (Morris, 1982; Sutherland, et al., 1982; Olton, et al., 1979; Packard, et al., 1989). There are many different variations to the classic MWT however a primary component involves animals learning to swim in a large circular pool to a platform (visible or submerged under the water) by using distal visual cues to navigate towards the goal (Sutherland, et al., 1983; Bast et al., 2009). Early MWT research highlighted the importance of hippocampal input with damage to hippocampal areas (i.e., dentate gyrus, CA3, etc.) producing impairments in escape latencies, swim heading, and platform location (Sutherland, et al., 1983). RAM consists of an elevated platform with 4-8 outbound radial arm. Tasks involve animals learning to associate cues in relation to the arms with positive or negative stimuli (McDonald & White, 1993). Common RAM tasks implement either win-stay or win-shift strategies to task demands. Essentially in a win-stay task, animals must return to arms with previously reinforced stimuli to receive a reward; whereas win-shift tasks require the animal to visit previously non-reinforced stimuli to receive the reward. Early RAM research investigating the involvement of multiple memory systems (HPC, amygdala, and dorsal striatum) in three different tasks found that rats with HPC damage were impaired in a win-shift version of the task (McDonald & White, 1993).

HPC dysfunctions via permanent damage or temporary inactivation cause impairments in discrimination between or retrieval of context-specific environmental cues, implicating HPC contributions to context-specific episodic memory (Good & Honey, 1991; Antoniadis & McDonald, 2000; Phillips & Ledoux, 1992; Wiltgen, et al., 2010). In addition to its role in spatial and context learning and memory, the HPC is integral for the ability to make discriminations based on configurations of stimuli (Eichenbaum et al., 1988; Rudy & Sutherland, 1989). An early paper from Rudy & Sutherland (1989) observed that the HPC formation was needed for the acquisition and retention of configural representations of compound stimuli but was not necessary for simple discriminations of individual elements. Another early demonstration of the interplay of HPC involvement in complex and simple configural discriminations showed HPC dependence in high but not low ambiguity configural tasks (McDonald, et al., 1994). Here, it was found that radial arm maze discriminations based on adjacent arms were dependent on the HPC, but not separated arms (McDonald, et al., 1994). This suggests that tasks with high ambiguity caused by higher levels of cue overlap necessitate HPC involvement. These early findings provided an important theoretical frame to design the experiments detailed in this thesis; titrating ambiguity and examining HPC participation.

The HPC plays a fundamental role in providing detailed representations for the separation of ambiguous cues encompassing spatial, temporal, and contextual information (Tulving, 1972; O'Keefe & Nadel, 1975; Moser & Moser, 1998; O'Reilly & Rudy, 2001; Hirsh, 1980; Sutherland & McDonald, 1990). Increasing cue ambiguity or amount of overlapping stimuli effectively increases the level of interference and potentially the necessity of hippocampal involvement. Pattern separation, or orthogonalization, is characterized by creating distinct, non-redundant representations in an attempt to reduce the likelihood of interference during retrieval (O'Reilly & McClelland, 1994; Rudy, 2001; Yassa & Stark, 2011). Interference, in part, is compensated for by orthogonalization or pattern separation through HPC circuits (McDonald & White, 1995; Gruber & McDonald, 2012). The complexity of associations necessary for correct distinctions in highly ambiguous situations is thought to be extremely difficult to achieve by independent structures outside of the HPC (Rudy & Sutherland, 1989; Antoniadis & McDonald, 2000; O'Reilly & Rudy, 2001; McDonald & Hong, 2013). A better understanding of the complexity of contextual conditioning and the specific role of the hippocampus may be achieved by increasing the level of cue overlap of the discrimination.

Hippocampal Connections

Through connections with the perirhinal and entorhinal cortices (EC), the HPC receives sensory input from all cortical sensory association areas, which in turn are sent back to the neocortex by HPC efferents through the subiculum and EC (Amaral & Witter, 1995, Amaral & Lavenex, 2007). Sensory information is encoded throughout the septal and temporal extent of the HPC in a distributed manner (O'Reilly & McClelland, 1994; Save, et al., 2000; Bast, et al., 2009). The septal and temporal poles of the HPC possess distinct neuronal connectivity patterns, with the former connected with brain regions associated with navigational and exploration cognitive processes (Moser & Moser 1998; McDonald et al., 2006), and the latter mainly contributing to context-specific inhibitory associations (McDonald, et al. 2001). The temporal pole of the HPC has also been associated in emotional reactions (Maren, 1999; Fanselow & Dong, 2010). Perirhinal projections are more extensive to the temporal pole of the HPC (ventral HPC, or VHPC), conveying information about individual objects or items (Lavenex & Amaral, 2000; Cenquizca & Swanson, 2007). Among the previously detailed connectivity, the VHPC also projects to the medial prefrontal cortex, amygdala, and ventral striatum, suggestive of a role in contextual specificity, latent and conditioned inhibition which has been confirmed empirically (Honey & Good, 1993; McDonald, et al., 2001, 2006; Voorn et al., 2004). Latent and conditioned inhibition are types of learning in which an animal stops attending to any stimuli that have never been associated with reinforcement. Conditioned inhibition occurs when an animal is trained to discriminate between a reinforced cue and a non-reinforced cue, with an excitatory response being accrued to the reinforced stimulus and a conditioned inhibitory response towards the non-reinforced stimulus (McDonald et al., 2001; McDonald et al., 2006). Early research into contextual learning and inhibition processes found that not only did specific learned associations transfer from one context to another (Hall & Honey, 1989), but also that HPC lesions produced impairments in the ability to use contextual cues from two different contexts to retrieve associative information (Good & Honey, 1991). In this seminal work, it was shown that habituation of unconditioned responses to stimuli presented in one context were intact when the stimuli was presented in another context; but also that latent inhibition was attenuated when the pre-exposure to the context and training sessions occurred in different contexts (Hall & Honey, 1989). The VHPC has also been found necessary for modulating the suppression of responding to irrelevant cues in a context dependent manner as damage to the VHPC disrupted contextual specificity of conditioned inhibition (McDonald, et al., 2001, 2006).

Postrhinal projections are greater to the septal pole of the HPC (dorsal HPC), conveying spatial and contextual information (Lavenex & Amaral, 2000; Cenquizca & Swanson, 2007; Agster & Burwell, 2013). The anterior and posterior cingulate cortices, which have a potential role in using spatial memories to navigate, also receive a large input from the dorsal HPC (DHPC) and neocortical projections (Sutherland, et al., 1989). As mentioned previously, the DHPC is a critical in encoding spatial information and necessary for spatial navigation, aided by the mass of spatially tuned cells (i.e., place cells, head direction cells, etc.) located in the DHPC and its projected output areas (Moser, et al., 1993; O'Keefe and Speakman, 1987; McNaughton et al., 1983). Although the distinction is not complete as VHPC has been found to participate in spatial abilities, though not as efficiently as dorsal HPC (Ferbinteanu & McDonald, 2000). Also of note is how the DHPC and VHPC contribute differently to fear conditioning tasks with the DHPC involved in fear conditioning to context, whereas the VHPC participation is needed for both fear conditioning to context as well as to explicit cues (Maren, et al., 1999; Bast, et al., 2001; Gruber & McDonald, 2013). It is important to understand the interactions within the HPC as a whole, as well as its segregated domains, to better infer about proper HPC function. The experiments in this thesis examine how HPC dependent tasks are performed with and without a functioning HPC, while also providing a paradigm to examine HPC subregions.

Anterograde and Retrograde Amnesia

Specific memory tasks utilizing varying types of relational information, (such as spatial, temporal or contextual), have been shown dependent on proper HPC function for accurate encoding, processing, or storage of information needed for task demands (Tulving, 1972; O'Keefe & Nadel, 1975; Moser & Moser, 1998; O'Reilly & Rudy, 2001; Hirsh, 1980; Sutherland & McDonald, 1990). When HPC function is compromised due to damage or inactivation, impairments in encoding new information or fractured storage of previously learned information can result. Retrograde amnesia (RA) occurs when memories are lost due to damage or inactivation of the neural structure dependent on storage (Gervais, et al., 2014; Phillips & Ledoux, 1992; Good & Honey, 1991), which has been observed in humans and non-human animals with HPC damage (Scoville & Milner, 1957; McDonald, et al., 2007). Anterograde amnesia (AA) affects the learning of new information after neural damage, showing essential participation of the damaged neural structure in the encoding or processing of specific types of information (McDonald, et al., 2007 & 2010; Clark, et al., 2002; Rudy, et al., 1998). For example, tasks such as object context pairing (Mumby, et al., 2002), paired association learning (Tse, et al., 2007), and hidden platform version of the water task (Sutherland, et al., 1982) all produce profound AA following HPC damage. These tasks are dependent on HPC input for accurate encoding or processing of information, with the absence of HPC input producing AA without compensation through alternate learning and memory systems. It is thought that the AA in these tasks is due to the inability to pair details and associations into a unified conjunctive representation. The HPC contributes essential contextual representations pairing details and associations into a unified conjunctive representation, and the loss of HPC participation results in an inability to form complex relational representations (Sutherland & Rudy, 1989).

Both retrograde and anterograde amnesia for certain types of memory following HPC damage are observed in animal models, but some memory tasks show a marked difference demonstrating one type of amnesia and not the other (Sutherland, et al., 2001; Lehmann, et al., 2009; Driscoll, et al., 2005). This may occur through compensation from multiple memory systems (McDonald, et al., 2004).

There are a number of memory tasks that display immense RA without AA, including fear potentiated startle reflex (Lehmann et al., 2010), learned fear to discrete tone (Sutherland et al., 2008), contextual fear conditioning (Maren et al., 1997), visible beacon memory (Clark et al., 2007), discrimination memory (Broadbent et al., 2007) and object recognition (Gaskin et al., 2003) indicating that profound RA but not AA implies that the damaged or inactivated HPC has an essential role in the primary storage. Non-HPC memory systems, such as the amygdala or striatum, can compensate and contribute different forms of learned behaviour in the absence of HPC input (McDonald & White, 1993). Learning early on in memory task training is often dominated by HPC input as it inhibits other systems immediate involvement (White, et al., 2013). Specific non-discriminative fear conditioning tasks are among those that, in the absence of HPC input, alternate memory systems can acquire representations of the task with adequate training (Lehmann, et al., 2009)., In order to confirm whether the HPC is involved in the encoding or storage of specific types of information, it is necessary to understand how a task is performed with a normally functioning HPC, as well as a damaged or inactivated HPC before training. It is clear that tasks with high feature overlap seem to be more dependent on HPC involvement and learning tasks involving configural representations of cues or context discriminations are an ideal platform for examining these interactions (McDonald, et al., 1994, 1997; Rudy & Sutherland, 1989). Context discrimination tasks with high feature overlap are thought to be particularly sensitive to HPC involvement during the initial encoding and processing training sessions as proper separation of cues is essential for learning. It is expected then that removal of HPC participation before training occurs would result in impairments on these types of tasks resulting in AA. Pattern separation presents a possible solution as orthongonalizing overlapping context and cue elements would be essential for proper context discrimination.

The use of context details becomes more difficult as cue elements degrade, are absent, or as different contextual features overlap between separate experiences. Pattern separation, or orthogonalization, is characterized by creating distinct, non-redundant representations in an attempt to reduce the likelihood of interference during retrieval (O'Reilly & McClelland, 1994; Rudy, 2001; Yassa & Stark, 2011). On a computational level, orthogonalization occurs through competitive sparse neural activity input through participating structures to generating distinct representations with a low probability of overlap (Leutgeb & Leutgeb, 2007; Leutgeb, et al., 2005; Hunsaker & Kesner, 2013). Pattern separation and/or completion processes may be the underlying computation allowing a subject to discriminate ambiguously defined contexts or experiences. Pattern completion occurs when a subset of cues from a previous experience can activate (retrieve) the stored pattern representing that experience (Marr, 1971; McNaughton & Morris, 1987; O'Reilly & McClelland, 1994; O'Reilly & Rudy, 2001). If a similar context contains only partial or degraded cues from a prior experience, the original input pattern is recalled, making a generalization of the expected outcome. The anatomical organization mediating information flow between the HPC and cortex via the EC which projects to the dentate gyrus and Cornu Ammonis 3 and 1(CA3 and CA1 respectively) subregions of the HPC for further processing reflects this process (O'Reilly & Rudy, 2001; Hunsaker & Kesner, 2013). This circuit is also auto associative, with dentate cells projecting to CA3, and CA3 cells back projecting onto themselves (Amaral, et al., 1990; Leutgeb, et al., 2007). Through the auto associative circuit small changes in input to the network of cells can allow for the retrieval of similar patterns that have already been stored (Leutgeb, et al., 2007). A competitive network processing incoming information produces sparse and distinct patterns along with CA3 unique contributions and rapid automatic encoding (Leutgeb & Leutgeb, 2007; Yassa & Stark, 2011). Reducing overlapping patterns with distinct ensembles alleviates interference among representations and allows for more accurate discrimination. It seems possible then that discrimination of highly ambiguous contexts with overlapping features may rely on pattern separation processes and associated HPC participation.

Current model

The production of appropriate behaviours is strongly influenced by the complex relational associations formed in certain learning and memory related neural structures. Highly ambiguous contexts or situations become increasingly difficult to interpret when cue overlap produces representational interference and potentially subsequent behavioural patterns. HPC contributions are probably required in order to separate or orthogonalize context and cue representations during encoding, as a means to decrease interference between potentially conflicting representations formed in different neural structures.

Context discriminations with high cue overlap places a high demand on pattern separation/completion processes in order to separate out distinct features or to connect partial details back to a previously experienced context. For example, being able to recall a previous experience (ie, an unsafe context), from a subset of cues present in a new experience, could result in more cautious behaviour or treating the context as unsafe. Using a discriminative conditioning task also avoids many confounds common to non-discriminative paradigms such as being influenced by the greater global context (ie, generalized fear to the training room). Also advantageous of discriminative conditioning is the inclusion of multiple measures of fear such as passive and active behavioural responses (freezing and avoidance, respectively). A well designed context discrimination paradigm will provide essential data on the effects of specific types of cognitive processing on behavioural output of complex relational information, and the potential role of the HPC in these processes.

Chapter 2

Discriminative Fear Conditioning to Context with High Feature Overlap

Introduction

Discriminating contexts with overlapping cues can be difficult. For example, cue or contextual ambiguity occurs with similarity or overlap among features included in situations with different outcomes (in Context A, a white square with a banana scent paired with the presentation of a tone= shock; in Context B, a white square with a eucalyptus scent paired with the presentation of a tone= no shock). Therefore, the meaning of a cue can be dependent on its relationship to other cues, expressed individually or in conjunction with at least one other cue (in which both contexts consist of white squares but; tone+banana=shock, tone +eucalyptus= no shock). The information acquired through contextual cues becomes essential to develop appropriate responses, especially in situations where many overlapping or common cues may be present in situations with different outcomes.

The effects of context ambiguity on learning and memory processes are not well understood. A common confound associated with many context conditioning paradigms is a lack of accounting for conditioning to contextual cues that are part of the larger training context but are not part of the experimentally manipulated context environment. For example, during fear conditioning to context experiments, other cues are also associated with the aversive event including the researcher, transport to and from the apparatus, housing and differences in training/testing rooms or apparatus. This non-chamber conditioning is a particular problem with non-discriminative paradigms because it can lead to animals to develop generalized fear or sensitization to a nonessential element of the task. With these phenomena the expression of fear is generalized to other cues in addition to those that were associated with the initial fearful experience. Experiments with loosely defined environments or paradigm parameters may neglect which elements are being utilized by the experimental subject, and how neural systems implicated in learning and memory processes may participate to influence behavioural output. The two contexts utilized in a contextual discrimination task that consist of many common cues but are different on one cue dimension may elucidate how cues are represented and utilized in these types of learning situations. Another potential issue arises in animal behavioural tasks that utilize cues with different sensory modalities. Olfactory, visual, spatial, geometrical and tactile cues are often used interchangeably as stimuli in learning and memory experiments, even though the ability of animals to use each sensory modality may be influenced by species specific bias (Muller & Kubie, 1987; Jeffery et al., 2007; Lee, et al., 2014). Designing experiments that specifically take into account the influence of task variables may further our understanding of how rodents use different sensory cues while learning context discriminations, as well as understanding how certain contextual elements are represented in the brain.

The specific aims of the following experiment include*:* 1) assessing the ability of rats to acquire a discriminative fear conditioning to task with medium feature overlap as a basis for comparison 2) assessing the ability of normal rats to acquire ambiguous context discriminations when the unique cue is visual, olfactory, or geometric shape. For aim 2, we hypothesized that the ambiguous context discrimination task with a unique olfactory cue would be the easiest to resolve given the sensory representation in the brain and the behavioural reliance of rats on olfactory information.

Methods & Design

Animals and handling

All animal housing, surgical and behavioural procedures were approved by the University of Lethbridge Animal Welfare committee and complied with guidelines set by the Canadian Council on Animal Care guidelines. Long-Evans male rats (N=43) weighing approximately 300-350g were used for the entirety of the study. Upon arrival, animals were housed in pairs in standard Plexiglas cages in a vivarium maintained at a 12 h light/dark cycle (lights on at 7:30am) with *ad libitum* access to food and water. All animals were then given one week to acclimatize before behavioural training or surgical procedures were performed. After the acclimation period, all rats were handled for five minutes each day by the experimenter for 4-7 days prior to experimental training.

Discriminative Fear Conditioning to Context (DFCTC) Training and Testing

Pre-Exposure

On the first day of conditioning, subjects were brought individually to the testing room to undergo pre-exposure. Each subject was placed in the middle alleyway connecting the two contexts and given free access to explore for 10min. Prior to training and based on pre-exposure data, rats were counterbalanced and assigned to their paired and unpaired contexts, to ensure that any preference displayed during testing is a result of the context-stimulus pairing and not from any initial pre-exposure biases. In addition to this, rats were counterbalanced such that half of the rats were exposed to the paired context on the first day of training and the other half the rats were exposed to the unpaired context on the first day of training. Cage mate pairs always received exposure to the paired or unpaired context on the same training day to avoid any instances of bystander stress transference in the home cage.

Training

For all training and testing; rats were brought individually from their home cages in a clear Plexiglas transport tub. During training, the animals are confined for 5 minutes to either the paired (shock) or unpaired (no shock) context. In the paired context, animals received three 0.6mA footshocks at the 2-, 3-, and 4-minute mark of the training session. Throughout training, defecation and urination were recorded as measures of unconditioned and conditioned fear. Training continued for 8 days, with 4 paired and 4 unpaired trials for each animal. Based on the counterbalanced schedule, training days alternated between either paired/unpaired or unpaired/paired for each subject.

Testing

Freezing - After 8 training days, subjects received 2 days of testing in which no shocks were given, and freezing behaviour was recorded for each subject in the paired and unpaired contexts. An animal was considered freezing when there was a complete lack of motion, except for breathing and vibrissae whisking. All freezing sessions were scored live and video recorded for verification.

Preference – Twenty-four hours after the second freezing session test, subjects were once again placed into the middle alleyway and given access to both contexts. This preference test provides an active demonstration of a learned association through avoidance behaviour of the paired context. Dwell time in each context was used to assess the overall group's context preference (paired or unpaired) after the training regime. Each subject's preference test was scored live as well as video recorded.

DFCTC – Apparatus

Experiment 1.1 Medium Ambiguity DFCTC

In the medium ambiguity DFCTC task, two contexts were different on three dimensions: shape, colour, and odour. As the basis for all the context manipulations to follow, we thought it prudent to include a group tested on what we will call the Standard DFCTC. All apparati were constructed at the University of Lethbridge. Both context chambers were made of acrylic Plexiglas walls and lid, with grid floors of stainless steel rod measuring approximately 0.32cm in diameter. A square prism chamber (41 cm long x 41 cm wide x 29 cm high) made with white acrylic is connected by a grey middle alley (16.5 cm x 11 cm x 11cm), to a triangle prism chamber (61 cm long x 61 cm wide x 30 cm high) made with black acrylic. Features common to both contexts included an identical grid floor, clear transparent lids, pill bottle scent cues, and the same acrylic Plexiglas material walls. The alley way allowed movement between the contexts during pre-exposure and preference tests. A pill bottle with air holes situated in the top corner of each chamber provided the olfactory cue through the application of either banana (amyl-acetate) or eucalyptus (Vicks Vapo-Rub) into the inside of the bottle. The banana scent cue was always paired in the black triangle chamber, and the eucalyptus scent cue was always associated with the white square chamber to prevent overlap of scents between contexts. The apparatus was situated in the center of the room atop a transparent table, with an angled mirror (45°) underneath for visualization by the experimenter. A video camera placed 2 feet in front of the mirror allowed the experimenter to record testing and training days. All training and testing was conducted within the same designated experiment rooms, and performed by the same experimenter. There was a designated shock room in which all paired trials were held, and a no-shock room in which all unpaired trials occurred. All testing occurred in the no-shock room to demonstrate that any learned associations are specific to the context, and not to external cues such as the training room.

Highly Ambiguous DFCTC

The apparatus for all training consisted of contexts that were either black or white, square or triangle prisms, with either eucalyptus or amyl acetate scent cues. Different combinations of the colour, shape or odour were used for different behavioural tasks. For the experiments aimed at studying visual cue and olfactory discrimination (Experiments: 1.2, 1.3, 2.1, 2.2) two identical white square prisms were connected by a middle alley allowing free access during pre-exposure and preference testing. Shape discrimination training utilized one black square and one black triangle prisms connected by the middle alley. Each chamber had a grid floor made of stainless steel rods. A pill bottle with air holes situated in the top corner of each chamber provided the olfactory cue through the application of either banana (amyl-acetate) or eucalyptus (Vicks Vapo-Rub) to the inside of the bottle. The apparatus sat in the center of the room atop a transparent table, with an angled mirror (45°) underneath for visualization by the experimenter. A video camera placed 2 feet in front of the mirror allowed the experimenter to video tape testing and training days. All training and testing was conducted within the same designated experiment rooms, and performed by the same experimenter (for each individual group). There was a designated shock room in which all paired trials were held, and a no-shock room in which all unpaired trials occurred. All testing is to occur in no-shock room to demonstrate that any learned associations are specific to the context, and not to external cues such as the training room. Specific details on the apparatus variants will follow below.

Experiment 1.2, 2.1- Visual Cue Discrimination - The two white square chambers were connected by the middle alley for the task designed for visual cue discrimination. The fundamental component to this task was the specific visual cue present in each of the two identical contexts; a solid black triangle (19.4cm x 19.4cm x 19.4cm) for one, and a zebra striped square (16.4cm x 16.4cm) for the other (See Figure 1). Visual cues were cut from a sheet of removable contact paper, and proportionally sized in relation to the context chamber wall. Each visual cue was placed inside the context chamber in the center of the inner northern wall, relative to chamber position. Eucalyptus scent cue was present in both of the context chambers, increasing the overlap and thereby ambiguity of the task. Scent cues were refreshed before each day's training/testing and stored in an odour-concealed container in a separate room. The common/overlapping elements to the visual cue task included: the shape, colour and scent of the contexts as well as the experimenter, time of day, transportation, and training and testing procedures. The only distinct and unique features to the contexts and procedure were the visual cues present in the subject's designated paired and unpaired contexts.

Experiments 1.3, 2.2- *Olfactory Cue Discrimination* - For this experiment, two previously unused white square chambers were used to prevent the presence of any lingering scent cues from past experiments influence learning and/or behaviour. The two scent cues used were eucalyptus and banana as both have been previously shown to be particularly effective in discrimination tasks. Scent cues were refreshed before each day's training/testing, and stored in an odour-concealed container in a separate room. After handling each scent cue, the researcher changed both gloves and gown so that no lingering scents were present during the subjects' individual handling and training/testing. Inside each of the two contexts a solid black triangle visual cue (19.4cm x 19.4cm x 19.4cm) was situated in the center of the northern wall. The common/overlapping elements to the olfactory discrimination task included: shape, colour and visual cue of the context as well as the experimenter, time of day, transportation, and training and testing procedures (Figure 2). The only distinct/unique feature to the task was the particular scent cue present in each of the two contexts.

Geometric Shape Discrimination

Experiment 1.4 - Square vs Triangle - An alley for this shape discrimination task connected one black square chamber and one black triangle chamber. The zebra striped square visual cue was fixed to the center of the northern wall, relative to the inside of the chambers. The salient banana scent cue was present in both of the context chambers and refreshed daily before training or testing. The common/overlapping elements to this task included: the colour, visual cue, and scent within the context, as well as the experimenter, time of day, transportation, and training and testing procedures. The only distinct/unique feature to the geometric shape discrimination task will be the particular shape of each of the two contexts (square, or triangle; see Figure 3).

Experiment 1.5 and 2.3 - Square vs Octagon – An additional shape discrimination task was included to provide a more distinct contrast between contexts as the square and triangle contexts possessed similar visual characteristics such as wall length. An alley for this shape discrimination task connected one black square chamber and one black octagon chamber (18cm x 18cm x 32cm). The zebra striped square visual cue was fixed to the center of the northern wall, relative to the inside of the chambers. The salient banana scent cue was present in both of the context chambers and refreshed daily before training/testing. The common/overlapping elements to this task included; the colour, visual cue, and scent within the context, as well as the experimenter, time of day, transportation, and training and testing procedures. The only distinct/unique feature to the spatial geometrical discrimination task was the shape of each of the two contexts (square, or octagon, see Figure 3). A more detailed account of the specifics of each contextual task are outlined in Figure 4.

Statistics

All statistics were conducted with IBM SPSS Statistics 21 (IBM, Armonk, New York), and all graphs were made with GraphPad Prism software (GraphPad Prism 7, La Jolla, CA). All statistical tests for normal animals were two-tailed and tested at an α level of 0.05. Rats were excluded if their freezing or preference behaviour in either the paired or unpaired context was greater than two standard deviations from the mean.

For all of the ambiguous discriminative conditioning, paired t-tests were used to compare freezing/dwell time in the paired and unpaired contexts during freezing and preference tests, respectively. For the lesion experiments, mixed model ANOVAs were used to compare freezing and preference behaviours between the groups. Planned comparisons were used to assess freezing and preference performance for each group, as we had the *a priori* assumption that following training rats would show less fear and prefer the safe context.

Results

Experiment 1.1 Medium Ambiguity DFCTC

Freezing - Paired samples t-tests revealed a marginally significant effect (*t* (7) = 2.326, p=.053) for animals (N = 8) to freeze more in their respected paired context compared to the unpaired context (Figure 5).

Preference - Paired samples t-tests revealed a significant main effect of context (*t* (7) = -2.389, *p* = .048) for animals. When animals were given a choice between paired and unpaired contexts in a preference test, animals spent more time in the unpaired context compared to the paired (Figure 5).

Experiment 1.2 – Visual High Ambiguity DFCTC

Freezing - Paired samples t-tests revealed that in the Visual high ambiguity DFCTC task there was not a significant effect for animals (N = 8) to freeze more in their respected paired contexts, (*t* (7) = 2.161, *p* = .068; Figure 6).

Preference – There was not a significant effect for animals to spend more time in their respective unpaired versus paired contexts (*t* (7) = -2.052, *p* = .079; Figure 6). Rats were not able to discriminate between visual cues in context discriminations with high feature overlap in the preference test.

Experiment 1.3 – Olfactory High Ambiguity DFCTC

Freezing – There was a robust main effect of context (*t* (7) = 3.978, *p* = .005) with animals (N = 8) spending a significant amount of time freezing more in the paired context compared to the unpaired context (Figure 7). Thus, animals learned to associate the paired context with the aversive stimulus and responded with defensive freezing behaviour.

Preference – There was a not a significant effect for animals to spend more time in their respected unpaired versus paired contexts (*t* (7) = -2.060, *p* = .078), (Figure 7).

Experiment 1.4 – Shape Variant 1 (Square-Triangle) of High Ambiguity DFCTC

Freezing - A paired samples t-test indicated that animals (N = 7) did not display more freezing in their respected paired contexts, (*t* (6) = 1.146, *p* = .295; Figure 8)

Preference - Animals were unable to distinguish between contexts in a preference test (Figure 8). Paired samples t-tests (*t* (6) = -.824, *p* = .442) revealed that animals did not spend more time in their respected unpaired versus paired contexts (square or triangle).

Experiment 1.5 Shape Variant 2 (Square-Octagon) of High Ambiguity DFCTC

Freezing – A paired samples t-test indicated that animals (N = 12) did not display more freezing in their respected paired contexts (*t* (11) = 1.905, *p* = .083; (Figure 9).

Preference - Animals showed active avoidance for the paired context, while spending more time in the unpaired context (t (11) = 2.778, p = .018; Figure 9). Thus, animals were able to discriminate between ambiguous contexts distinct only by their shape (square or octagon).

Discussion

Standard DFCTC

A medium feature overlap DFCTC paradigm was altered into several high feature overlap variants designed to investigate the efficacy of context discriminations when only one feature differs between the contexts. Olfactory, visual, and geometric shape constituted the variable sensory feature contextual cues in each task. Experimental results indicated that the specific sensory feature used was the key feature determining normal rat's ability to process ambiguous contextual information under these training conditions. It was found that normal rats could accurately distinguish high ambiguity context discriminations based on olfactory and visual information, and context shape (square vs octagon). Rats were unable to complete discriminations of distinct contexts shaped as square or triangle.

Visual High Ambiguity DFCTC Behaviour

Experiment 1.2 was designed to examine whether rats could discriminate between highly ambiguous contexts distinct only by an explicit visual cue within the context. It was found that animals were unable to discriminate the visual cues associated with their respected paired/unpaired contexts. It is possible that the visual cues do not provide salient enough information to stand out clearly among the many other overlapping sensory features. Rats may preferentially process to more species specific sensory information, such as olfactory or tactile cues. We believe that increasing the training schedule and sample size would produce a more robust significant effect in the highly ambiguous visual task.

Olfactory High Ambiguity DFCTC Behaviour

Of the different sensory cue features, unsurprisingly, unique olfactory information embedded in a context consisting of overlapping cues allowed the rats to show discriminative behaviour. It is surprising that normal rats were able to exhibit a robust effect in conditioned freezing, but not in preference. The performance on the preference test does not appear to be from an inability to discriminate between the cues, as they accurately did so in freezing, but it is possible that this may be the result of a particular design feature of the apparatus. The results and implications of the olfactory discrimination will be discussed in more detail in the general discussion.

Geometric Shape-High Ambiguity DFCTC Behaviour

An interesting finding emerged from the two versions of the shape tasks. In shape variant 1, rats were unable to discriminate between contexts differing only in shape, either square or triangle (Experiment 1.4). However, in shape variant 2 rats learned the correct association and were able to discriminate between the square and octagon, with a significant effect in preference testing (Experiment 1.5). It is surprising that rats were unable to distinguish between the square and triangle as an assortment of previous data have found effective place cell coding of spatial location for context chambers of various geometric shapes (McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995; Leutgeb, et al., 2004, 2007). Rat place cells may be encoding and mapping to the spatial geometry of an environment however the cognitive information may not be translated into a behavioural response in challenging highly ambiguous discriminations. A question also arises from the finding that rats were able to correctly perform in the preference test but not freezing. Including two behavioural measures allows researchers to examine both passive and active responses in the freezing and preference tests, respectively. The freezing tests consist of two sessions with individual exposure to the two contexts. This is a challenging task as animals must recall the relational information of not just the context that they are currently in, but also the other context they have experienced, and associate the correct context with the paired stimuli. The preference test allows animals to have a direct exposure of both contexts, possibly making for a more easily accessible discrimination. Therefore, the variability in the freezing and preference test results for the normal rats in the shape discrimination (square vs octagon), may be that the discriminative freezing is difficult in contexts with high feature overlap.

Chapter 3

Hippocampal Lesions

If the HPC is responsible for the separation or orthogonalization of similar experiences it makes sense to assume that detailed relational representations via hippocampal circuits may decrease susceptibility to interference in certain types of context discriminations through the formation of complex configural associations (Sutherland & Rudy, 1989; Cohen & Eichenbaum, 1993). Increasing the level of cue ambiguity is thought to recruit the HPC as a means to form independent configural associations between stimuli that are not possible through non-hippocampal memory systems. A task designed with high cue ambiguity should be sensitive to HPC dysfunction, possibly impairing subsequent acquisition and encoding of relational information. ***Specific aims: investigate the effects of pre-training HPC lesions in the different (olfactory, visual, geometric shape) ambiguous context discriminations.***

We predict that intact rats should be able to adequately distinguish between environments with high feature overlap, but in the absence of hippocampal function, performance should be impaired in initial acquisition. However, I note that it is possible that complete removal of the HPC prior to highly ambiguous DFCTC task could produce either; enhanced performance, impaired performance, or no effect to the task. A brief explanation of these alternative predictions can be found below.

Enhanced Performance

Enhanced task performance would be caused by the elimination of competitive interactions between the HPC and other areas of the brain implicated in learning and memory processes. The absence of hippocampal input could allow for heightened attention towards or processing of specific details of the task and the disregard of overlapping cues.

Impaired Performance

Impaired performance, I would infer be caused by eliminations in encoding, consolidation of information processing of the HPC. Without the detailed relational representations dependent on an intact HPC, tasks with high ambiguity should be increasingly difficult to discriminate between.

No Effect to Performance

If No effect of hippocampal lesions was found in these experiments I would infer was the result of non-hippocampal learning and memory systems providing an alternative solution to the task utilizing information in a different way. Non-hippocampal memory systems can support the formation of context memories when exposed to extensive training after hippocampal inactivation or damage (White & McDonald, 2002). Whether these memory systems would be able to form detailed context representations in a highly ambiguous situation without essential encoding and orthogonalization from the HPC is possible but highly unlikely based on current evidence.

Methods and Design

Animals and handling

All animal housing, surgical and behavioural procedures were approved by the University of Lethbridge Animal Welfare committee and complied with guidelines set by the Canadian Council on Animal Care guidelines. Long-Evans male rats (82) weighing approximately 300-350g were used for the entirety of the study. Upon arrival, animals were housed in pairs in standard Plexiglas cages in a vivarium maintained at a 12 h light/dark cycle (lights on at 7:30am) with *ad libitum* access to food and water. All animals were then given one week to acclimatize before behavioural training or surgical procedures were performed. After the acclimation period, all rats were handled for five minutes each day by the experimenter for 4-7 days prior to experimental training.

Highly Ambiguous DFCTC

All training and testing procedures, as well as training apparati were identical to those previously mentioned in Chapter 2.

Lesion Experiment Training and Testing

For the experiments examining hippocampal involvement in a highly ambiguous DFCTC task, subjects were separated into 2 groups. The lesion groups received complete hippocampal lesions prior to all training and testing in each of the discriminative conditioning variants. The second group received sham surgeries. The training and testing to follow were the same as previously detailed. These experiments were designed to explore hippocampal involvement in the acquisition of these DFCTC tasks

Surgeries

For the olfactory and visual tasks (Experiment 2.1, 2.2), rats were divided into groups: complete hippocampal lesion (N = 12) and sham controls (N = 12). The rats used in the geometric shape discrimination task (Experiment 2.3) were divided into complete hippocampal lesion (N = 9) and sham controls (N = 9). Rats were anesthetized with isoflurane anaesthesia (4% with 2 L/min of oxygen for induction and 2% after surgical plane was established); as well as with intraperitoneal (i.p.) injections of phenobarbital (65mg/kg body weight). The hair from the top of the head was removed with an electric shaver, and the scalp was sterilized with alcohol and betadine. During surgery, an ocular lubricant gel was applied to the animals' eyes for protection. Animals were placed into a stereotaxic apparatus for all lesions. Neurotoxic lesions of the complete: dorsal (AP: -3.1, -3.1, -4.1; L: ±(1.0, 2.0, 2.0); V:-3.6, -3.6, -4.0), intermediate (AP: -4.1, -5.0, -5.0; L: ±(3.5, 3.0, 5.0); V:-4.0, -4.1, -5.0), and ventral hippocampus (AP: -5.0, -5.8, -5.8, -5.8; L: ±(5.2, 4.4, 5.1, 5.1); V:-7.3, -4.4, -6.2, -7.5) were produced by injecting a 5 mg/mL solution of N-methyl-D-asparate in pH-balanced phosphate buffer, through a 30-gauge cannula attached to a Harvard mini-pump (McDonald et al., 2006). Injections were infused at a rate of 0.2μL/min for approximately 1 min and 20s, with cannulae left in the brain tissue for a 2min diffusion period. Diazepam (0.1mL i.p.) was administered following the surgery to prevent the development of seizures in hippocampal lesioned animals (McDonald et al., 2006). A second diazepam injection was utilized if any lingering post-surgical animal seizure-like behaviour (i.e., rigid intense posture and shaking, curled limbs, clenched teeth, etc.) emerged within 15 minutes following the initial injection.

Following surgery, each animal was monitored until awake and active. Animals were held in the surgical suite overnight for monitoring and then returned to their home cages the following day. All animals were kept in a separate cage overnight before being returned to their cage mate as the lesions resulted in initial hyperactivity and sensitivity to others. Animals were given 1 week to recover from surgery before training.

Histology

After behavioural training, rats were administered a lethal dose of sodium pentobarbitol (euthansol) and then perfused transcardially with a mixture of buffered physiological saline (1% PBS) followed by 4% paraformaldehyde (PFA). Removed brains were post-fixed in a solution of 30% sucrose in 4% PFA after 24hrs. Brains were frozen on a cryostat at -21°C, and sectioned coronally at 40-µm thickness. Sections were mounted and stained in Cresyl violet (0.1%) to determine cell loss and gliosis resulting from the lesions.

Lesion Quantification

The extent of complete HPC (DG, CA1, and CA3) damage volumes were calculated using the Cavalieri method (Schmitz & Hof, 2005) via Stereo Investigators ® (MicroBrightField Inc., 2013, Version 10). A grid size 120µm was used with a Zeiss AxioImager M1 microscope (Zeiss, Jena, Germany). Every twelfth section was counted with an average of 16 sections assessed for each animal. In each section, the total number of points in contact with HPC tissue were counted. The number of points per section were then analysed by multiplying the area associated with each point, section thickness (40µm), and the section sampling fraction. Percent of HPC damage in each lesion animal was calculated by dividing the quantified spared tissue volume by the average HPC volume of the control group, multiplied by

100. One-samples t-tests were used to compare the lesion sizes of each group to threshold.

Results

Experiment 2.1 Visual High Ambiguity DFCTC with HPC Lesion

Freezing – Repeated measures ANOVA showed that there was a main effect of context (*F* (1, 21) = 5.616, *p* = .027), but there was no significant effect of group (*F* (1, 21) = .1.595, *p* = .220), nor was there a significant interaction (*F* (1, 21) = .020, *p* = .889). Planned comparisons indicated that the freezing times were similar in both the paired and unpaired contexts for the HPC Lesion (N = 12), shown in Figure 10A. There appeared to be a trend for Sham control animals (N = 11) to freeze more in their respected paired contexts (Figure 10A).

Preference - There was no significant effect of context (*F* (1, 21) = .178, *p* = .678), nor was there a significant interaction (*F* (1, 21) = .332, *p* = .571) determined by a repeated measures ANOVA. There was a marginally significant group effect (*F* (1, 21) = 4.061, *p* = .057) with Sham control rats showing a stronger preference for unpaired context. Planned comparisons indicated that the HPC Lesion rats did not show a preference for either context, as depicted in Figure 10B.

Experiment 2.2 Olfactory High Ambiguity DFCTC with HPC Lesion

Freezing – Repeated measures ANOVA showed that there was a main effect of context (*F* (1, 22) = 7.867, *p* = .010), but no significant effect of group (*F* (1, 22) = 2.005, *p* = .171) or a significant interaction (*F* (1, 22) = 1.595, *p* = .750) was observed. Planned comparisons indicated that Sham control (n=12) group froze more in the paired context, but not HPC Lesion (n=12) (Figure 11A).

Preference - There was no effect of context (*F* (1, 2) = 543, *p* = .469), no effect of group (*F* (1, 22) = .867, *p* = .362), nor was there a significant interaction effect (*F* (1, 22) = 334, *p* = .569). Planned comparisons indicated that neither group showed a preference of context, as depicted in Figure 11B.

Experiment 2.3 Geometric Shape High Ambiguity DFCTC with HPC Lesion

Freezing – There was no effect of context (*F* (1, 16) = 4.155, *p* = .058), no effect of group (*F* (1, 16) = .507, *p* = .487), and there was not significant interaction (*F* (1, 16) = .029, *p* = .868). Planned comparisons indicated that freezing times did not differ in both the paired and unpaired contexts for the HPC Lesion (N = 9) and Sham control (N = 9) groups as determined by a repeated measures ANOVA (Figure 12A). Therefore, neither the HPC lesion nor sham controls could discriminate between their paired/unpaired contexts in the freezing test.

Preference – There was a main effect of context (*F* (1, 16) = 20.171, *p* < .0001), no effect in group (*F* (1, 16) = 1.234, *p* = .283), and also there was not a significant interaction (*F* (1, 16) = 1.220, *p* = .286). Planned comparisons indicated that HPC Lesion and Sham control rats avoided the paired context during the preference test (Figure 12B). Both groups were able to correctly identify the cue (shape) signalling the aversive stimuli, and showed a preference for the unpaired context.

Lesion Quantification

The NMDA injections produced extensive cell loss in all of the principal subfields (CA1-CA3) of the HPC and the DG. Unbiased stereology estimated 81.52% of the HPC was damaged (SD= 4.15881; Min= 72.89; Max=87.1) for animals in the visual high ambiguity DFCT; 77.4% of the HPC was damaged (SD= 3.69; Min= 72.1; Max=82.91) for animal in the olfactory high ambiguity DFCTC; and 74.9% of the HPC was damaged (SD= 4.14; Min= 70.0; Max=80.1) for animals in the geometric shape high ambiguity DFCTC. There were no animals estimated below 70% HPC damage, therefore no animals were removed from analysis. A One-Way ANOVA indicated that there was a difference between lesion size in groups (F (2, 11) = 7.430, p = .002). Each groups lesion size was also compared to the threshold HPC damage of 70% and one-samples t-tests indicated that every group was significantly different from the threshold. Therefore, all lesion groups were above the necessary criterion to be included. No noticeable damage was found in surrounding regions (i.e., amygdala, thalamus, etc.).

Discussion

When rats with HPC lesions were trained in tasks with high feature overlap, different effects were revealed based on the distinct cue used. In the olfactory high feature overlap DFCTC task, intact animals were able to discriminate between olfactory cues with many other overlapping features present whereas animals with HPC lesions were impaired. It was also found that animals were unable to discriminate visual cues in the highly ambiguous DFCTC task. Animals with HPC lesions displayed poor performance in the visual cue highly ambiguous DFCTC with animals showing almost equal numbers in both fear measures, indicating that they could not discriminate between contexts. Surprisingly, animals with HPC lesions were not impaired at discriminating geometric shapes in contexts with high feature overlap. Intact animals in the geometric shape task were also able to perform the task, although not as efficiently as the HPC lesion animals. In the following paragraphs, I will describe these findings and possible explanations briefly, with more detail to follow in the general discussion.

Visual High Ambiguity DFCTC with HPC Lesions

Similar to normal rat data for the Visual high ambiguity DFCTC task, the sham group were unable to discriminate between contexts; HPC lesion rats could not discriminate between visual cues under conditions with high feature overlap (Experiment 2.1). The inability for the groups to show this form of context conditioning is unlikely due to visual acuity in rats as previous research has shown that rats can make visual discriminations (Fenton, et al., 2000; Kim, et al., 2012). The data suggest that the poor performance in the Visual high ambiguity DFCTC task of HPC lesion is not due to visual acuity but possibly an inability to efficiently separate overlapping features in the ambiguous task. In regards to the non-significant trend in effect size in the sham controls, it is likely that this would be alleviated with an increase in both training sessions and sample size. This is supported by the consistently close trend for normal rats to discriminate the visual cues in contexts with overlapping features. The visual high ambiguity task will be discussed in further detail in the general discussion.

Olfactory High Ambiguity DFCTC with HPC Lesions

Data obtained from the Olfactory high ambiguity DFCTC task revealed that complete HPC lesions impaired performance in both testing measures, whereas rats in the sham group showed the correct learned association discriminating between contexts in the freezing test (Experiment 2.2). Thus, the HPC appears to be more important for making context discrimination with many overlapping cues but unique olfactory cues. It is possible that the impairment in HPC lesioned rats is due to HPC processing of complex configural representations and discriminations, and not olfactory information processing as the rodent brain has a wide dispersion of olfactory representation outside of the HPC (Fortin et al., 2002; Kesner, et al., 2002). This is unlikely however as partial lesions in a VHPC group also produced impairment in the preliminary data for the Olfactory high ambiguity DFCTC task (unpublished data from our lab). The VHPC shares a bidirectional projection directly with the olfactory bulb and other primary olfactory cortices (Cenquiza and Swanson, 2007; Fanselow & Dong, 2010), and VHPC lesions impair olfactory dependent memory tasks (Fortin, et al., 2002; Hunsaker, et al., 2008). It is possible that damage to the VHPC eliminates the flow of olfactory information into the HPC, preventing hippocampal encoding and processing. This is also supported by increased anatomical projections from the dorsolateral band of EC, which primarily carries olfactory info from olfactory and perirhinal cortices, to the VHPC as compared to the DHPC (Steffenach, et al., 2004). The data finding that both full HPC and VHPC (data not shown) lesions impair highly ambiguous olfactory discriminations suggests that the HPC has a fundamental role in the processing and disambiguation of olfactory information embedded within other overlapping contextual cues.

Geometric Shape High Ambiguity DFCTC with HPC Lesions

The HPC lesion group in the Shape high ambiguity DFCTC task produced some surprising results (Experiment 2.3). Contrary to a mass of HPC spatial research showing deficits in spatial abilities with HPC dysfunction/inactivation (Moser & Moser, 1998; Pothuizen, et al., 2004; McDonald, et al., 2006; McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995), the HPC lesion group was not impaired. Both HPC lesion and sham groups were able to discriminate between contexts in the preference test, with HPC lesion animals showing an enhancement compared to controls. It is possible that HPC inactivation lessened its inhibition of other neural structures, such as retrosplenial cortex or posterior parietal for example, allowing spatial geometric cues to become more salient (Burwell & Amaral, 1998; Vann, et al., 2009). Or perhaps absent HPC output resulted in reduced interference from overlapping cues, leading to an enhanced ability to focus on the distinct cue. However, without essential HPC input providing representations of spatial contextual information or place cell spatial mapping to support complex relational representations, spatially defined context discriminations should not be expected (Moser & Moser, 1998; Pothuizen, et al., 2004; McDonald, et al., 2006; McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995; Gruber & McDonald, 2012). The effects of lesions and relationship to contexts with high feature overlap will be analyzed further in the general discussion.

Chapter 4

Pattern Completion

Introduction

Environmental context and individual cue elements have direct influences on how an organism learns to produce situation appropriate behaviours. Contextual cues can be combined into configural representations encapsulating the context as a whole or hold individual meaning when presented in situations with different outcomes. Previous research has explored these ideas using configural tasks such as negative pattern discriminations. Training on these operant conditioning tasks consists of the presentation of a tone that if the rat presses a lever will be reinforced with food. On other trials a light is presented and lever pressing is also reinforced. A third type of trials also occurs on this task in which the compound of the tone and light are presented. On these trials the compound is not reinforced. This is considered a configural task because for the rat to solve the problem they must learn to treat the elements and the compound differently even though the compound consists of the reinforced elements. The idea is that the HPC creates a unique configural cue of the compound that will have a different reinforcement history than the elements that are represented elsewhere in the brain. (Sutherland & McDonald, 1990; Sutherland et al., 1989). However, processing and interpretation of the context details becomes more difficult as cue elements become degraded or absent, or conversely as different contextual features begin to overlap between experiences. Pattern separation and/or completion processes may underlie the computations necessary for discriminations of ambiguously defined contexts and experiences.

Pattern separation, or orthogonalization, is characterized by creating distinct, non-redundant representations in an attempt to reduce the likelihood of interference during retrieval (O'Reilly & McClelland, 1994; Rudy, 2001; Yassa & Stark, 2011). Pattern completion occurs when a subset of cues from a previous experience can retrieve the stored pattern representing that experience (Marr, 1971; McNaughton & Morris, 1987; O'Reilly & McClelland, 1994; O'Reilly and Rudy, 2001). If a similar context contains only partial or degraded cues from a prior experience, the original input pattern is referred back to make a generalization of what to expect next.

Research from the Honey lab have recently examined how animals represent patterns of stimulation and the formation on links between elements into configural representations (Iordanova, et al., 2011; Lin, et al., 2016). Their experiments were designed with the theory that future encounters involving the presentation of any one element, could be resolved by retrieving the entire previously experienced pattern sequence. To test this, rats were exposed to contexts with compound configurations of elements consisting of; time of day exposure (day or night), visual (checkered or dotted context walls), and auditory (clicks or tones) stimuli. Rats received exposure to a variation of combinations in the pre-exposure, training, and testing sessions. For example, in a configural memory experiment rats were pre-exposed to context AX (dotted context with presentation of an auditory tone) and context BY (checkered context with presentation of auditory clicks) in the morning; followed by exposure to context AY (dotted context with clicks), and BX (checkered context with tone) in the afternoon (Iordanova, et al., 2011). The midday training session removed the visual component (no checkered or dotted walls), and featured pairing the auditory stimuli with an aversive footshock (i.e., tone paired with shock, clicks unpaired) (Iordanova, et al., 2011). This was followed by the critical testing session measuring freezing, whereby rats were again placed in the contexts in morning and afternoon trials with the visual stimuli (checkered or dotted walls), but without the presentation of the auditory stimuli. It was found that pre-exposure to specific stimuli facilitated learning with more time freezing in the contexts that were previously combined with the auditory stimuli that was paired with the footshock. That is, presentation of the visual cue alone (which was previously presented with the auditory stimuli in pre-exposure) provoked marked freezing in the rats although the visual cue was never actually paired with the aversive footshock. This suggests that the pre-exposed patterns had been encoded configurally and the test patterns retrieved a configural memory (Iordanova, et al., 2011). Their research goes on to find that damage to the HPC before training impairs rats ability to perform these complex configural memory tasks (Iordanova, et al., 2011; Lin, et al., 2016). The findings from the Honey lab provide an important link between research examining configural memory, the HPC, and pattern completion processes; an area of research which is currently still solidifying.

By definition, pattern separation and completion are separate points on a spectrum, with separation occurring at the initial encoding phase and completion occurring at retrieval. However, this explanation is an over simplification, as elements of initial encoding and later retrieval should be required for both processes. Pattern completion may occur during retrieval processes, but the initial encoding of the input pattern is essential for the establishment of representations to which a comparison can be made. Conversely with pattern separation, operationally orthogonalizing features before encoding helps prevent overlap and interference at the onset of exposure but would still need to be accessed during later discriminations. Pattern separation and completion processes facilitate difficult discriminations, forming complete representations from a degraded input extract distinct details from overpopulated input.

Evidence for pattern separation and completion are supported by a variety of methodologies; including electrophysiology and computational models (Hebb, 1949; Marr, 1971; McNaughton & Morris, 1987; Rolls, 1989; McClelland, McNaughton, & O'Reiller, 1995; Leutgeb, et al., 2007; Brandon, et al., 2014; Mizumori 2004, 2007; Knierim & Neunuebel, 2016), immediate early genes (Guzowski et al., 2004; Vazdarjanova & Guzowski, 2004; Pevzner, et al., 2012; Arias et al., 2015), and also neuroimaging with human subjects (Pidgeon & Morcom, 2016; Yassa & Stark, 2011). Much of the pattern separation and completion research using behavioural models seem misguided in translating computational pattern terminology into a functional test and subsequent behavioural correlate. In other words, many behavioural models misinterpret the terminology and design paradigms which neglect key components to pattern completion and separation operations (Gold and Kenser, 2005; Hunsaker, et al., 2008; Langston & Wood; Morris, et al., 2013; Kesner, et al., 2016). In my view, behavioural models of pattern completion need to establish an initially feature rich environment which becomes degraded over training or only present partial features during testing in order for pattern completion processes to occur.

Here, we designed a behavioural task and model to be representative of the computational framework of pattern separation and completion. Pattern completion terminology emphasizes the retrieval of a stored pattern representation from a degraded or partial subset of cues. The pattern completion procedure was defined with an initial overlap of several different sensory modality cues, which gradually decreased in exposure during training and testing. Three different sensory modalities (visual, tactile, and olfactory) were used in combination for each version of the pattern completion task. The '3-2-1' label details the amount of cues available for the pre-exposure, training, and testing, respectively (detailed in Figure 13 and Figure 14). Pre-exposure included all three sensory modality cues to establish the training environment with many distinct features. The training pairings had two of the sensory cues, with the specific cue of interest absent. The preference test had only a single sensory cue available to the rat which was the cue of interest that had been removed from the training. The initial exposure session should be the critical period for sensory information to be encoded into the brain to establish a configural representation strong enough to be called upon during a retrieval session with only a subset of the original cues available. We aimed to develop a new paradigm designed as a behaviourally appropriate model for assessing actual pattern separation/completion processes. A pattern completion task should by definition involve an initially feature rich experience which becomes degraded over training sessions to only a subset of original cues available during the retrieval test.

We hypothesize that providing a minimal amount of contextual cues during a retrieval test should constitute a challenging discrimination, only achievable by recalling the previous configural representation, via pattern completion processes. Each variant should be challenging although the pattern completion task using olfactory information for its retrieval cue should be the most salient for rats providing a robust and salient input representation to be referred back to, given the results reported in Chapter 1.

Methods & Design

Animals and handling

All animal housing and behavioural procedures were approved by the University of Lethbridge Animal Welfare committee and complied with guidelines set by the Canadian Council on Animal Care guidelines. Long-Evans male rats (N=27) weighing approximately 300-350g were used for the entirety of the study. Upon arrival, animals were housed in pairs in standard clear Plexiglas cages in a vivarium maintained at a 12 h light/dark cycle (lights on at 7:30am) with *ad libitum* access to food and water. All animals were given one week after arrival to acclimatize before behavioural training or surgical procedures were performed. After one week to acclimate, all rats were handled for five minutes each day by the experimenter for 5 days. Animals were given a single cookie (Chips Ahoy, Nabisco) during each handling session to serve as pre-exposure to the food reward before training. Prior to behavioural training, animals were food deprived to 90% their *ad libitum* weight. For the duration of the experiment, animals were weighed every day to ensure that there were no drastic or hazardous decreases in weight. All rats increased weight throughout the food deprivation period.

Apparatus

Four identically sized square prism chambers (41 cm long x 41 cm wide x 29 cm high) served as apparatus for the pattern completion task. Two of the square chambers were made from black acrylic Plexiglas, and the other two square chambers were made with white acrylic Plexiglas. Of the two black chambers, one was associated with the banana (amyl-acetate) olfactory scent cue, whereas the other black chamber was constructed new without ever having scent cue contact. One white chamber was associated with the eucalyptus (Vicks Vapo-Rub) scent cue, whereas the other white chamber was also constructed new without ever having scent cue contact. A grey middle alley was connected to allow free access between two chambers during pre-exposure and preference tests. All context chambers had a grid floor made from stainless steel rod (0.32cm in diameter). A sheet of fluorescent light covers (Clear plastic polycarbonate lighting diffusers, Home Depot, CA.) were sized to the bottom interior of the contexts and were fitted under the grid floor to serve as a tactile texture cue. Tactile inserts had two distinct textures which could be flipped over for each variant. One side of the tactile insert was studded and rough, while the other side was sanded smooth. Special context chamber lids were created to allow the attachment of infrared cameras (4 HD 4MP Dome Infrared Cameras) that were connected to a nearby computer programmed with recording software (Swann™ Advanced Security Systems8-channel NVR with 2TB HDD and SwannView Plus, 2014). All testing was video recorded on the computer and used for scoring and analysis. Armasight Night Vision Goggles (Ninox Gen 2) were used to aid the researcher during dark training or testing sessions.

Pre-Exposure

In the pre-exposure phase, the two contexts were distinct in three modalities; visual, tactile, and olfactory. In the visual and olfactory versions of the task; Context A was a white square chamber, with a smooth tactile floor mat and a eucalyptus scent cue. Context B had a black square chamber, with a rough spiked floor mat and a banana scent cue. The tactile version differed included counter balancing such that half of the rats were exposed to the smooth mats with the white square context and rough with the black triangle context, while the other half to the rough mats with white square context and the smooth mats with black triangle context. All three sensory modality cues were present during the pre-exposure sessions. In the olfactory version, for example, animals experienced two distinct contexts differing in three sensory modalities; visual, tactile, and olfactory. To ensure optimal exposure to the full gauntlet of cues, animals were given two 20min pre-exposure sessions on Day 1 and Day 2 of training. During pre-exposure and subsequent behavioural training, animals were brought in pairs via transport containers on a trolley cart, to the designated training and testing room. All animals remained in the room on the transport cart during training and testing to create a relatively controlled, enclosed environment. Individually, animals were placed in the middle alley and allowed to freely explore between the two contexts. Prior to training and based on pre-exposure data, rats were counterbalanced and assigned to their paired and unpaired contexts, as was described previously (Chapter 1). One constant was that cage mate pairs always received exposure to the paired or unpaired context on the same training day to avoid any instances of bystander stress or exposure to the food reward scent in the home cage.

Training

For the duration of training, the available sensory cues were reduced to two, as the particular sensory cue of interest was omitted from training. In the olfactory pattern completion task, there were no olfactory cues present during training. In the visual pattern completion task, training was done in complete darkness to eliminate any visual cues. Animals were placed individually in either their paired or unpaired context for 30min sessions in which they are allowed to freely explore the closed context. In the paired context, animals encountered a full sized chocolate chip cookie, weighing approximately 10grams. Any remaining cookie not consumed in the training session was weighed and recorded. In the unpaired context, there were no food reward present. Training persisted for 8 days, containing 4 paired and 4 unpaired trials for each animal. Based on the counter balanced schedule, training days alternated between either paired/unpaired, or unpaired/paired for each subject. After being returned to the home cage, animals were not fed for approximately 2hrs, in an attempt to distinguish the food reward in the paired sessions from normal feeding.

Preference Test

Twenty-four hours after the last training session, available sensory cues were reduced; the only cue present was that which was absent during training. In the olfactory pattern completion task, for example, animals were tested in the dark and with tactile mats taken out. The only cue available was the olfactory cue previously omitted from training. Context A had only the eucalyptus scent cue present, with no visual or tactile cues available. Context B had only the banana scent cue present, with no visual or tactile cues available. Test subjects were once again placed into the middle alley way and given open access to both contexts. Dwell time in each context was used to assess the overall group’s context preference (paired or unpaired) after the training regime. Preference tests were video recorded for scoring and reference. In order to eliminate the visual component to the Olfactory and Tactile 3-2-1 preference test, all animals were tested in the dark.

Statistics

All statistics were conducted with IBM SPSS Statistics 21 (IBM, Armonk, New York), and all graphs were made with GraphPad Prism software (GraphPad Prism 7, La Jolla, CA). All statistical tests were two-tailed and tested at a significance of $\alpha = .05$. For all of the pattern completion tasks, paired t-tests were used to compare dwell time in the paired and unpaired contexts during preference tests.

Results

Experiment 3.1 - Olfactory 3-2-1 Pattern Completion

There was a significant main effect of context (t (5) = 2.746, p = .041). Figure 15 shows that during a preference test, animals (N = 6) had significantly longer dwell times in their respected paired contexts. This suggests that rats were able to recall the initial pre-exposure session with all three cues available, solely from the presentation of the olfactory cue and compare it to the training sessions which had the olfactory cue absent.

Experiment 3.2 - Visual 3-2-1 Pattern Completion

There was no effect of context (t (7) = .817, p = .441). Figure 16 shows the total dwell times for animals (N = 8) during the preference test, revealing no differences. Rats were unable to use the single visual cue present in testing to recall the initial input pattern from pre-exposure.

Experiment 3.3 - Tactile 3-2-1 Pattern Completion

There was no effect of context (*t* (7) = -.425, *p* = .684). Figure 17 shows the total dwell times for animals (N = 8) during the preference test. Tactile cues did not provide adequate information for rats to recall the pre-exposure input pattern from a degraded subset of cues.

Discussion

In order to establish an appropriate behavioural measure for pattern separation/ completion processes, experiments must be designed to reduce the amount of relevant cues to a testing phase consisting of only a partial subset of cues from the original exposure. Results showed that the 3-2-1 pattern completion task proved exceptionally challenging to naïve rats, as they were unable to accurately discriminate contexts in both visual and tactile paradigms. It is unlikely that poor performance is due to rats inability to use the different sensory modalities as research has shown rats can reliably be trained on olfactory, visual, and tactile pattern discriminations (Weeden, et al., 2014; Kim, et al., 2012; Grion, et al., 2016). The olfactory based version of the 3-2-1 pattern completion task was the only one which produced a significant effect in a preference test. It is unsurprising given the immense representation of olfactory information in the rodent brain that the olfactory variant of the task would provide the best behavioural performance (Cenquiza and Swanson, 2007; Fanselow, 2010).

The 3-2-1 pattern completion procedure was designed to challenge rats' abilities to recall previously experienced stimuli from a degraded availability of contextual information. Correct discrimination between contexts in this task would only be achievable through pattern completion processes. Essentially, animals are exposed to cues ABC, trained on AB, and tested on C. Therefore, any gained preference to a cue in the testing phase must be connected back to the pre-exposure as the cue present in testing was never paired with the positive stimuli. We believe that the underlying computation to this discrimination is pattern separation/completion processes dependent on the HPC.

Caveats

There are other considerations to the task particulars which may have affected performance in each variant of the experiment. It is possible that compared to olfactory information, visual and tactile are not as salient stimuli or well represented in memory systems providing a less reliable representation for recall. Another possibility is that the sample sizes were not large enough or the there was not enough pairing session to induct an effect in this appetitive task. To keep the experiment as an appetitive task it would benefit future research to increase both sample size and paired sessions. Changing the reinforcer stimulus from appetitive to aversive (ex. Shock) may also support better conditioning as it has previously been shown that conditioned responses to an appetitive reinforcer was diminished between different contexts; whereas an aversive reinforcer was not (Hall & Honey, 1989). Pattern separation/completion research will be discussed in further detail in the general discussion.

Chapter 5

General Discussion

Summary of Findings

The experiments in this thesis were designed to understand how rats learn and remember highly ambiguous context discriminations with a single distinct sensory cue among many overlapping features. Pilot experiments indicated that the key feature determining a normal rat's ability to process and discriminate between ambiguous contextual information was dependent on the sensory modality of the unique cue. Normal rats learned about the significance of different contexts when they were defined by olfactory but not visual information. High ambiguity geometric shape discriminations produced different results based on the specific shapes used. When the distinct shape cues used were a square and triangle, rats were unable to discriminate between contexts. However, when a square and octagon were used, rats accurately discriminated between contexts in a preference test. The results suggest that a major determinant of normal animal behaviour in tasks that utilize sensory stimuli for context or cue discriminations is the sensory modality implemented and the innate relevance to the animal. When examining HPC involvement in these tasks it was found that rats with HPC lesions were impaired in the olfactory task and no effect in the visual high ambiguity task. Surprisingly, rats with HPC lesions were enhanced in the shape high ambiguity DFCTC (square vs octagon). For the purposes of the general discussion, I will provide a brief background revisiting the previously discussed literature detailing contextual conditioning and HPC involvement; and the results and interpretations of each of the experiments outlined in the thesis.

Context Conditioning and the HPC

Research in rodent behaviour has long relied on sensory stimuli to serve as cues to differentiate contexts or to signal task demands (for example,:Rudy and Sutherland, 1995; Eichenbaum, et al., 1998; Antoniadis & McDonald, 1999; Lenck-Santini, et al., 2005; Fenton, et al., 2000; White & McDonald, 2002; Kim, et al., 2012; Pothuizen, et al., 2004). Rodents can efficiently learn, utilize, manipulate and discriminate between different sensory modalities, including olfactory (Eichenbaum, et al., 1998;1999; Lenck-Santini, et al., 2005; Fortin, et al., 2002; Otto & Poon, 2006; Hunsaker, et al., 2008; Law & Smith, 2012), visual (Fenton, et al., 2000; Lenck, et al., 2005; Potvin, et al., 2009; Kim, et al., 2012; Lee, et al., 2014), geometric/spatial (McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995; Ferbinteanu & McDonald, 2001; Jeffery, et al., 2007) and tactile (Lee, et al., 2014; Grion, et al., 2016) stimuli. Certain associations learned in one context can be transferred to other, new and different contexts (Hall & Honey, 1989). Rodents can form and store configural representations into conjunctive relationships among the cues in an environment (McNaughton et al., 1983; Gruber & McDonald, 2012; O'Keefe and Speakman, 1987); binding together various contextual cues into a unified representation allows for more accurate recall during ambiguous situations. The present data suggests that although rodents discriminated between different sensory modalities, there were innate biases, which influenced the ability to recognize and utilize different sensory stimuli in contexts with overlapping features.

The HPC has been identified as an important system for the discrimination between or retrieval of context-specific environmental cues, implicating HPC input in context-specific episodic memory (Good & Honey, 1991; Antoniadis & McDonald, 2000; Phillips & Ledoux, 1992; Wiltgen, et al., 2010). Specific memory tasks utilizing varying types of relational information, (such as spatial, temporal or contextual), have been shown dependent on proper HPC function for accurate encoding, processing, or storage of information required for task demands (Tulving, 1972; O'Keefe & Nadel, 1975; Moser & Moser, 1998; O'Reilly & Rudy, 2001; Hirsh, 1980; Sutherland & McDonald, 1990). In addition to its role in spatial associations, the HPC is integral for the ability to make discriminations based on configurations of stimuli (as reviewed in Gruber & McDonald, 2012; Rudy & Sutherland, 1989). The HPC seems critical for forming contextual representations pairing details and associations into a unified conjunctive representation and the loss of HPC participation results in an inability to form complex relational representations (Sutherland & Rudy, 1989).

There are however some exceptions to HPC dependence in contextual conditioning tasks, as seen in specific non-discriminative fear conditioning which delegate alternate memory systems in the absence of HPC input (Lehmann, et al., 2009). However, contextual discriminations in the absence of the hippocampus only occur in the anterograde direction and require extended training. Although some non-discriminative fear conditioning tasks defined by unambiguous details or simple configurations possess information that can be acquired by non-hippocampal memory systems, without necessitating hippocampal involvement as needed in complex configural tasks (Sutherland & Rudy, 1989; McDonald, et al., 1997).

Similarly, the HPC is associated with the formation of internal context representations and goal oriented behavioural output in tasks involving motivation and memory (Kennedy & Shapiro, 2004; Kennedy, et al., 2009). In Kennedy's (2009) experiment, researchers examined the influence of motivation states (ie thirst or hunger) on both HPC dependent context memory retrieval. It was found that for HPC-independent random foraging distinct CA1 representations responded to motivational states when they signaled memory discriminations but not for foraging. Therefore, the HPC combined internal motivations with external cues to guide behaviour based on the representation of the whole context formed by the HPC. Thus, the HPC is critically involved in encoding and storing conjunctive representations related to varying forms of relational information.

The HPC creates conjunctive representations to join individual features or contextual elements together and by connecting the relations of these features through configural processes. Configural representations allow limited or degraded information to be predictive of outcomes based on a schema. For example, in an experiment designed to limit the amount of visuospatial information available, olfactory cues compensated and contributed to place cell firing (Save et al., 2000). Thus, place cells selectively fire in relation to external and internal cues, which support multiple forms of sensory information contributing to place field stability (Save et al., 2000).

Another valuable aspect to distinguishing environmental features involves processing temporal components of events, which has also been associated with HPC function. For example, in a sequential ordering task, designed to examine the ability to distinguish between overlapping olfactory cues over varying time frames, rats were presented with food wells of varying scent cues and selected the previously presented scent to receive a food reward. An impairment was observed when HPC damage was combined with a temporal delay in the presentation of cues (Fortin et al., 2002), highlighting the critical role of the HPC in temporal ordering and disambiguation of overlapping cues. Likewise, HPC dysfunction impaired performance in pattern separation of both overlapping and unique distal visual cues (Potvin, et al., 2009); as rats with DHPC lesions were impaired at a RAM task discriminating between adjacent arms featuring matching distal visual cues and separate arms with distinct cues. The experiments in this thesis were designed to examine HPC involvement in context discriminations with high feature overlap, under the hypothesis that complex contextual representations would be HPC dependent. Rats with complete HPC lesions were tested in olfactory, visual, and geometric variants of the highly ambiguous DFCTC task and it was found that HPC involvement varied in some interesting ways.

Visual-High Ambiguity DFCTC

Normal and intact rats were unable to discriminate high ambiguity contexts that were the same except for distinct visual cues inside the contexts. A possible reason for the visual discrimination no effect could be that among the plethora of overlapping sensory stimuli, visual information takes a backseat to sensory information more relevant to rodents (ex. Olfactory cues) interfering with their ability to resolve the discrimination. Another possibility is that the training schedule was not extensive enough for animals to learn the relationship between the visual cue and the aversive stimulus. An extended training regiment may allow more experience/exposure with the visual cues, aiding in the ability for rats to recognize the importance in signalling the paired aversive stimulus. Increasing the sample size of the animals included in this experiment would be another method that would likely produce a significant effect. Changing the aversive stimulus to appetitive may also allow for a better discrimination as it may encourage exploration with reduced risk to choosing the wrong context.

In the visual discrimination, HPC lesion rats were similar to sham and normal rats, showing no inclination of the discrimination between visual cues in contexts with high feature overlap. It is unlikely that this is due to poor visual acuity in rats as similar visual discriminations have been shown effective behaviourally (Fenton, et al., 2000; Sharp, et al., 1996; Lenck, et al., 2005; Potvin, et al., 2009; Kim, et al., 2012; Lee, et al., 2014). Electrophysiology research has shown that place field ensemble activity reacts to changes in the position and orientation of cue cards (Fenton, et al., 2000). This suggests that visual cue information is being encoded by place cells, however in the current high ambiguity discrimination, this information may not be sufficient to produce an appropriate behavioural response. On the other hand, olfactory and spatial cues may be more salient to rats, possibly being more easily accessible for cognitive processing. The visual discrimination may be more easily resolved in these kinds of ambiguous context discriminations with less other salient stimuli also present. Another possibility is that the visual cue was simply not big enough relative to context chamber walls. The visual cues placement was centered relative to context walls however, roughly 50% of the wall was still visible. In future experiments it may aid to have the visual cue constitute an entire wall or if an identical visual cue was present on all internal walls. A main interpretation of the visual high ambiguity DFCTC impairment is that the challenging context discrimination with many overlapping features was to difficult to resolve for normal rats and rats with HPC lesions. The absence of enhanced performance in HPC lesion rats in the visual high ambiguity DFCTC also suggests that the HPC is not inhibiting other participating memory systems.

Olfactory-High Ambiguity DFCTC

Complex olfactory discriminations were found to be dependent on HPC function. There is a clear bias for olfactory information supported anatomically by the extensive representation throughout the rodent brain (Fortin, et al., 2002; Hunsaker, et al., 2008). The olfactory task (Experiment 1.3) showed that normal rats were able to utilize only olfactory information and make context discriminations amidst other overlapping sensory elements. There was an unexpected finding in which normal rats and sham controls (in the HPC lesion group) more accurately discriminated between the contexts in the freezing tests, compared to the preference. I believe that the reason for this is due to limitations in apparatus design, and not an inability to discriminate between unique olfactory cues. During the freezing test, animals are isolated to the individual contexts, with a single olfactory cue presented amongst the many other overlapping cues. The finding that normal animals and sham controls distinguished each olfactory cue in the freezing tests shows an ability to associate the respected paired or unpaired contexts, and olfactory cues which define them, with the aversive stimulus. It is possible that during the preference test, as the contexts are connected by an open alley way, that the scent cues mix causing interference in the rats ability to separate each cue. A human observer may not see the effect of crossover between olfactory cues, but to an animal that has proven to focus a great deal of attention to olfactory information (Experiments 1.3, 2.2, Unpublished data) it could have interfered with their ability to express learned behaviour. Support for this theory can be found in the other experiments in this thesis where rats demonstrated a preference without any conflicting olfactory information (Experiment 1.5, 2.2). Further research is required to assess these ideas.

Complete and partial HPC lesion data also emphasize the sensitivity of the olfactory task as it was the variant with the best performance (for normal rats), and was impaired by only partial HPC dysfunction (data not shown). Other HPC dependent behaviour tasks have found that rats with partial HPC lesions (DHPC) were unimpaired in the MWT with sparing little as 26% of HPC (Moser, et al., 1995). Another study examining HPC sub regions found that incomplete lesions of the HPC isolated to DHPC, intermediate HPC, or VHPC resulted in minimal impairments in a spatial navigation task (Jarrard, et al., 2012). An effect of lesion was only seen with complete HPC lesions (Jarrard, et al., 2012), suggesting that even spared sub regions of the HPC can efficiently contribute to a task otherwise thought HPC dependent. Of the entirety of the HPC, the VHPC receives the main input from olfactory information systems (Eichenbaum, et al., 1998;1999; Lenck-Santini, et al., 2005; Fortin, et al., 2002; Hunsaker, et al., 2008). It is possible that VHPC inactivation caused an absence of olfactory information input to the HPC, resulting in an inability to properly process olfactory stimuli. Also as complete and partial HPC lesions did not lead to an enhancement or null effect in olfactory discriminations, it can be concluded that the HPC is both an active participant in the processing olfactory information, but also that the HPC is not inhibiting any other participating memory systems. The HPC provides essential processing and potentially providing a disambiguation function supporting olfactory context discriminations with an excess of overlapping sensory features (Experiments 2.2, data not shown).

Shape-High Ambiguity DFCTC

An interesting contrast emerged when manipulating the geometric shape in highly ambiguous context discriminations. When the distinct geometric feature was a square or triangle (Chapter 2, Experiment 1.4) rats were unable to form the correct associations necessary for context discrimination. However, when the distinct geometric shapes differed by square or octagon shapes (Chapter 2 Experiment 1.5), the rats were able to show discriminative behaviour. One explanation for this could be the relative visual and spatial differences between square-triangle and square-octagon. Inside the context chambers, the square and triangle have similar profiles in terms of wall length, and corner position. The octagon chamber presents a very distinct local profile, with several short and prevalent corners and walls as compared to the square chamber.

Although cells in rat HPC have been shown to encode distinct geometric shapes (for example: square, rectangle and circle), it is possible that rats do not have access to translate these spatial representations into an appropriate behavioural response (Leutgeb, et al., 2007; Brandon, et al., 2014). The ability of rats to use geometric shape information for learning tasks has been debated with certain data suggesting a preferred method of favoring local matching processes and rejecting global matching of shape (Pearce et al., 2004); whereas an alternative proposes the use of global matching to constrain and guide the use of local cues (Cheng and Gallistel, 2005). In Pearce’s 2004 experiment, rats were trained to go to a hidden platform in a specific corner of a rectangular water maze, and then transferred to a water maze of different shape for testing. Navigation to the hidden platform was easier for rats when it was located in the corner that was geometrically equivalent, rather than the opposite corner where it had previously been (Pearce, et al., 2004). The experiment indicated that rats relied on local cues instead of the overall shape. Applying this logic to our findings is purely speculative conjecture as our geometric shape task with many overlapping features provokes a more challenging discrimination, although it does provide information on how rats may be attending to information in geometric based discriminations.

Spatial abilities have been directly associated with proper HPC function for many years (Morris, 1982; Sutherland, et al., 1982; McNaughton, et al., 1983; Muller and Kubie, 1987). It has also been shown that animals are able to perform spatial navigation with reference to the shape of their environment and that the ability depends on the capacity to navigate with respect to local cues (Cheng, 1986; Pearce, et al., 2001). As such, we expected that the geometric high ambiguity DFCTC would be severely impaired by HPC dysfunction. However, we were surprised to find that the opposite occurred with complete HPC lesions leading to an enhancement in spatial geometric discrimination (Experiment 2.2). How is it then that the removal of a neural structure critically linked with spatial abilities, improved performance on this version of a context discrimination.

There are certain experiments that have found varied hippocampal dependence in spatial memory when task requirements fit certain criteria, such as; egocentric based spatial representations (Eichenbaum, et al., 1990), or anterograde spatial memory involving home base navigation (Travis, et al., 2010). There are certain conditions to experiments finding HPC-independent spatial memory in that many of them feature task demands that are modified from classical spatial memory tasks. Home base navigation has been found to be HPC-independent in the anterograde but not in the retrograde direction, suggesting that the HPC is involved in the forming and storage of place memories while intact, however if HPC damage occurs before training and followed with additional training sessions afterwards, non-HPC can incrementally establish a spatial representation at a slower rate (Travis, et al., 2010; Bast et al., 2009). Researchers describing these phenomena have suggested that HPC overshadowing prevents other systems from processing spatial information, however in the absence of the HPC it is possible that a network of grid cells in EC, might be able to store sufficient spatial information to navigate some place tasks (Hargreaves et al., 2005; Knierim & Neunuebel, 2016).

As previously mentioned, there is support for specific types of memory tasks being hippocampal dependent in the retrograde but not anterograde direction (Lehmann et al., 2010; Sutherland et al., 2008; Maren et al., 1997; Clark et al., 2007; Broadbent et al., 2007; Gaskin et al., 2003). Although certain experiments have found hippocampal-independent memory varied in ways with other memory systems compensating with the absence of hippocampal processing, it was not thought possible in our tasks as hippocampal place cells are thought to be involved in the spatial map, context discrimination that is reliant on shape should not be possible. (Moser & Moser, 1998; Pothuizen, et al., 2004; McDonald, et al., 2006; McNaughton et al., 1983; Muller & Kubie, 1987; Moser, et al., 1995; Gruber & McDonald, 2012).

Of the two geometric shape-high ambiguity DFCTC variants, we decided to use the second, square vs octagon, to test HPC lesions as it was easier to resolve for HPC intact rats. The data provokes some interesting considerations to normal rats' abilities to discriminate between geometrical spatial contexts (Experiments 1.4-1.5). Previous data have shown that place cell ensembles can encode with precision multiple contexts with varying geometric properties (ie. Square, rectangle, circle) (Leutgeb, et al., 2007; Brandon, et al., 2014). Rat place cells may be encoding and mapping the spatial geometry of an environment however the cognitive information may not be translated into a behavioural response in difficult discriminations; similar to the possible reason for the visual cue impairment (Experiment 2.1) (Fenton, et al., 2000).

Normal rats were unable to correctly discriminate square vs triangle contexts, but they were able to efficiently discriminate between square vs octagon (Experiments 1.4-1.5). A possible explanation would be to examine the local profiles of these different geometric contexts from the inside. The square and triangle contexts have similar wall length and corner position; whereas the octagon provides an explicitly different environment from the square with smaller walls and considerably more prevalent and distinct corners. It is possible that rats were able to visually associate context details without necessitating spatial abilities.

In an experiment investigating the influence of the shape of the arena for a water maze task, it was found that rats gave preferential bias to local cue features over global shape (Pearce, et al., 2004). There is further support for this interpretation as a previous experiment found that non-spatial contextual cues (ie odour or colour) were able to aid in disambiguation of otherwise similar geometries leading to accurate environmental context discrimination when presented in combination, but not individually (Anderson & Jeffery, 2003). In the experiment by Anderson & Jeffery, 2003, as rats explored the environment, simultaneously recorded place cells in the DHPC did not all receive the same information about individual context changes, however a large majority responded (remapped) to a combination of the elements (odour and colour). Place cells were recorded in the DHPC as animals freely foraged for food scattered in square contexts which were manipulated with variations of colour and scent cues combinations. Researchers interpreted this finding as heterogeneous input of contextual elements being sent to the HPC for creating a configural representation of the context, thereby constructing a representation of the spatial environment (Anderson & Jeffery, 2003). This finding also highlights how different sensory modality information can influence configural representations and subsequent behavioural output. It is possible that the elimination of hippocampal input changes the saliency or interpretation/encoding of the different sensory modalities; possibility shifting reliance on other cues (ie. Scent). Only further investigation into HPC involvement in ambiguously defined context discriminations will be able to provide definitive answers. In specific regards to the results of the shape discrimination with HPC lesioned rats, future research should focus on examining HPC subregions and both medial and lateral EC inputs participation and corresponding behaviour.

Pattern Completion

The 3-2-1 pattern completion procedure was designed to challenge rats' abilities to recall previously experienced stimuli from a degraded availability of contextual information. This task was seen as an excellent exemplar of an episodic memory task (Tulving, 1972) dependent on pattern separation/completion computations mediated by the hippocampus. Certain types of information can be learned in one context, and transferred to a different context (Hall & Honey, 1989). Formative research in behavioural pattern completion has shown how animals use previously learned pattern representations in the guidance of future experiences with degraded information (Iordanova, et al., 2011; Lin et al., 2016). These experiments highlighted the importance of an information rich pre-exposure, in which a configural representation must be formed in order to correctly retrieve the initial input pattern during the test session with partial cues. It was also found that lesions to the HPC prior to training abolished pattern memory that required configural processes, but had no effect on simple elemental processes (Iordanova, et al., 2011). The behavioural pattern completion work from the Honey lab detailing the interplay between pre-exposure, training, and testing sessions was an integral component in designing our new task. The experiment was designed to feature a pre-exposure session with all the cues available, training sessions with a single cue absent, and the testing session with only a single cue from the pre-exposure available. Only the cues in the training phase are paired with the stimulus; therefore, any preference demonstrated to a cue in the testing phase is dependent on the ability to recall the cue from the pre-exposure session and associate it with the elements from the paired training sessions. Learning this difficult association is reliant on the ability to use impoverished information to recall the initial input and connect that back to the experience during training. Rats recalled the initial input pattern when olfactory information was the key feature, but not when it was visual or tactile. Olfactory information has proven to be the most effective retrieval cue of the original event. (Experiments 1.2-1.6, 2.1-2.3, 3.1-3). This shows that it is not strictly the presence of sensory information but a crucial link between the pre-exposure and the testing block. Correct discrimination between contexts in this task would only be achievable through pattern separation/completion processes.

Pattern separation and completion processes have been studied using a variety of approaches including; computational and electrophysiology models (McNaughton & Morris, 1987; Rolls, 1989; McClelland, McNaughton, & O'Reiller, 1995; Leutgeb, et al., 2007; Brandon, et al., 2014; Mizumori 2004, 2007; Knierim & Neunuebel, 2016), and IEG's (Guzowski, et al., 2004; Czernlawski & Guzowski, 2014; Vazdarjanova & Guzowski, 2004; Pevzner, et al., 2012; Arias et al., 2015), however there appears to be a disparity in how to properly examine and interpret these processes. Pattern separation/completion research can be divided into work done by computational neuroscientist that adhere to adhering to a strictly computation definition, and researchers using this computational terminology to support loosely based behavioural models and tasks (Gold & Kesner, 2005; Hunsaker, et al., 2008; Langston & Wood, 2010). Experiments which accurately define and examine pattern completion processes use a union between computational definitions and translating it into an appropriate behavioural model and task/s. In computational studies, pattern completion is very strictly defined as the ability of a network to retrieve from memory a complete pattern of activity when presented with incomplete or degraded input patterns, or more simply a network in which activity patterns can become associated with themselves (Knierim & Neunuebel, 2016). Research focused on the electrophysiology approach emphasize the auto-associative binding properties in area CA3 (Hebb 1949; Marr, 1971; McNaughton & Morris, 1987; Rolls, 1989). In this way, cell activity outputs can be made more similar than the inputs when the changes to input are due to a subset of partial or degraded elements of a representation (pattern completion), or small differences in representations of legitimately similar inputs (generalization) (Knierim & Neunuebel, 2016). Similarly, the IEG approach focuses on activity in areas such as CA3 or CA1, but in an attempt to correlate specific IEG (such as activity regulated cytoskeleton-associated protein, or ARC) with context exposure (Guzowski, et al., 2004; Pevzner, et al., 2012). Certain pattern completion studies implementing IEG are approaching the design in a way that marries the computational and behavioural elements, although they often lack any behavioural test to assess the effect of degraded cues on pattern completion processes (Vazdarjanova & Guzowski, 2004).

Many behavioural tasks designed to assess pattern separation and/or completion use spatial change detection as a measure of pattern separation or completion (Hunsaker, et al., 2008; Gold & Kesner, 2005). Spatial detection in these tasks consist of objects within a context being manipulated by varying spatial distances and animal exploration is used as a measure of behavioural pattern separation (Hunsaker, et al., 2008; Gold & Kesner, 2005). The issue with spatial detection tasks is that the majority do not involve aspects which would constitute pattern separation or completion (i.e., Retrieval of whole representation from partial or degraded cues), creating orthogonalized representations in contexts with increasingly overlapping features. Instead, these spatial detection tasks rely on an animal's ability to investigate objects that have been manipulated only by minuscule degrees without altering any local or global cue elements. The very definitions of pattern completion and separation involve either the degradation or overrepresentation of cues, while spatial detection tasks feature neither. Inferring that an animal has successfully pattern separated behaviourally by analyzing objects which have simply been moved slightly is an experimental misconception (Gold & Kesner, 2005).

A more appropriate behavioural assay of pattern separation/completion processes would include the presence of local or background contextual cues being manipulated into an overlap or absence (see Iordanova, et al., 2011; Lin, et al., 2016). The 3-2-1 pattern completion paradigm proposed was designed to represent a more accurate behavioural model for computationally defined pattern completion. Any preference displayed in the testing session cannot be from any direct association with the paired positive stimulus as they were never presented together. Therefore, any learned association or preference for a cue in the testing phase is dependent on the ability to recall previous presentations of the cue in combination of other cues, and linking that to the cues that were actually paired with the appetitive stimulus. The task proved extremely challenging to rats although they were able to pattern complete when olfactory information served as the essential sensory cue providing good evidence that it may be used as a model for behavioural pattern completion.

The olfactory pattern completion task detailed in this thesis mends the incongruence between computational and behaviour models and tasks of pattern separation/completion. Our new paradigm provides an exciting and unique approach to implement computational definitions of pattern completion in a behaviourally relevant model. There are numerous avenues to utilize our model in studying learning and memory systems and specific neural structures thought to be involved in pattern processes (i.e., HPC, specifically CA3 and CA1, DG, etc.).

Conclusions

The goal of this thesis was to explore the theories and ideas presented in our labs previous work, and build from the framework established from those experiments (Hall & Honey, 1989; Good & Honey, 1991; McDonald, et al., 1994, 1997; Sutherland & McDonald, 1990; Antoniadis & McDonald, 1999, 2000). Recently findings have shown that certain context discriminations (based on task demands or procedural variations) are not dependent on HPC function (Lehmann, et al., 2009). Our olfactory high ambiguity discriminative fear conditioning to context task has been found to require the HPC, and is sensitive enough to produce impairments with even partial damage to the HPC. A primary finding from the experiments is that olfactory information is the modality of the only predictive cue that allows the animal to overcome the cue overlap. Olfactory information proved the most valuable and reliable sensory cue available in both the high ambiguity discrimination and pattern completion tasks. We have also designed a unique pattern completion task that, to the best of our knowledge, is the only one of its kind. The 3-2-1 Olfactory Pattern Completion task should provide an exciting new model to explore pattern completion, context discriminations, or configural representations in addition to investigating specific learning and memory systems.

References

Agster, K.L., and Burwell, R.D. (2013). Hippocampal and subicular efferents and afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. Behav. Brain Research, 245, 50-64.

Ahn, J. R. and I. Lee (2014). Intact CA3 in the hippocampus is only sufficient for contextual behavior based on well-learned and unaltered visual background. Hippocampus 24(9): 1081-1093.

Amaral, D.G., Ishizuka, N., and Claiborne, B. (1990). Neurons, numbers and the hippocampal network. Prog. Brain Res. 83: 1–11.

Amaral, D. G., and Lavenex, P. (2007). Hippocampal neuroanatomy. The Hippocampus Book, eds P. Andersen, R. Morris, D. Amaral, T. Bliss, and J. O'Keefe (New York, NY: Oxford University Press, Inc), 37–114

Amaral, D., and Witter, M. (1995). "Hippocampal formation," in The Rat Nervous System, 2nd Edn.ed G.Paxinos (Sydney, NSW: Academic Press),443–485.

Anderson, M.I., and Jeffery, K.J. (2003). Heterogeneous Modulation of Place Cell Firing by Changes in Context. The Journal of Neursci. 23(26): 8827-8835

Antoniadis, E. A., and McDonald, R. J. (2000). Amygdala, hippocampus and discriminative fear conditioning to context. *Behav. Brain Res.* 108, 1–19.

Arias, N., et al. (2015). The recognition of a novel-object in a novel context leads to hippocampal and parahippocampal c-Fos involvement. Behav Brain Res 292: 44-49.

Bouton M (1993). Context, time, and memory retrieval in the interference paradigms of pavlovian learning. Psych Bull; 114:80–99.

Brandon, M. P., Koenig, J., Leutgeb, J. K., Leutgeb, S. (2014). New and distinct hippocampal place codes are generated in a new environment during septal inactivation. Neuron 82(4): 789-796.

Broadbent, N. J., et al. (2007). Rats depend on habit memory for discrimination learning and retention. Learn Mem **14**(3): 145-151.

Burwell, R.D., and Amaral, D.G. (1998). Cortical Afferents of the Perirhinal, Postrhinal, and Entorhinal Cortices of the Rat. J. Comp. Neurology 398:179-205.

Clark, R.E., Broadbent, N.J., Zola, S.M. & Squire, L.R. (2002) Anterograde amnesia and temporally graded retrograde amnesia for a nonspatial memory task after lesions of hippocampus and subiculum. J. Neurosci., 22, 4663–4669.

Clark, R. E., Broadbent, N. J., & Squire, L. R. (2007). The hippocampus and spatial memory: Findings with a novel modification of the watermaze. Journal of Neuroscience, 27, 6647–6654.

Cenquizca, L.A., and Swanson, L.W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res. Brain Res. Rev. 56, 1–26.

Cheng, K. (1986). A purely geometric module in the rat's spatial representation. Cognition, 23, 149–178.

Cohen NJ, Eichenbaum H. (1993). Memory, amnesia, and the hippocampal system. Cambridge, MA: MIT Press.

Czerniawski, J., and Guzowski, J.F. (2014). Acute Neuroinflammation Impairs Context Discrimination Memory and Disrupts Pattern Separation Processes in Hippocampus. J. Neurosci., 34(37): 12470-12480.

Driscoll I, Howard SR, Prusky GT, Rudy JW, Sutherland RJ. (2005). Seahorse wins all races: Hippocampus participates in both linear and nonlinear visual discrimination learning. Behav Brain Res 164: 29–35.

Eichenbaum, H.B., Fagan, A., Mathews, P., Cohen, N.J., (1988). Hippocampal system and odor discrimination learning in rats: impairment or facilitation depending on representational demands. Behav. Neurosci. 102 (3), 331–339.

Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space? Neuron 425:184–188.

Fanselow, M.S., and Dong, H.W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65, 7–19.

Fanselow, M.S., and Tighe, T.J. (1988). Contextual Conditioning With Massed Versus Distributed Unconditional Stimuli in the Absence of Explicit Conditional Stimuli. J. Exp. Psych: Animal Behav Pro, Vol. 14, No. 2, 187-199.

Ferbinteanu, J., McDonald, R.J., (2001). Dorsal/ventral hippocampus, fornix, and conditioned place preference. Hippocampus 11, 187–200.

Fenton, A.A., Csizmadia, G., Muller, R.U. (2000). Conjoint Control of Hippocampal Place Cell Firing by Two Visual Stimuli, I. The Effects of Moving the Stimuli on Firing Field Positions. J. Gen. Physiol. 116 (191-209).

Fortin, N.J., Agster, K.L., and Eichenbaum, H.B. (2002). Critical role of the hippocampus in memory for sequences of events. Nat. Neurosci. 5, 458–462.

Gaskin, S., Tremblay, A. & Mumby, D.G. (2003) Retrograde and anterograde object recognition in rats with hippocampal lesions. Hippocampus, 13, 962–969.

Gervais, N. J., Barrett-Berstein, M., Sutherland, R. J., and Mumby, D. G. (2014). Retrograde and anterograde memory following selective damage to the dorsolateral entorhinal cortex. Neurobiology of Learning and Memory (116): 14-26

Gold, A. E. & R. P. Kesner (2005). The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat. Hippocampus 15(6): 808-814.

Good M. & Honey RC. (1991). Conditioning and contextual retrieval in hippocampal rats. Behav Neurosci 105:499 –509.

Grion, N., Akrami, A. Zuo, Y. Stella, F. Diamond, M. E. (2016). Coherence between Rat Sensorimotor System and Hippocampus Is Enhanced during Tactile Discrimination. PLoS Biol 14(2): e1002384.

Gruber, A.J., and McDonald, R.J. (2012). Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behaviour. Frontiers in Behav. Neurosci., 50, 1-26.

Guzowski, J. F., Knierim, J. J., Moser, E. I. (2004). Ensemble dynamics of hippocampal regions CA3 and CA1. Neuron 44(4): 581-584.

Hargreaves EL, Rao G, Lee I, Knierim JJ. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science 308:1792–1794.

Hall, G. & Honey, R.C. (1989). Contextual Effects in Conditioning, Latent Inhibition, and Habituation: Associative and Retrieval Functions of Contextual Cues.

Hebb, D. (1949). The organization of behavior. New York: Wiley.

Hirsh, R. (1974). The hippocampus and contextual retrieval of information from memory: A theory. Behavioral Biology, 12, 421-444.

Hirsh, R. (1980). The hippocampus, conditional operations and cognition. Physiological Psychology, 8, 175-182.

Holland, P.C. and Bouton, M.E. (1999). Hippocampus and context in classical conditioning. Current Opinion in Neurobiology, 9: 195-202.

Hunsaker, M. R. and R. P. Kesner (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. Neurosci Biobehav Rev 37(1): 36-58.

Hunsaker, M. R., Rosenberg, J. S., Kesner, R. P. (2008). The role of the dentate gyrus, CA3a,b, and CA3c for detecting spatial and environmental novelty. Hippocampus 18(10): 1064-1073.

Iordanova, M.D., Burnett, D.J., Good, M., Honey, R.C. (2011). Pattern Memory Involves Both Elemental and Configural Processes: Evidence From the Effects of Hippocampal Lesions. Behav. Neurosci. Vol. 125, No. 4, 567-577.

Jarrard, L. E., Luu, L. P., Davidson, T. L. (2012). A study of hippocampal structure-function relations along the septo-temporal axis. Hippocampus 22(4): 680-692.

Kennedy, P.J., and Shapiro, M.L. (2004). Retrieving memories via internal context requires the hippocampus. J. Neurosci. 24, 6979–6985.

Kennedy, P.J., Shapiro, M.L., and Squire, L.R. (2009). Motivational States Activate Distinct Hippocampal Representations to Guide Goal-Directed Behaviors. PNAS Vol. 106, No. 26, 10805-10810.

Kesner, R. P., Gilbert, P. E., & Barua, L. A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. Behavioral Neuroscience, 116, 286–290.

Kesner, R. P., Kirk, R. A., Yu, Z., Polansky, C., Musso, N. D. (2016). Dentate gyrus supports slope recognition memory, shades of grey-context pattern separation and recognition memory, and CA3 supports pattern completion for object memory. Neurobiol Learn Mem 129: 29-37.

Kim, J.J, Fanselow, M.S. (1992). Modality-Specific Retrograde Amnesia of Fear. Science, Vol. 256 (675-677).

Kim, S., Lee, J., Lee, I. (2012). The hippocampus is required for visually cued contextual response selection, but not for visual discrimination of contexts. Frontiers in Behav. Neurosci. Vol. 6: 1-10.

Knierim, J. J. and J. P. Neunuebel (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. Neurobiol Learn Mem 129: 38-49.

Langston, R. F. and E. R. Wood (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. Hippocampus 20(10): 1139-1153.

Lavenex, P., and Amaral, D.G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus* 10, 420–430.

Lee, H., Wang, C. Deshmukh, S. S., Knierim, J. J. (2015). Neural Population Evidence of Functional Heterogeneity along the CA3 Transverse Axis: Pattern Completion versus Pattern Separation. Neuron 87(5): 1093-1105.

Lee, K.J., Park, S., Lee, I. (2014). Elemental or contextual? It depends: individual difference in the hippocampal dependence of associative learning for a simple sensory stimulus. Frontiers in Behavioural Neuroscience Vol.8 (217) 1-9.

Lehmann, H., Lacanilao, S, and Sutherland, R. (2007). Complete or partial hippocampal damage produces equivalent retrograde amnesia for remote contextual fear memories. Eur J Neurosci **25**(5): 1278-1286.

Lehmann, H., Sparks, F.T., Spanswick, S.C., Hadikin, C., McDonald, R.J., and Sutherland, R.J. (2009). Making context memories independent of the hippocampus. Learn. Mem. 16, 417–420.

Lehmann, H., Sparks, F. T., O'Brien, J., McDonald, R. J., and Sutherland, R. J. (2010). Retrograde amnesia for fear-potentiated startle in rats after complete, but not partial, hippocampal damage. Neuroscience **167**(4): 974-984.

Lenck-Santini, P. P., Rivard, B., Muller, R. U., Poucet, B.. (2005). Study of CA1 place cell activity and exploratory behavior following spatial and nonspatial changes in the environment. Hippocampus 15(3): 356-369.

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. Science 305(5688): 1295-1298.

Leutgeb, S., Leutgeb, J. K., Barnes, C. A., Moser, E. I., McNaughton, B. L., Moser, M. B (2005). Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science 309(5734): 619-623.

Leutgeb, S. and J. K. Leutgeb (2007). Pattern separation, pattern completion, and new neuronal codes within a continuous CA3 map. Learn Mem 14(11): 745-757.

Leutgeb, J. K., Leutgeb, J. K., Moser, M. B., Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science 315(5814): 961-966.

Lin, T-C.E., Dumigan, N.M., Good, M., Honey, R.C. (2016). Novel sensory preconditioning procedures identify a specific role for the hippocampus in pattern completion. Neurobiology of Learning and Mem. 130, 142-148.

Maren, S. (1999). Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats. Behav. Neurosci. 113, 283–290.

Maren, S., Aharonov, G., and Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. Behav.BrainRes. 88, 261–274.

Marr, D., (1971). Simple memory: a theory for archicortex. Proc. R. Soc. Lond. B Biol. 262 (841), 23–81.

McClelland, J.L., McNaughton, B.L., O'Reilly, R.C., (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol. Rev. 102, 419–457.

McDonald, R. J., Devan, B. D., Hong. N. S. (2004). Multiple memory systems: the power of interactions. Neurobiol Learn Mem **82**(3): 333-346.

McDonald RJ, Hong NS. (2000). Rats with hippocampal damage are impaired on place learning in the water task even when over trained under constrained behavioral conditions. Hippocampus 10:153–161.

McDonald, R.J., King, A.L., and Hong, N.S. (2001). Context-specific interference on reversal learning of a stimulus-response habit. Behav. Brain Res. 121, 149-165.

McDonald, R. J., Lo, Q., King, A. L., Wasiak, T. D., and Hong, N. S. (2007). Empirical tests of the functional significance of amygdala-based modulation of hippocampal representations: evidence for multiple memory consolidation pathways. Eur J Neurosci **25**(5): 1568-1580.

McDonald, R.J., Jones, J., Richards, B., and Hong, N.S. (2006). A double dissociation of dorsal and ventral hippocampal function on a learning and memory task mediated by the dorso-lateral striatum. Eur.J. Neurosci. 24, 1789–1801.

McDonald, R.J., Murphy, R.A., Guarraci, F.A., Gortler, J.R., White, N.M., and Baker, A.G. (1997). Systematic comparison of the effects of hippocampal and fornix-fimbria lesions on acquisition of three configural discriminations. Hippocampus 7, 371-388.

McDonald, R. J. and N. S. Hong (2013). How does a specific learning and memory system in the mammalian brain gain control of behavior? Hippocampus **23**(11): 1084-1102.

McDonald RJ, White NM. (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 107:3–22.

McNaughton, B.L., Barnes, C.A., and O'Keefe, J. (1983). The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp.BrainRes.* 52, 41–49.

McNaughton, B.L., Morris, R.G.M., (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends Neurosci. 10 (10), 408–415.

Milner B. (1959). The memory defect in bilateral hippocampal lesions. Psychiatr Res Rep 11:43–52.

Mizumori, S. J., Smith, D. M., Puryear, C. B. (2007). Hippocampal and neocortical interactions during context discrimination: electrophysiological evidence from the rat. Hippocampus 17(9): 851-862.

Mizumori, S. J., Yeshenko, O., Gill, K. M., Davis, D. M. (2004). Parallel processing across neural systems: implications for a multiple memory system hypothesis. Neurobiol Learn Mem 82(3): 278-298.

Morris, A. M., Weeden, C. S., Churchwell, J. C., Kesner, R. P. (2013). The role of the dentate gyrus in the formation of contextual representations. Hippocampus 23(2): 162-168.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O'Keefe, J., (1982). Place navigation impaired in rats with hippocampal lesions. Nature 297 (24), 681–683.

Moser, M.B., and Moser, E.I. (1998). Functional differentiation in the hippocampus. Hippocampus 8, 608–619.

Moser, M.B., and Moser, E.I., and Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J. Neurosci. 13, 3916-3925.

Moser, M.B., Moser, E.I., Forrest, E., Andersen, P., and Morris, R.G. (1995). Spatial learning with a minislab in the dorsal hippocampus. Proc. Natl. Acad. Sci. USA 92, 9697–9701.

Muller, R.U., and Kubie, J.L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *J. Neurosci.* 7, 1951–1968.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. Learning & Memory, 9(2), 49–57.

Nadel, L., Willner, J., (1980). Context and conditioning: a place for space. Physiol. Psychol. 8 (2), 218–228.

O'Keefe, J., and Nadel, L. (1978). The Hippocampus as a Congnitive Map. Oxford: Oxford University Press.

O'Keefe, J., Nadel, L., Keightley, S., and Kill, D. (1975). Fornix lesions selectively abolish place learning in the rat. Exp. Neurol. 48, 152–166.

O'Keefe, J., and Speakman, A. (1987). Single unit activity in the rat hippocampus during a spatial memory task. *Exp. Brain Res.* 68, 1–27.

Olton, D.S., Becker, J.T., Handelmann, G.E. (1979). Hippocampus, space and memory. Behav. Brain Sci. 2: 313-365.

O'Reilly, R.C., McClelland, J.L., (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. Hippocampus 4, 661–682.

O'Reilly, R.C., and Rudy, J.W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychol. Rev. 108, 311–345.

Otto, T., & Poon, P. (2006). Dorsal hippocampal contributions to unimodal contextual conditioning. Journal of Neuroscience, 26, 6603–6609.

Packard, M.G., Hirsh, R., and White, N. M. (1989). Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. *J. Neurosci.* 9, 1465–1472.

Pavlov, I. (1927). Conditional Reflexes: Dover Publications, New York.

Pearce, J. M., Good, M. A., Jones, P. M., & McGregor, A. (2004). Transfer of spatial behavior between different environments: Implications for theories of spatial learning and for the role of the hippocampus in spatial learning. Journal of Experimental Psychology: Animal Behavior Processes, 30, 135–147.

Pearce, J. M., Ward-Robinson, J., Good, M., Fussell, C., & Aydin, A. (2001). Influence of a beacon on spatial learning based on the shape of the test environment. Journal of Experimental Psychology: Animal Behavior Processes, 27, 329–344.

Pevzner, A., Miyashita, T., Schiffman, A. J., Guzowski, J. F. (2012). Temporal dynamics of Arc gene induction in hippocampus: relationship to context memory formation. Neurobiol Learn Mem 97(3): 313-320**.**

Pidgeon, L. M. and A. M. Morcom (2016). Cortical pattern separation and item-specific memory encoding. Neuropsychologia 85: 256-271.

Phillips RG, LeDoux JE. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285.

Pothuizen, H.H., Zhang, W.N., Jongen-Reˆ lo, A.L., Feldon, J., and Yee, B.K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. Eur. J. Neurosci. 19, 705–712.

Potvin, O., Dore, F.Y., and Goulet, S. (2009). Lesions of the dorsal subiculum and the dorsal hippocampus impaired pattern separation in a task using distinct and overlapping visual stimuli. Neurobio. Learn. Mem. 91,287-297.

Rivard, B., Li, Y., Lenck-Santini, P.P., Poucet, B., Muller, R.U. (2004). Representation of Objects in Space by Two Classes of Hippocampal Pyramidal Cells. J. Gen. Physiol. Vol. 124 (9-25).

Rudy, J.W. (2009). Context representations. Context functions, and the parahippocampal-hippocampal system. Learn. Mem. 16:573-585. Doi:10.1101/lm.1494409.

Rudy, J. W., & O'Reilly, R. C. (1999). Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. Behavioral Neuroscience, 113(5), 867–880.

Rudy, J.W., O'Reilly, R.C., (2001). Conjunctive representation, the hippocampus, and contextual fear conditioning. Cogn. Affect. Behav. Neurosc. 1 (1), 66–82.

Rudy, J.W., and Sutherland, R.J. (1989). The hippocampal formation is necessary for rats to learn and remember configural discriminations. Behav. Brain. Res. 34, 97–109. doi:10.1016/S0166-4328(89)80093-2

Rolls, E. T. (1989). Functions of neuronal networks in the hippocampus and neocortex in memory. In J. H. Byrne & W. O. Berry (Eds.), Neural models of plasticity: Experimental and theoretical approaches (pp. 240–265). San Diego: Academic Press.

Save, E., Nerad, L., and Poucet, B., (2000). Contribution of Multiple Sensory Information to Place Field Stability in Hippocampal Place Cells. Hippocampus 10:64-76.

Schmitz C, Hof P (2005) Design-based stereology in neuroscience. Neuroscience 130: 813–831.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery & Psychiatry, 20, 11–21. doi:10.1136/jnnp.20.1.11

Sharp, P.E., Blair, H.T., and Brown, M. (1996). Neural network modeling of the hippocampal formation spatial signals and their possible role in navigation: a modular approach. Hippocampus 6, 720–734.

Steffenach, H., Witter, M., Moser, M., Moser, E.I. (2004). Spatial Memory in the Rat Requires the Dorsolateral Band of the Entorhinal Cortex. Neuron, Vol. 45, 301-313.

Sutherland, R.J., Lehmann, H., 2011. Alternative conceptions of memory consolidation and the role of the hippocampus at the systems level in rodents. Curr. Opin. Neurobiol. 21 (3), 446–451.

Sutherland, R. J., Kolb, B., & Whishaw, I. Q. (1982). Spatial mapping: Definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neuroscience Letters, 31, 271–276.

Sutherland, R. J., Whishaw, I. Q., and Kolb, B. (1983). A behavioural analysis of spatial localization following electrolytic, kainate- or cochicine-induced damage to the hippocampal formation in the rat. Behav. Brain Res. 7, 133–153. doi: 10.1016/0166-4328(83)90188-2

Sutherland, R.J., and McDonald, R. J. (1990). Hippocampus, amygdala, and memory deficits in rats. *Behav. Brain Res.* 37, 57–79.

Sutherland, R.J., McDonald, R.J., Hill, C.R., and Rudy, J.W. (1989). Damage to the hippocampal formation in rats selectively impairs the ability to learn cue relationships. Behav. Neural Biol. 52, 331–356.

Sutherland, R. J., & Rudy, J.W.(1989). Configural association theory: The contribution of the hippocampus to learning, memory, and amnesia. Psychobiology, 17(2), 129–144.

Sutherland, R.J., Sparks, F.T., and Lehmann, H. (2010). Hippocampus and retrograde amnesia in the rat model: A modest proposal for the situation of systems consolidation. Neurpsychologia, 48, 2357-2369.

Sutherland, R. J., O'Brien, J., & Lehmann, H. (2008). Absence of systems consolidation of fear memories after dorsal, ventral, or complete hippocampal damage. Hippocampus, 16, 417–420.

Sutherland, R.J., Weisend, M.P., Mumby, D., Astur, R.S., Hanlon, F.M., Koerner, A., Thomas, M.J., Wu, Y., Moses, S.N., Cole, C. & others (2001). Retrograde amnesia after hippocampal damage: recent vs. remote memories in two tasks. Hippocampus, 11, 27–42.

Thorndike, E. L. (1911). Animal intelligence: Experimental studies. New York: Macmillan

Tolman, E.C. (1932). Purposive Behavior in Animals and Man. New York, NY: Appleton.

Travis, S.G., Sparks, F.T., Arnold, T., Lehmann, H., Sutherland, R.J., Whishaw, I.Q., (2010). Hippocampal damage produces retrograde but not anterograde amnesia for a cued location in a spontaneous exploratory task in rats. Hippocampus 20, 1095–1104.

Tse, D., Langston, R. F., Kakeyama, M., Bethus, I., Spooner, P. A., Wood, E. R., et al. (2007). Schemas and memory consolidation. Science, 316(5821), 76–82.

Tulving E (1972) Episodic and semantic memory. In: Organization of memory (Tulving E, Donaldson W, eds), pp 382– 403. New York: Academic.

Tulving E (2002) Episodic memory: From mind to brain. Annu Rev Psychol 53:1-25.

Vann, S.D., Aggleton, J.P., and Maguire, E.A. (2009). What does the retrosplenial cortex do? Nature Reviews. Vol 10: 792-802.

Vazdarjanova, A., Guzowski, J.F., (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. J. Neurosci. 24 (29), 6489–6496.

Voorn, P., Vanderschuren, L.J., Groenewegen, H.J., Robbins, T.W. and Pennartz, C.M. (2004). Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci. 27, 468-474.

Weeden, C. S., Hu, N. J., Ho, L. U., Kesner, R. P. (2014). The role of the ventral dentate gyrus in olfactory pattern separation. Hippocampus 24(5): 553-559.

White, N. M., & McDonald, R. J. (1993). Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behavioural Brain Research, 55, 269–281. doi:10.1016/0166-4328(93)90122-7.

White, N.M., and McDonald, R.J. (2002). Multiple parallel memory systems in the brain of the rat. Neurobiol. Learn. Mem. 77, 125–184.

White, N.M., Packard, M.G., and McDonald, R.J. (2013). Dissociation of Memory Systems: The Story Unfolds. Behav. Neurosci. 127, (6): 813-834/

Wiltgen, B.J., Zhou, M., Cai, Y., Balaji, J., Karlsson, M. G., Parivash, S. N., Li, W., and Silva, A. (2010). The Hippocampus Plays a Selective Role in the Retrieval of Detailed Contextual Memories. Curr. Biol., 20, 1336-1344.

Yassa, M.A., and Stark, C.E.L. (2011). Pattern separation in the hippocampus. Trends in Neurosciences, 34, 10: 515-525.

Figures

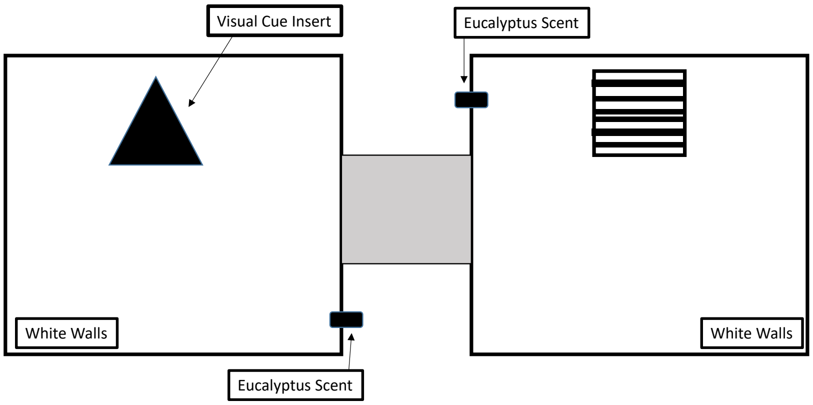

Figure 1: Diagram depiction of the visual cue variant to the highly ambiguous discriminative fear conditioning to context task. The two context squares are connected by a grey alleyway in the middle. A solid black triangle (left) and a zebra striped square (right) served as visual cues. Eucalyptus scent cues were present in both of the contexts.

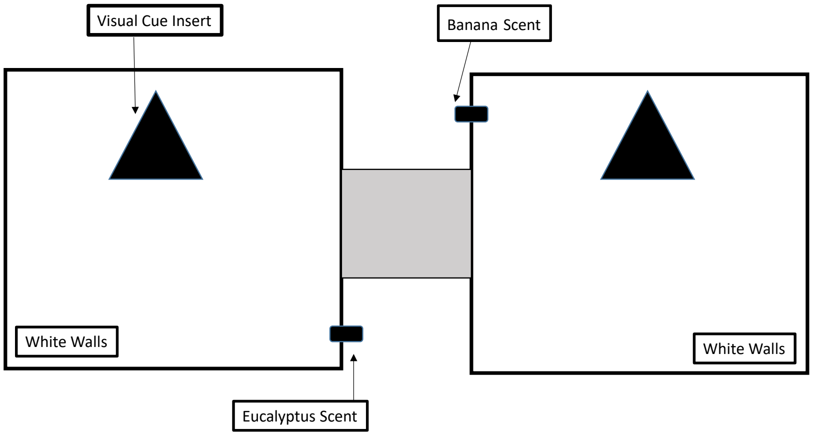

Figure 2: Diagram depiction of the olfactory cue variant to the highly ambiguous discriminative fear conditioning to context task. The two context squares are connected by a grey alleyway in the middle. A eucalyptus scent cue (Vicks Vapo-Rub, left) and a banana scent cue (amyl-acetate, right) served as visual cues. 2 solid black triangles served as overlapping visual cues.

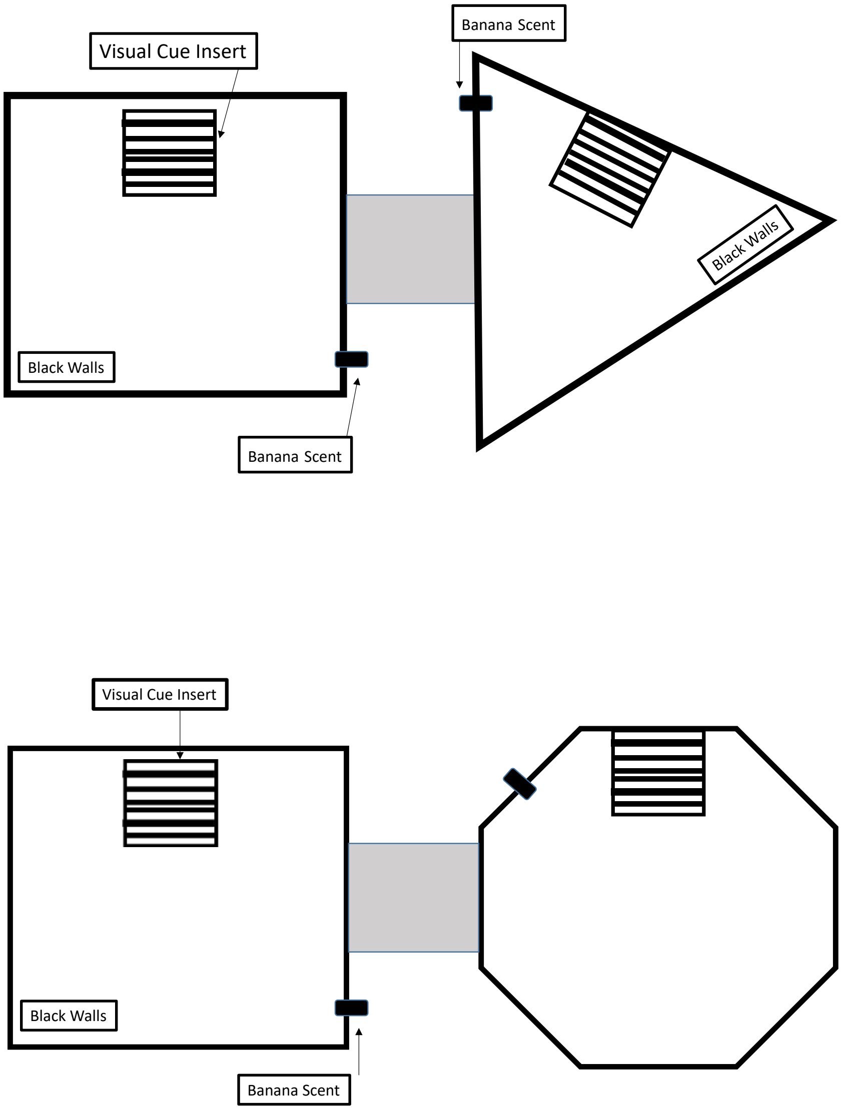

Figure 3: Diagram depiction of the geometric shape variants to the highly ambiguous discriminative fear conditioning to context task. The two context squares are connected by a grey alleyway in the middle. In geometric shape variant 1 (top) the two contexts differ in shape with a square and a triangle. Geometric shape variant 2 (bottom) a square and an octagon served as the key cue. Both variants utilized overlapping banana scent cues and zebra square visual cues in both contexts.

Experiment	Task	Constant	Different
1.1	Standard DFCTC	-	Shape, Scent, Colour
1.2 2.1	Visual Highly Ambiguous DFCTC	Shape, Scent, Colour	Visual Cue Insert
1.3 2.2	Olfactory Highly Ambiguous DFCTC	Shape, Colour, Visual Cue	Scent
1.4	Geometric Shape Highly Ambiguous DFCTC Variant 1	Colour, Scent, Visual Cue	Shape (square-triangle)
1.5 2.3	Geometric Shape Highly Ambiguous DFCTC Variant 2	Colour, Scent, Visual Cue	Shape (square-octagon)

Figure 4: A detailed description of all the highly ambiguous discriminative fear conditioning to context tasks, showing the constant and different variables to each task.

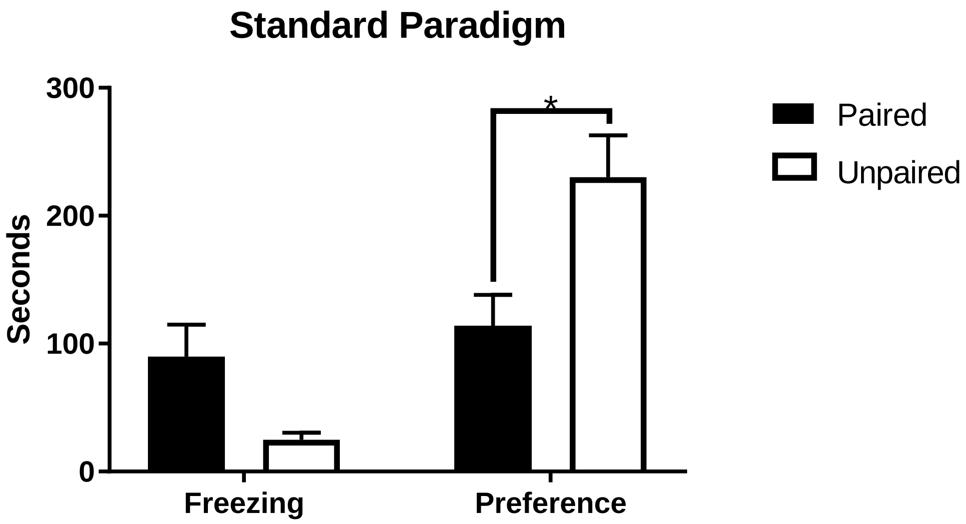

Figure 5: Results of the Freezing and Preference tests for Experiment 1.1. Total freezing and preference times in both paired and unpaired contexts for standard model of the discriminative fear conditioning to context tasks.

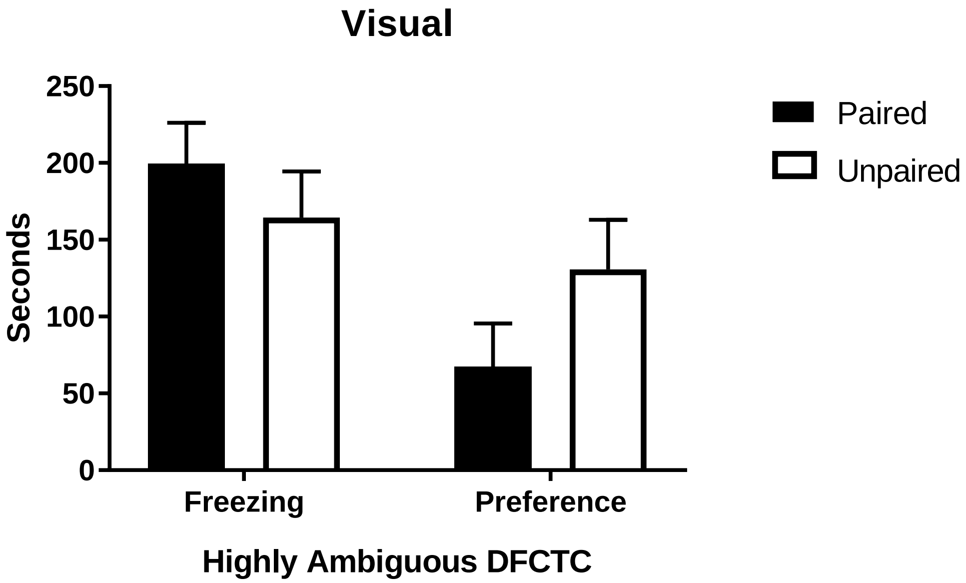

Figure 6: Results of the Freezing and Preference tests for Experiment 1.2. Total freezing and preference times in both paired and unpaired contexts for the Visual high ambiguity variant of the discriminative fear conditioning to context tasks.

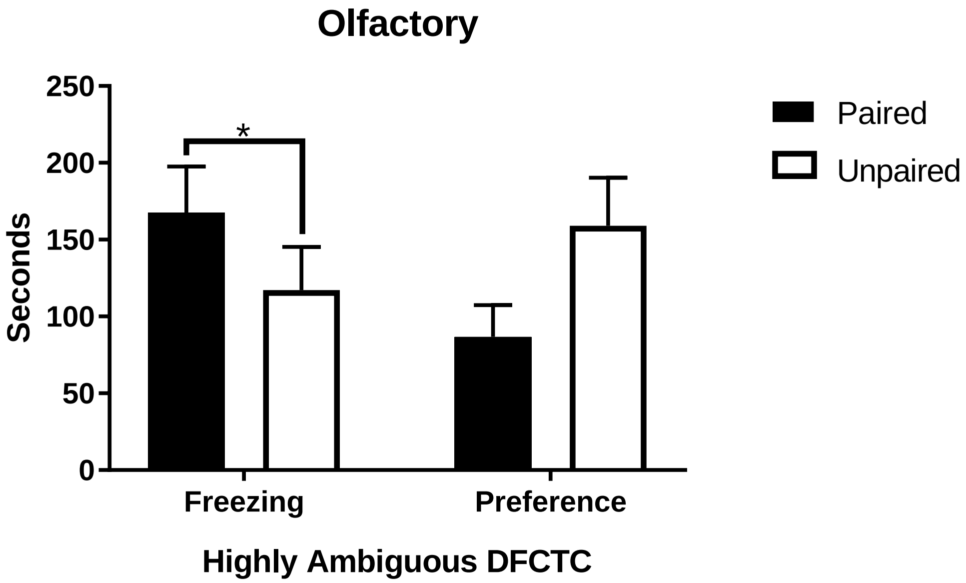

Figure 7: Results of the Freezing and Preference tests for Experiment 1.3. Total freezing and preference times in both paired and unpaired contexts for the Olfactory high ambiguity variant of the discriminative fear conditioning to context tasks.

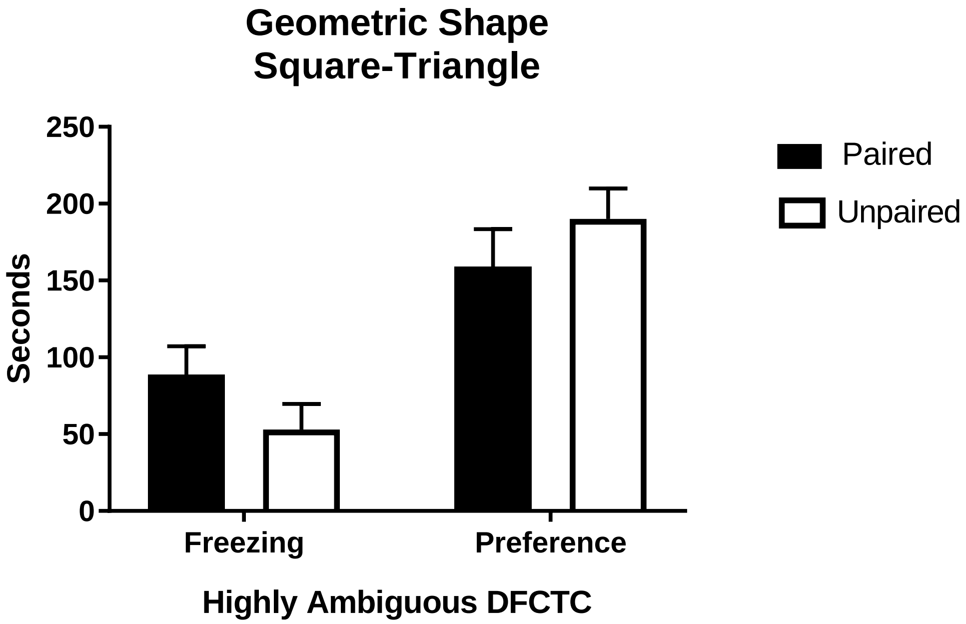

Figure 8: Results of the Freezing and Preference tests for Experiment 1.4. Total freezing and preference times in both paired and unpaired contexts for the first Geometric Shape high ambiguity variant (square vs triangle) of the discriminative fear conditioning to context tasks.

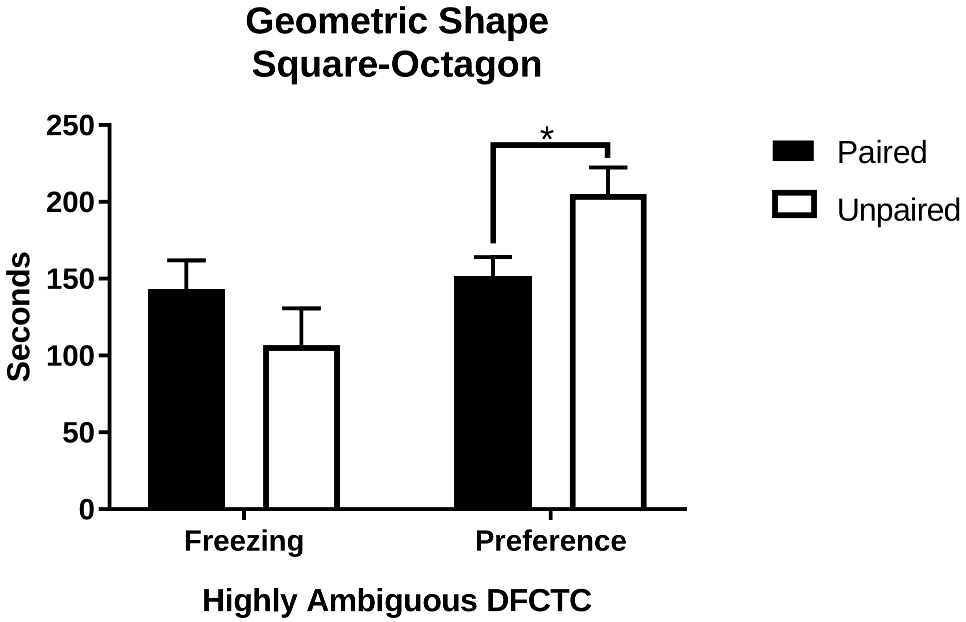

Figure 9: Results of the Freezing and Preference tests for Experiment 1.5. Total freezing and preference times in both paired and unpaired contexts for the second Geometric Shape high ambiguity variant (square vs octagon) of the discriminative fear conditioning to context tasks.

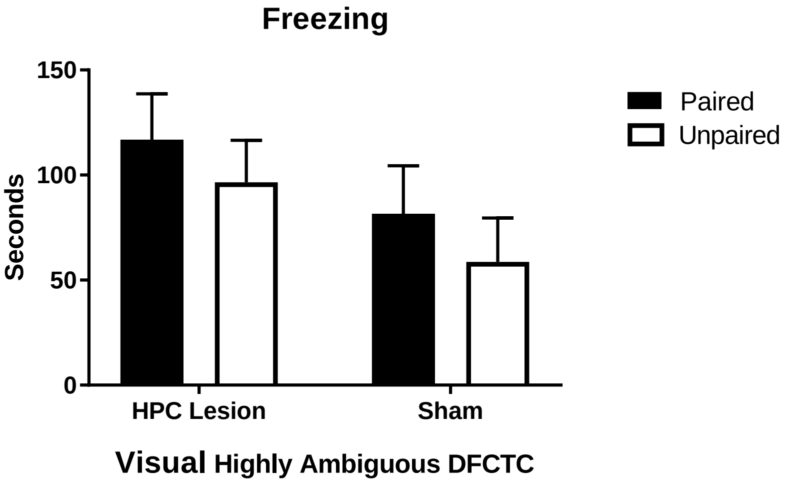

Figure 10A: Results of Experiment 2.1 Freezing Tests. Hippocampal lesion and sham surgery animals trained in the Visual Cue variant of the discriminative fear conditioning to context task.

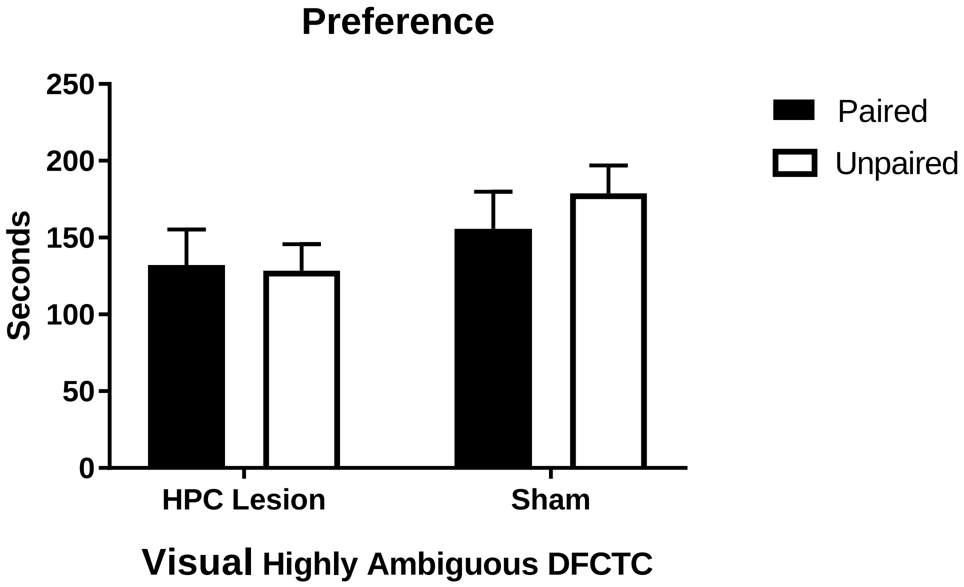

Figure 10B: Results of Experiment 2.1 Preference Test. Hippocampal lesion and sham surgery animals trained in the Visual Cue variant of the discriminative fear conditioning to context task.

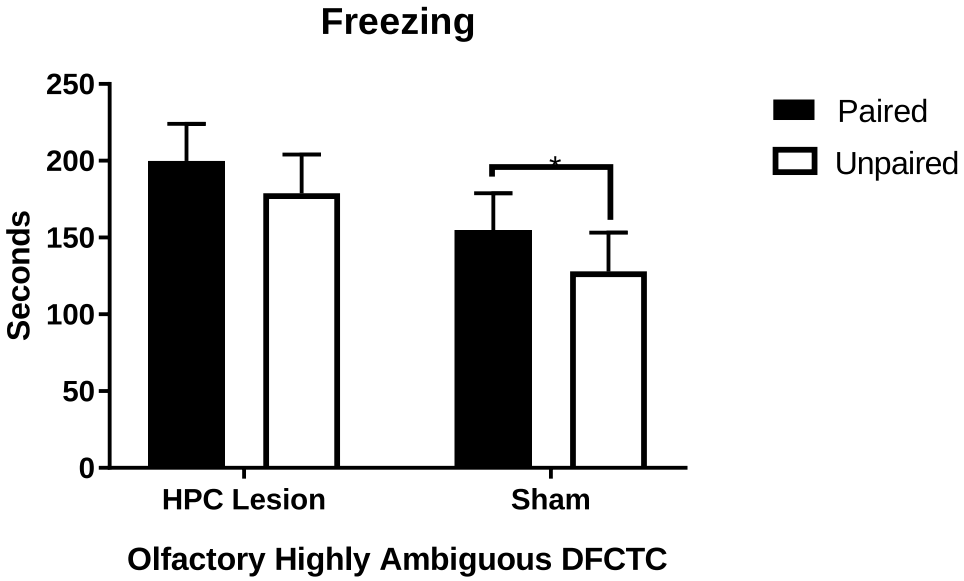

Figure 11A: Results of Experiment 2.2 Freezing Tests. Hippocampal lesion and sham surgery animals trained in the Olfactory Cue variant of the discriminative fear conditioning to context task.

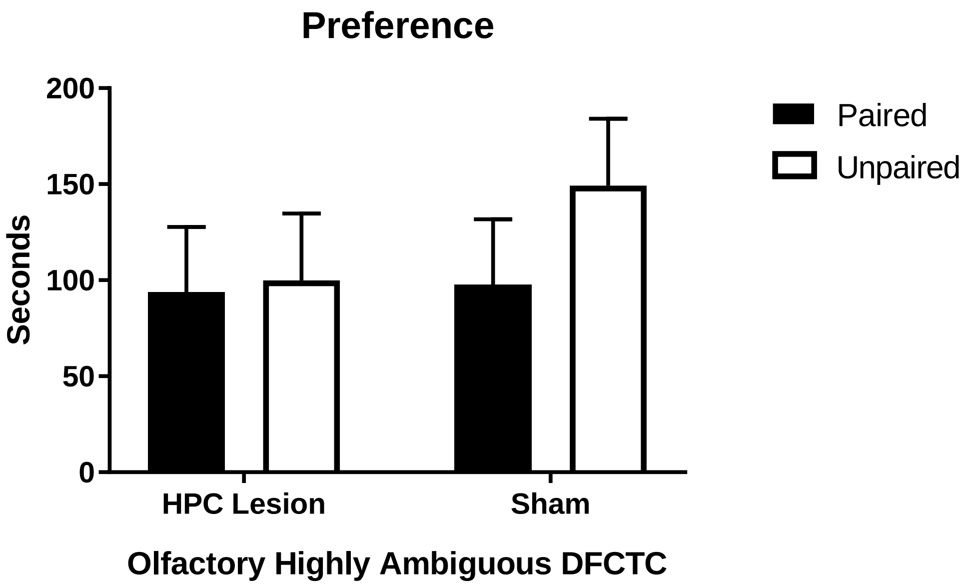

Figure 11B: Results of Experiment 2.2 Preference Test. Hippocampal lesion and sham surgery animals trained in the Olfactory Cue variant of the discriminative fear conditioning to context task.

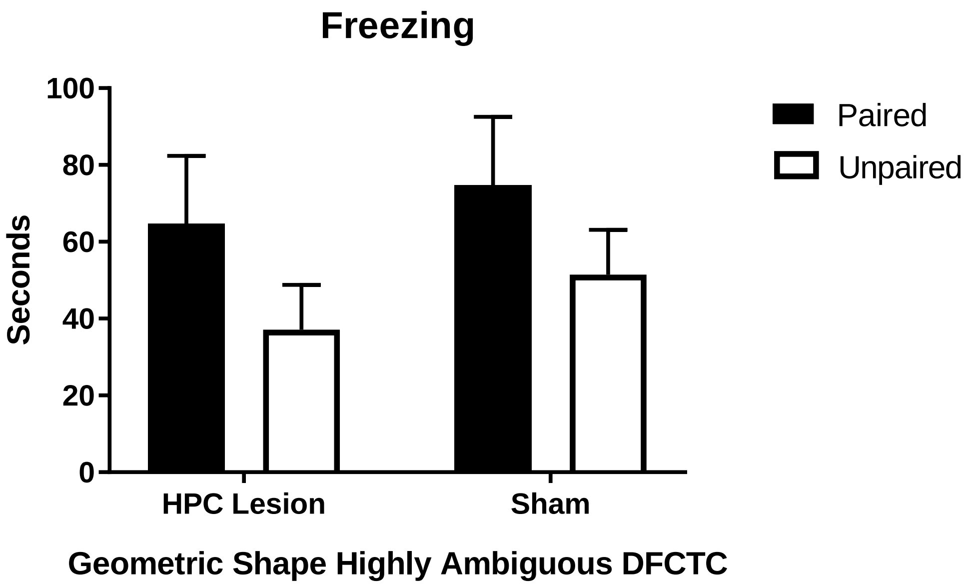

Figure 12A: Results of Experiment 2.3 Freezing Tests. Hippocampal lesion and sham surgery animals trained in the Geometric shape variant of the discriminative fear conditioning to context task.

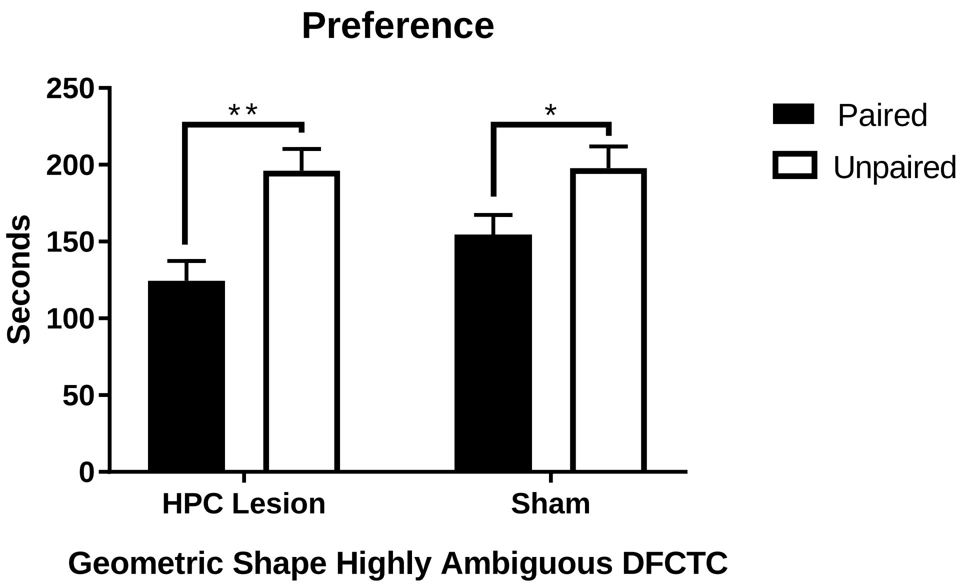

Figure 12B: Results of Experiment 2.3 Preference Test. Hippocampal lesion and sham surgery animals trained in the Geometric shape variant of the discriminative fear conditioning to context task.

Experiment	Pre-Exposure	Training	Preference Test
Olfactory 3.1	Olfactory + Visual + Tactile	Visual + Tactile No Olfactory	Olfactory Only No Visual or Tactile
Visual 3.2	Visual + Olfactory + Tactile	Tactile + Olfactory No Visual	Visual Only No Olfactory/Tactile
Tactile 3.3	Tactile + Olfactory + Visual	Visual + Olfactory No Tactile	Tactile Only No Olfactory/Visual

Figure 13: A detailed description of the 3-2-1 Pattern Completion task, showing the constant and different variables to each task.

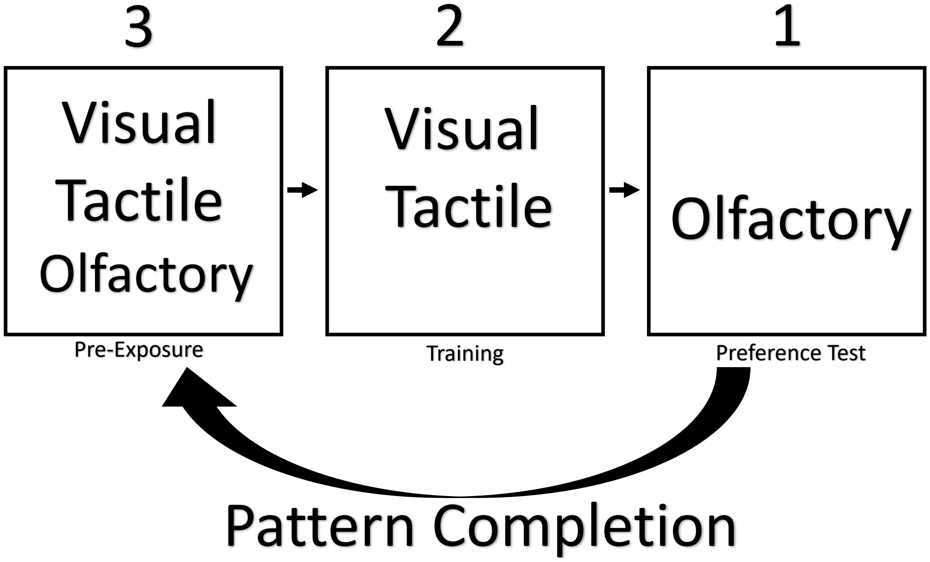

Figure 14: A visual guide to the 3-2-1 Pattern Completion procedure; pre-exposure, training, and testing.

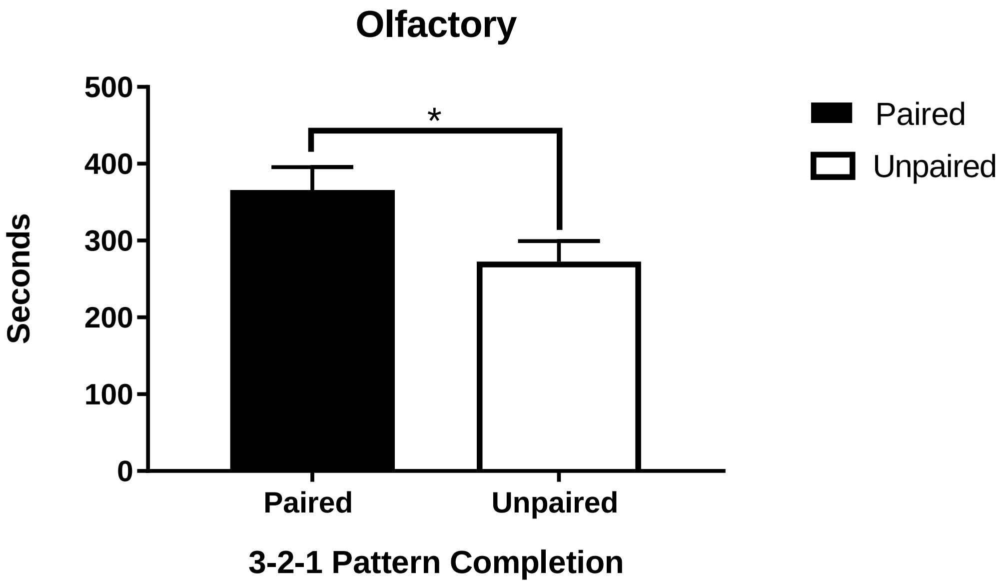

Figure 15: Results of Experiment 3 Pattern Completion Preference Tests. Rats in the olfactory version of the 3-2-1 Pattern Completion tasks were able to accurately complete the task.

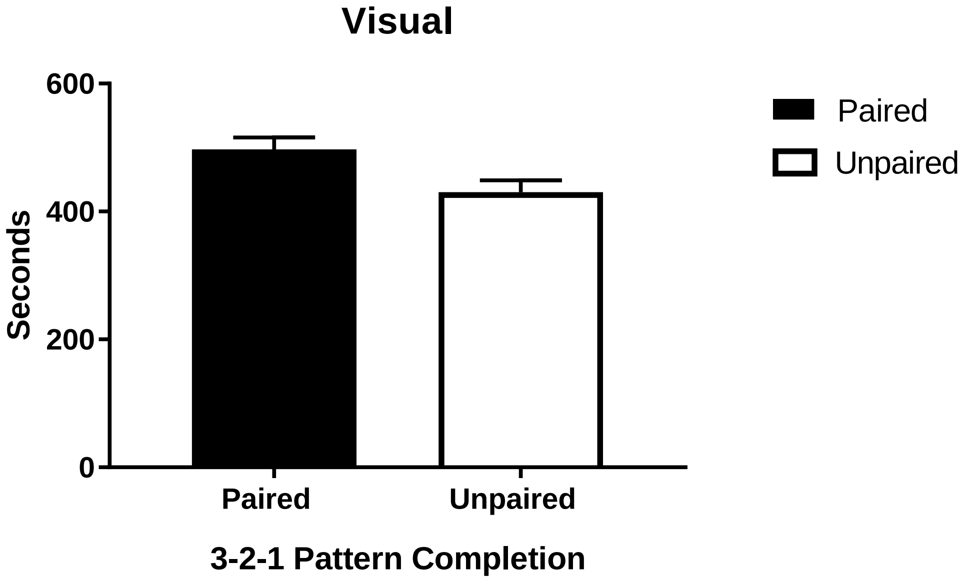

Figure 16: Results of Experiment 3 Pattern Completion Preference Tests. Rats in the visual version of the 3-2-1 Pattern Completion tasks were unable to accurately complete the task.

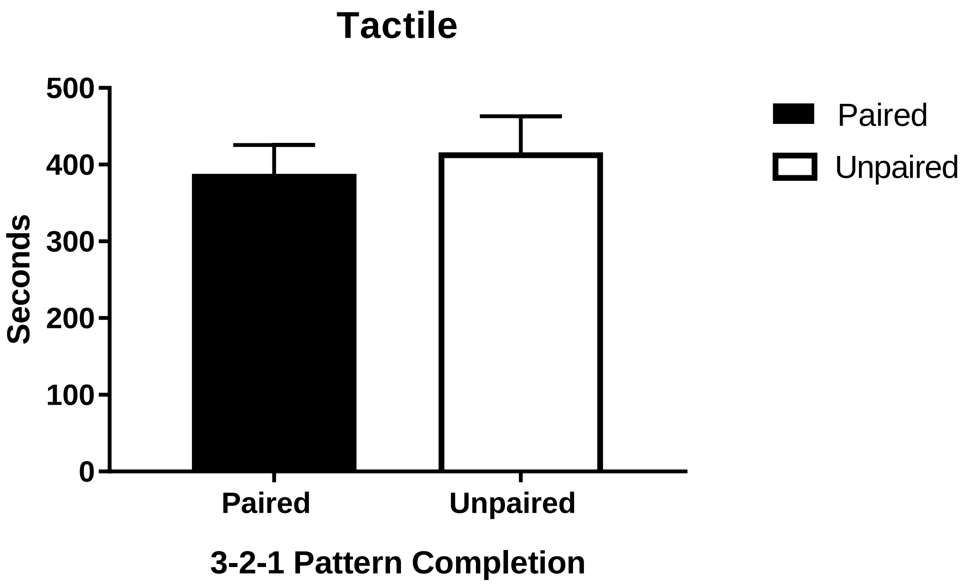

Figure 16: Results of Experiment 3 Pattern Completion Preference Tests. Rats in the tactile version of the 3-2-1 Pattern Completion tasks were unable to accurately complete the task.