

**A BEHAVIOURAL ANALYSIS OF VISUAL PATTERN SEPARATION ABILITY
BY RATS: EFFECTS OF DAMAGE TO THE HIPPOCAMPUS**

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Dedication

To my mother; without your unwavering support and kind words this work could not have been completed. Your balanced approach to life has provided me with an ability to see the bigger picture in all of this.

ABSTRACT

Different events usually contain similar elements that can contribute to interference during memory encoding and retrieval. The hippocampus (HPC), a structure that is critically involved in some forms of memory, has been hypothesized to reduce interference between memories with overlapping content, thus facilitating correct recall. Pattern separation is one hypothetical process whereby input ambiguity is reduced. Here we test the hypothesis that the HPC and/or dentate gyrus (DG) are important for pattern separation by measuring performance by rats with damage in tasks that require discrimination between visual stimuli that share systematically varying numbers of common elements. Rats with HPC damage were slower to resolve discriminations with minimal degrees of overlap. Lesions of the DG did not affect the ability of rats to deal with overlap, suggesting a dissociation between the HPC and DG. Our results provide partial support for the idea that the HPC contributes to the pattern separation process.

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It is unfortunately necessary for me to disacknowledge the Microsoft programmers who are responsible for the Office X version of Excel. A definition, Excel “to be better than; to be very good at something”. I find that your program does none of these things and ask that you please rename it.

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ABBREVIATIONS

ADX	adrenalectomy
ANOVA	analysis of variance
CCAC	Canadian Council on Animal Care
CDZ	chlordiazapoxide
DG	dentate gyrus
EC	entorhinal cortex
HPC	hippocampus
ip	intraperitoneal
MWT	Morris water task
NIH	National Institute of Health
NMDA	N-methyl-D-aspartate
PFA	paraformaldehyde
S-	negative stimulus
S+	positive stimulus
SEM	standard error of the mean
VWT	visual water task

CHAPTER ONE

The Hippocampus and Memory

Work by Scoville and Milner (1957) on memory deficits suffered by patients who had sustained bilateral medial temporal lobe resection has provided a basis for research into the nature of memory and its physical location within the brain. Scoville and Milner's (1957) findings suggest that structures within the medial temporal lobe, especially the hippocampus, play an important role in the storage and recall of certain memories. Further investigation by Milner, Corkin and Teuber (1968) revealed a dissociation between at least two types of memory in regards to the amnesia produced by lesions limited to the temporal lobe. Specifically, patient HM (who had previously undergone bilateral temporal lobectomy) demonstrated amnesia for episodic memories, yet presented with an intact practice effect and priming ability. These initial findings have led to further experimentation and dissociation between differing forms of memory and their location within the brain.

Squire and Cohen (1980) distinguish between declarative and nondeclarative forms of memory. Declarative memories can be classified as those that are consciously recalled. Episodic (knowledge of specific events) and semantic (knowledge of facts) memories both fall within this category (Kandel, Kupfermann & Iversen, 2000). Declarative memory is thought to be dependent upon the HPC (Cohen and Squire, 1980, see also Cohen and Eichenbaum, 1993). Discussions of nondeclarative forms of memory usually focus on procedural elements (see Kandel, Kupfermann & Iversen, 2000 for a discussion of other forms of nondeclarative memory). Nondeclarative memories are typically acquired gradually and can be thought of as knowledge of *how* to perform a

given task. The skill of riding a bicycle can be thought of as a type of nondeclarative memory. However, remembering *where* you rode your bicycle on a particular occasion falls under the declarative category. Research on these differing memory systems suggests that lesions limited to the HPC can produce profound deficits in declarative memory while leaving other memory processes intact (see Milner, Squire and Kandel, 1998 as well as Eichenbaum, 1999).

The exact role that the HPC plays within the memory process is still a highly controversial topic. The sheer number of differing theories in relation to HPC function that have been proposed within the scientific literature supports this statement. A brief synopsis of a few of these theories will provide examples of the diverse thinking in regards to HPC function.

Based upon a review of the clinical literature, Squire, Cohen and Nadel (1984) support Milner's original suggestion that the HPC plays a temporally limited role in memory. That is, recent, but not remote, memories require an intact hippocampus. The HPC is critical for the process of stabilizing long-term memories in the neocortex, a process now commonly referred to as *between-systems consolidation*. Squire, Cohen and Nadel (1984) conjecture that the storage and recall of certain types of memory is initially dependent upon the HPC. Over a given period of time (ranging from days to years) these memories become HPC independent.

There has been widespread scientific investigation in an attempt to support between-systems consolidation theory (Reed and Squire, 1998; Wiltgen, Brown, Talton and Silva, 2004) and much of the research does seem to suggest a temporal gradient that is present for certain forms of memory. Reed and Squire (1998) present information on

several human subjects, two of which (patients A.B. and L.J.) presented with temporally graded amnesia as a result of damage limited mainly to the HPC. Reed and Squire (1998) also report on two more subjects (G.T. and E.P.) who demonstrated a flat temporal gradient. These patients suffered more extensive damage to the medial temporal lobe, suggesting older memories may be consolidated in medial temporal lobe structures adjacent to the HPC. The exact processes underlying between-systems consolidation are still uncertain (see Alvarez and Squire, 1994 for possible mechanisms). Despite this, between-systems consolidation theory remains one of the more widely held accounts of HPC function (Nadel and Moscovitch, 1997; McGaugh, 2000).

The configural association theory proposed by Sutherland and Rudy (1989) states that the HPC is responsible for building configural representations of the environment within which a given organism operates. More specifically, Sutherland and Rudy (1989) posit that the HPC binds together elementary stimulus events, creating unique configural representations that are distinct from the stimulus events themselves. They suggest that the HPC is responsible for acquisition, storage and recall of these configural representations. Conflicting experimental data has required the configural association theory to undergo revision (Rudy and Sutherland, 1995). Modifications to the theory suggest that the HPC still plays a role in configural processing but may not be the locus of the circuitry responsible for encoding associations as originally proposed. In their reappraisal of configural association theory, Rudy and Sutherland (1995) suggest that the HPC is responsible for highlighting differences between configural representations.

Alvarado and Rudy (1995) provide experimental support for configural association theory in their analysis of the ability of rats with HPC lesions to resolve the

transverse-patterning problem. The transverse-patterning problem is a unique form of discrimination in that each stimulus is ambiguous. Typically the transverse-patterning problem is performed as such: A+ vs. B-, B+ vs. C-, and C+ vs. A-. Rats with HPC damage were unaffected in their ability to resolve discriminations in which an elemental solution was present (for example, A+ vs. B-, C+ vs. D-, and E+ vs. F-). However, when the solution required a configural association (as in transverse-patterning) rats with HPC lesions were impaired relative to controls.

In their publication, *The Hippocampus as a Cognitive Map* (1978) O'Keefe and Nadel posit that the HPC contains and employs the cognitive maps first suggested by Tolman (1948). O'Keefe and Nadel build on Tolman's theory, by suggesting the HPC as the location for these cognitive maps, focusing exclusively on spatial navigation. The presence of neurons within the hippocampus that respond specifically to an animal's location within the environment (place cells) provides additional support for the cognitive map theory (O'Keefe and Dostrovsky, 1971; Muller, 1996).

Muller, Kubie and Ranck (1987) provide experimental support for the presence of place cells in an assessment of rats foraging for food in an open field. Rats were trained to search for food pellets scattered on the floor of a cylindrical environment and place cell activity was measured through the use of chronically implanted electrodes. Muller, Kubie and Ranck (1987) report that the activation of place cells is highly correlated with a rats location in a given environment. These place cells are stable over time as long as the environment remains relatively constant.

It is clear that the controversy surrounding the exact function of the HPC in relation to memory function remains. There are numerous theories explaining HPC

function, a limited number of which have been briefly discussed here. A complete review is well beyond the scope of this paper. Perhaps a little less obvious is the fact that a great many of the memories that we encode often contain a degree of overlap. That is, during our lifetime we acquire memories that contain variable amounts of overlap or common elements. This is mirrored in many of the experimental paradigms that are employed to investigate memory function in non-human subjects.

In recalling a specific episode or event, a person is required to differentiate between any number of possibilities before retrieving the correct memory. When asked what you ate for dinner last Thursday, there is an immense amount of overlapping or ambiguous information available, ranging from the time of day, the location of the meal, what you had to drink and so on. There will be in fact memories for multiple meals, many of which share a large number of common features or elements.

This overlap in episodic memory is also demonstrated in experimental paradigms assessing memory function. The Morris Water Task (MWT) provides a good example. The MWT requires a rat to locate a hidden platform in a swimming pool through the use of distal cues around the room. Rats are often trained on multiple sequential platform locations in a given experiment. Despite markedly different platform locations, a high degree of overlap is present (cues, the pool, room lighting, the presence of the investigator, etc). Thus, a given rat is required to temporally differentiate between episodes in a single context, where slightly different views of the identical cues are critical for accurate memory retrieval.

Overlap is also apparent in situations in which configural representations have been constructed. It is often the case that some of the individual elements that produce a

given configural representation are similar if not identical. This is seen in the transverse-patterning problem, A+ vs. B-, and B+ vs. C- share 50% of their individual elements. Configural overlap is not limited to the transverse-patterning problem; for example, semantic information often carries with it a high degree of overlap. The configural representation of a penguin (consisting of elements such as “is a bird,” “lays eggs,” “has wings,” “is flightless,” etc) has much in common with the representation of a black oystercatcher. It is however doubtful that many of us would have any difficulty in discriminating between the two kinds of birds, despite the many elements in common between the two species.

We are now able to make two basic assertions about memory and the HPC:

1. Regardless of differences in theoretical approaches, there is general agreement that the HPC plays a role in certain forms of memory.
2. Memories often share a degree of overlap. This degree of overlap is variable and is dependent upon a multitude of factors, including but not limited to: context, group membership and temporal similarity.

Thus far we have discussed a neural system that is involved in the storage and recall of certain forms of memory. We have also made the claim that many of the memories that the HPC system must encode contain a degree of overlap. In order to deal with this overlap the HPC should facilitate both the storage and recall of information in a manner that minimizes interference. Thus, the HPC should employ mechanisms that increase the efficiency of storage and the accuracy of recall of said information. Pattern separation is a candidate mechanism that serves to directly decrease interference due to common features.

Pattern Separation

Many of the declarative memories we encode contain a degree of overlap that can potentially lead to interference. This ensuing interference can result in faulty encoding as well as the incorrect recall of previously stored information. The idea of interference due to the storage or recall of multiple items that share a degree of overlap is by no means novel and has been well documented in the scientific literature (Marr, 1971; McCloskey and Cohen, 1989; Shapiro and Olton, 1994).

Within the connectionist community the problem of interference has been widely investigated. It has been suggested that overlapping representations within a given neural network may produce what has been termed *catastrophic* interference (Marr, 1971). Thus, in the case of a complex memory we can see that a number of elements need to be encoded. For example, memory for a meeting at work that occurred last week would require you to recall time of day, people present, topic of discussion, location, etc. As has been previously discussed this representation is likely to share some of its elements with other episodic memories. It is this sharing of elements that directly contributes to catastrophic interference. An element shared between two or more representations will increase the likelihood of incorrect recall. As representational overlap increases, so too does interference, eventually leading to a situation in which it is impossible to successfully discriminate between any memory representations.

At the behavioural level, the process of pattern separation allows a given individual to distinguish between two or more events that share a degree of overlap or commonality. In other words, the process of pattern separation serves to directly mitigate the deleterious effects of interference. In order to successfully pattern separate the

differences between similar or overlapping inputs must be highlighted (referred to as orthogonalization). This pattern separation or orthogonalization is typically assumed to occur through strong inhibitory connections and sparse connectivity within the HPC (Gilbert, Kesner and DeCoteau, 1998).

Multiple theories have suggested the HPC as a structure that might serve to reduce interference (Marr, 1971; Shapiro and Olton, 1994; McClelland, McNaughton and O'Reilly, 1995; Rolls, 1996). All of these theories rely on sparse activity of individual neurons within the HPC to produce separated representations resulting in a reduction of interference. The HPC itself contains relatively few active neurons at any given time (O'Reilly and McClelland, 1994). It is this sparseness of activity that these theories contend is responsible for interference reduction. To see why this might be so, it is necessary to discuss hippocampal structure and some of the distinctive properties that differentiate it from other medial temporal lobe areas.

Located in the medial temporal lobe (Figure 1) the HPC receives inputs from a variety of unimodal and polymodal cortical association areas (Kandel, Kupfermann & Iversen, 2000). Many of the inputs into the HPC are channeled through the entorhinal cortex (EC). In turn, the EC projects into the dentate gyrus (DG) via the perforant path. The DG is the main point of entry into the HPC and has been referred to as the "gatekeeper" of HPC input (Westbrook, 2000). The DG projects to the CA3 field via the mossy fibers. From CA3 the Schaffer collaterals project to the CA1 field. Neurons in the CA1 field have projections that terminate in subiculum. Projections arising in the subiculum then project back to entorhinal cortex (via the fornix) and multiple other

subcortical regions (Eichenbaum, 2002). These regions (mainly entorhinal and perirhinal cortices) then project back to the cortical association areas.

Within HPC, connections are largely unidirectional in nature (Amaral, 1995). This is useful in distinguishing the HPC from surrounding structures. Barnes, McNaughton, Mizumori, Leonard and Lin (1990) show that activity levels within the HPC are lower than surrounding cortices. They note that the spontaneous firing activity of cells within the HPC is typically below 1 Hz. Barnes, McNaughton, Mizumori, Leonard and Lin (1990) argue that this relatively low firing rate is suggestive of a functional difference between HPC and surrounding cortical areas. McClelland and Goddard (1996) build on this assertion, stating that lowest activity patterns occur within the DG itself. Activity in the HPC ranges from 0.4% of total neurons active in the DG (at any given time), to 2.5% in CA1 and CA3. In comparison to entorhinal cortex activity (7% of total neurons active), HPC activity is much lower and more sparse. This distinction allows us to further discriminate HPC function from other structures within the medial temporal lobe. For the purposes of this discussion we will consider the HPC as the DG, CA fields and the subiculum.

Shapiro and Olton (1994) point to numerous reports of increased susceptibility to interference in the absence of an HPC. There is evidence from both human and non-human research noting the negative effects of increasing interference. Patient HM, who underwent bilateral resection of the medial temporal lobe (Scoville and Milner, 1957) is reported as successfully recalling lists of numbers by employing rehearsal as a mnemonic strategy. HM was successful at recall as long as interference was kept to a minimum;

increasing the level of interference during the rehearsal phase (thus interfering with his mnemonic strategy) led to forgetting of the sequence of numbers.

Shapiro and Olton (1994) discuss another amnesic patient (KC) who demonstrates similar deficits in tasks with increased levels of interference. KC's brain damage is extensive and includes bilateral lesions of the HPC. Patient KC has recently been subjected to an extensive battery of memory tests (Rosenbaum et. al, in press) including a semantic learning task in which the level of pre-experimental interference was varied. KC was taught to pair novel definitions with specific target words (for example, a talkative featherbrain – PARAKEET). He was then required to recall the target word when presented with the definition in question. Associative interference was varied as a function of the presence or absence of a pre-existing response to the given definition (thus, the presence of a pre-existing response increased the level of interference). Rosenbaum et al. (in press) report that as the levels of pre-experimental interference increase, the ability to successfully learn new semantic information decreases. Rosenbaum et al. (in press) note that this reasoning also applies to normal individuals. Findings from this study provide further evidence that recall is directly affected by experimental interference. Rosenbaum et al (in press) state that the critical factor in the ability of KC to successfully perform a given memory task seems to be the level of interference present during a given learning episode.

Similar findings have been reported in animal studies, and Shapiro and Olton (1994) provide examples of deficits in several tasks with increased levels of interference. They note that in delayed conditional discriminations, concurrent discriminations, and discrimination reversals, HPC damaged rats are impaired when levels of interference are

increased. In the case of delayed conditional discriminations Shapiro and Olton (1994) define interference as the number of stimuli presented during trial onset, noting that interference increases directly as a result of the number of stimuli that are required to be remembered. The correct response at the end of a given trial is dependent upon the initial presentation of the stimuli. With a single discrimination item, interference is minimal and HPC lesions exert little or no effect. As interference (the number of stimuli) increases so do the deleterious effects of HPC lesions. Concurrent discrimination paradigms provide more insight into the effects of interference in HPC lesion animals. Simply put, the more object discriminations that are learned the more interference that occurs. Shapiro and Olton (1994) note that in a one-pair object discrimination paradigm lesions of the HPC did little to affect discrimination ability in rats. However, in an eight-pair object discrimination (higher interference) choice accuracy was greatly reduced. Interference has also been used to explain the deficits suffered by rats with HPC damage during discrimination reversals. HPC lesions impair reversal ability in HPC damaged rats (Shapiro and Olton, 1994). Previous exposure to a set of stimuli increases interference during reversal, and it is this interference that is suggested to cause the impairments seen in HPC damaged rats. The combination of human and non-human findings highlights the possible role that the HPC may play in reducing interference.

Marr (1971) provides us with one of the first attempts to define the function of the HPC. Noting the potential for interference during recall of complex patterns of information, Marr (1971) suggested the HPC as a mechanism to rapidly encode information in a manner that would reduce overlap and avoid what he termed catastrophic interference. Marr (1971) saw the neocortex as the repository for memory

and the HPC as a structure that served to reduce interference during the encoding of information. In Marr's (1971) paper the HPC is regarded as integral for encoding complex patterns in a rapid, separated manner. Similarly, he suggests the HPC also assumes a pattern completion role, recalling entire patterns from partial inputs. Both of these processes permit successful recall from a neocortical network that contains multiple complex patterns of which many share a high degree of overlap. More recent accounts of HPC function have built upon Marr's original theory (for example, McClelland, McNaughton and O'Reilly, 1995; Rolls, 1996).

McClelland, McNaughton and O'Reilly (1995) believe the HPC and neocortex employ differing yet complementary forms of learning. These complementary learning systems work in concert to allow a given individual to encode and employ differing forms of information. They suggest that the HPC system employs a rapid, pattern separated learning rate suitable for encoding episodic memories. The neocortex in turn uses a slower mechanism (referred to as integration), employing overlapping representations that allow it to extract generalities from the environment. McClelland, McNaughton and O'Reilly (1995) argue that the slow integrative nature of neocortical learning allows for generalization across experiences and to novel situations.

O'Reilly and Rudy (2000) propose that pattern separation and the configural association theory originally put forth by Sutherland and Rudy (1989) are two related consequences of sparse encoding. Interestingly, in their reappraisal of configural association theory, Rudy and Sutherland (1995) discuss the ability of the HPC to decrease the similarity between configural units. Clearly, configural association theory and

McClelland, McNaughton and O'Reilly's (1995) complementary learning systems theory are assessing similar processes.

Despite a number of theoretical attempts to define the neural processes underlying pattern separation, limited behavioural research has been conducted on the topic. Much of the work attempting to assess pattern separation as a result of HPC function has been spatial in nature (Gilbert, Kesner and DeCoteau, 1998; Lee, Yoganarasimha, Rao and Knierim, 2004). Gilbert, Kesner and DeCoteau (1998) employed a delayed- match-to- sample paradigm using a dryland version of the MWT. Rats were trained to locate a food reward hidden underneath an object in a specific location in the MWT (sample phase). During the match phase rats were re-introduced to the MWT in which two identical objects were now present, the original (in the same location) and a second object (identical to the original, except for the lack of a food reward) which varied in its spatial separation from the original. Spatial location of the two objects in relation to each other was varied. Gilbert, Kesner and DeCoteau (1998) found that rats with HPC damage were impaired in cases in which the correct and incorrect objects were located in close proximity, suggesting an impairment in spatial pattern separation ability.

The investigation of spatial pattern separation carries with it an unfortunate confound, that of the role of the HPC in spatial function alone. That is to say, in spatial situations in which pattern separation is not explicitly tested the HPC is still critical (Sutherland, Kolb and Whishaw, 1982). To dissociate pattern separation ability from spatial ability during a spatial task is difficult at best. Thus, to isolate pattern separation ability from spatial navigation it is perhaps best to assess pattern separation in a non-spatial domain.

In an attempt to investigate the role of the HPC in pattern separation in a nonspatial task a series of three experiments were conducted. The first was designed to assess the ability of rats with complete lesions of the HPC to perform a visual discrimination in which the positive and negative stimuli varied in regards to cue overlap. The second experiment was conducted to determine if smaller lesions, limited to the DG, would result in a similar pattern of deficits in pattern separation ability in the same visual discrimination task. Due to computational and theoretical research the DG has been postulated to be an integral component of the pattern separation process (McClelland and Goddard, 1996; Norman and O'Reilly, 2003). Lesions limited to the DG should therefore produce deficits in a visual pattern separation task. The third experiment explicitly evaluated the possibility that task difficulty leads to deficits in rats with damage to the HPC or DG.

CHAPTER TWO

Experiment One: Pattern Separation In Rats With Lesions Of The Hippocampus

Previous analyses have suggested that the HPC is a structure suited to perform pattern separation (Marr, 1971; McClelland, McNaughton and O'Reilly, 1995; Rolls, 1996). This is often attributed to the ability of the HPC to produce rapid, separated representations of overlapping inputs (McClelland, McNaughton and O'Reilly, 1995). This rapid, separated encoding makes the HPC suitable for the storage and recollection of episodic memories. The relatively sparse coding by neurons within the HPC is the basis for the hypothesized pattern separation ability (Barnes, McNaughton, Mizumori, Leonard and Lin 1990).

Much of the research into pattern separation has been computational in nature, driven mainly by theoretical network models of HPC function (O'Reilly and Norman, 2002). Recent articles have examined the pattern separation process at the cellular level, assessing the ability of place cells within the HPC to discriminate between environments with overlapping features (Lee, Yoganarasimha, Rao and Knierim, 2004; Leutgeb, Leutgeb, Treves, Moser and Moser, 2004). Results from these studies suggest that place cells produce stable representations of a given environment and can differentiate between two environments that contain even a high degree of contextual ambiguity. Leutgeb, Leutgeb, Treves, Moser and Moser (2004) demonstrate that place cells in the CA3 field of the HPC can differentiate between environments that share a large number of common features. Thus, when tested in the same environmental chamber in differing rooms, different subsets of place cells in the CA3 field are activated, suggesting an ability to differentiate between two very similar contexts.

There has been limited analysis of pattern separation at the level of behaviour. Gilbert, Kesner and DeCoteau (1998) have provided one of the few behavioural descriptions of pattern separation in the spatial domain (see Chapter One). In a non-spatial domain, research by Agster, Fortin and Eichenbaum (2002) has assessed the ability of rats with HPC damage to disambiguate sequences of odours that contain a degree of overlap. Briefly, rats were trained on two odour sequences that overlapped in the middle odour items (both sequences contained the same odours for two trials). Control rats retained the ability to discriminate between the two overlapping sequences of events, while rats with HPC lesions were impaired at differentiating between them. Agster, Fortin and Eichenbaum (2002) contend that rats with HPC damage fail to discriminate between the two odour discrimination sequences as a consequence of the overlap present in the ambiguous middle discriminations. These results are interesting in that they demonstrate pattern separation is dependent upon the HPC in a nonspatial setting.

In order to evaluate the generality of the pattern separation in the behavioural domain, an experiment was designed to assess the behavioural effects of complete neurotoxic lesions of the HPC in a task that requires intact pattern separation ability. Given the previous theoretical, computational, cellular and limited behavioural analyses, we should expect to see deficits in a task that requires HPC damaged rats to discriminate between stimuli that share overlapping features.

Methods

Subjects

Subjects for this experiment were male, Long Evans Black Hooded rats (n = 15). At the outset of the experiment, rats were between 90 and 100 days of age and were experimentally naïve. Experimental subjects were obtained from the University of Lethbridge breeding colony. All rats were group housed (two to three animals per cage) in a 12-hour light/dark cycle. Rats had ad libitum access to food and water for the duration of the experiment. All behavioural testing occurred during the rats' light cycle. All experiments were conducted following the Canadian Council on Animal Care (CCAC) and the National Institute of Health (NIH) guidelines.

Apparatus

The Visual Water Task (VWT, Prusky 2000) was employed for all experimental training and testing sessions (Figure 2). The VWT requires a rat to discriminate between pictures presented on two computer monitors at the ends of the two arms of a Y-shaped swimming pool. One of the pictures is consistently paired with an invisible platform allowing the animal to escape from the water (positive stimulus, S+). Presentation of the pictures is pseudo-randomized such that the picture associated with escape from the pool is just as likely to be on either the left or right side. A constraint is placed on picture presentation such that a cue will be presented for no more than two consecutive trials on the same side. A 46 cm long barrier separates the two monitors at the end of which an invisible choice plane is set. A choice is considered incorrect if the rats' hind legs pass the choice plane in the arm in which the negative stimulus (S-) is presented.

Pre-Surgical Training

All rats were initially trained on a simple visual discrimination. The discriminanda consisted of two shapes. The shapes were displayed simultaneously, one on each monitor. S+ was a ten-point star, S- was a capsule shape (Figure 3A). The shapes were located in a central position on each monitor and took up approximately one third of the entire monitor area. One session was run per day, each session consisting of 10 trials (excluding the first two sessions). In order to introduce the rats to the apparatus, two five trial sessions (one session per day) were initially run. All sessions involved placing the rat in the end of the pool, facing away from the computer monitors. Rats were required to turn 180 degrees and swim toward the positive stimulus in order to escape from the pool. Rats were trained to a criterion of 90% correct per session for five sessions before surgery was performed.

Surgical Procedure

All surgical procedures took place under aseptic conditions. Surgery occurred within 24 hours of the last training session. All rats received 2 mg/kg intraperitoneal (ip) injections of Chlordiazapoxide (CDZ) 15 minutes prior to anesthetic induction. General anesthesia was induced using a 4% concentration of isoflurane and subsequently maintained at a 2% concentration (oxygen at 2 litres per minute) for the remainder of the surgery. Upon induction, the rats head was shaved and the animal was placed in a stereotaxic setup (KOPF® instruments). The scalp was swabbed three times alternately with Hibitane® (4% chlorhexidine gluconate) and alcohol (70% isopropyl) solutions. Using a number 10 scalpel a midline incision of the scalp was made. The periosteum was incised and blunt-dissected away from the skull. Holes were drilled through the skull

employing Bregma as a landmark. Sites were located as follows; 3.1, 4.1, 5, 5.3 and 6 mm posterior to Bregma, \pm 1.5, 3, 3, 5.2, 5 mm medial/lateral at each site respectively. Lesions of the HPC were made by microinjecting N-methyl-D-aspartate (NMDA) at a concentration of 10 mg/ml for 3.5 minutes (0.125 μ l/min) per site at five bilateral sites. Injection depths were determined using Bregma as a landmark. Depths were 3.6, 4, 4, 7.3 and 7.3 mm ventral from Bregma moving from anterior to the posterior of the animal. Injection cannulae were left in place post-infusion for an additional 3.5 minutes to allow for complete diffusion of NMDA away from the tip. Upon completion of HPC lesions, the incision was sutured closed (3-0 coated Vicryl, Ethicon Inc.). Post-surgical care involved the administration of CDZ to control seizure activity (2 mg/kg, as required). Buprinex® (buprenorphine HCl, 0.05 mg/kg) and Somnotol® (sodium pentobarbital, 15 mg/kg) were also administered to minimize pain and discomfort. Rats were allowed to recover in their home cage for a period of seven days before testing proceeded.

Shams

Sham rats received 2 mg/kg of CDZ ip 15 minutes prior to surgery. Sham surgery consisted of opening the scalp at the midline, scraping the periosteum back and clamping the incision open briefly. Animals were sutured closed and placed in recovery. Upon awakening all shams were given 0.05 mg/kg buprenorphine HCl for analgesia. Sham rats were allowed to recover in their home cage for a period of seven days before behavioural testing began.

Post-Surgical Training

Seven days after surgery, rats were reintroduced to the VWT using the original discriminanda. When criterion had been re-attained (90% correct for three consecutive

sessions) the cue size was decreased (Figure 3B). This cue size change was necessary to allow the surrounding cues required for the pattern separation task to fit on the monitors. Rats were retrained to a criterion of 90% correct for three consecutive sessions at which point four surrounding cues were faded in. The fade-in process was performed in five steps (Figure 4) requiring an individual rat to achieve a success rate of three sessions at or above a mean of 90% correct at each step. Upon completion of the fade-in process central cues were replaced such that each monitor now displayed five distinct cues (Figure 5). Rats were required to demonstrate 90% discrimination ability for three consecutive sessions before moving on to the pattern separation paradigm.

Pattern Separation

Pattern separation was divided into four stages with cue redundancy increasing in each successive stage (Figure 6). Criterion was pre-determined as five sessions at or above 90% correct, one session of which was allowed to be below (excluding session one). Pattern Separation One consisted of making the central cue redundant (Figure 6A). Rats were required to perform this discrimination at the pre-determined criterion before continuing on to Pattern Separation Two. Pattern Separation Two (Figure 6B) consisted of the redundant central positive cue and an additional redundant surrounding cue. Again, rats were required to achieve the pre-determined criterion before moving on to the following stage of pattern separation. This methodology was followed until Pattern Separation Four in which only a single point of discrimination remained.

Histology

Upon completion of behavioural testing all rats were perfused transcardially with physiological saline (0.9% NaCl, approximate volume 200 ml) followed by a 4%

Paraformaldehyde (PFA, approximate volume 200 ml) solution. Brains were extracted and stored in a 4% PFA, 30% sucrose solution. Sections were cut on a frozen microtome (Vibratome®) at a thickness of 50 microns. Every fifth section within the HPC was mounted on glass slides and stained with Cresyl Violet. Brains were assessed for both lesion size and location. Lesions varied in size from small to complete bilateral damage of the HPC (Figure 7). Extra-hippocampal structures suffered little damage as a result of the lesion technique.

Results

A total of 11 rats completed the experiment (six sham animals and five HPC damaged animals). Due to spontaneous seizure activity approximately a month and a half post-surgery four HPC damaged rats were dropped from the study. No animals exhibiting seizures were included in the data analysis. All means are reported as plus/minus standard error (SEM). An alpha level of 0.05 was used for all statistical analysis in this experiment.

Post-Surgical Retention

A one-way analysis of variance (ANOVA) revealed that HPC damaged rats required a significantly greater number of sessions to return to pre-surgical performance when compared to Sham rats, $F(1,9) = 11.024$, $p = 0.009$. Mean number of sessions to return to criterion was 1.4 (SEM \pm 0.2) for HPC damaged rats and 0.3 (SEM \pm 0.2) for Shams (Figure 8).

Post-Surgical Training

ANOVA revealed no performance difference between groups in relation to the cue size reduction, $F(1,9) = 2.438$, $p = 0.153$. On average HPC rats took 6 (SEM \pm 1.45) sessions to return to criterion with Shams taking a mean of 3.17 (SEM \pm 1.14) sessions to achieve criterion.

A significant difference was observed between Sham and HPC groups in regards to surround fade-in (Figure 9). HPC rats spent more sessions below criterion when compared with Shams, $F(1,9) = 8.532$, $p = 0.017$. HPC rats spent an average of 18.2 (SEM \pm 6.6) sessions below the criterion threshold, with Sham rats spending 0.8 (SEM \pm 0.3) sessions below threshold.

The groups did not differ significantly during central cue replacement, $F(1,9) = 0.982$, $p = 0.348$). HPC rats required a mean of 4 (SEM \pm 1.3) sessions to successfully resolve central cue replacement, whereas Sham rats required 2 (SEM \pm 1.48) sessions to meet criterion.

Pattern Separation

Pattern Separation One took HPC rats significantly more sessions to resolve than Sham rats, $F(1,9) = 5.945$, $p = 0.037$, with HPC damaged rats taking an average of 8.4 (SEM \pm 3.0) sessions and Sham rats requiring an average of 1.2 (SEM \pm 0.5) sessions (Figure 10A).

There was a trend for rats with HPC lesions to take more sessions to resolve Pattern Separation Two (Figure 11A), although these results were not significant, $F(1,9) = 3.864$, $p = 0.081$. During Pattern Separation Two HPC rats took on average 2.2 (SEM \pm 0.89) sessions to return to criterion and Sham rats spent 0.33 (SEM \pm 0.33) sessions

below criterion. This trend disappeared in Pattern Separation Three, $F(1,9) = 3.146$, $p = 0.11$ (Figure 12). HPC rats required a mean of 10.8 (SEM \pm 3.06) sessions to resolve Pattern Separation Three, with Shams taking 5.17 (SEM \pm 1.42) sessions to return to criterion.

To further elucidate the deficits observed in HPC damaged rats a repeated measures ANOVA was conducted to determine if mean discrimination ability differed significantly between groups over the first five days of each stage of pattern separation. The repeated measures ANOVA revealed a significant difference between groups during Pattern Separation One, $F(1,9) = 5.405$, $p = 0.045$, with Sham rats out performing HPC damaged animals (90.0 SEM \pm 1.9 and 79.6 SEM \pm 2.7 respectively, Figure 10B).

Repeated measures ANOVA comparing mean discrimination ability for Pattern Separation Two showed a trend for HPC damaged rats to perform worse than controls over the first five sessions, $F(1,9) = 3.57$, $p = 0.091$ (Figure 11B). However, this trend disappeared in Pattern Separation Three, $F(1,9) = 0.875$, $p = 0.374$. With the exception of a single HPC damaged rat, no animals were able to resolve Pattern Separation Four.

Discussion

After learning to discriminate between two composite visual arrays, rats with an intact hippocampus are unaffected by making identical one of the visual elements in arrays. Discrimination performance by rats with nearly complete neurotoxic lesions of the HPC are impaired by adding a common visual element. HPC damaged rats were able to eventually resolve the discrimination with one common visual element at the same level of performance as intact animals. This suggests that rapid and flexible responding to pattern ambiguity is dependent upon the HPC, but that other brain structures can

satisfactorily perform pattern separation function. These behavioural findings are counterintuitive to neural network models that suggest catastrophic interference in the absence of the HPC (Marr, 1971; French, 1999).

The same trend continued when the two arrays contained two identical elements, with Sham animals displaying a mild effect of increasing cue redundancy. HPC rats demonstrated a larger deficit although results were just beyond significance. Adding a third identical element affected performance by the two groups of rats equally. This lack of significance difference may be a result of the relatively small group size. It is important that in all cases of adding common cues, the rats with HPC damage were affected more than control rats.

An alternative hypothesis is that as rats become more experienced at the VWT, they become better at performing visually based discriminations. The extensive pre-surgical training, as well as previous experience with a pattern separation task may mitigate the effects of cue overlap during Pattern Separation Two and Three. What is important is that despite this pre-training, rats with complete HPC damage experience difficulties in situations involving feature ambiguity.

Statistical analysis revealed a small but significant impairment in the post-surgical retention of the initial visual discrimination. Rats with HPC damage were able to successfully relearn the initial visual discrimination as well as subsequent visual discrimination problems. This ability of rats to learn visual discriminations after HPC damage has been reported previously (Epp, Keith, Prusky, Douglas and Sutherland, 2004). These findings suggest that both the relearning of the initial discrimination and the ability to learn any subsequent visual discriminations are dependent upon extra-

hippocampal neural networks. Since this phenomenon is not the central focus of this study, a lack of appropriate follow-up experiments makes it difficult to make any concrete claims (see Sutherland et al., 2001 for a discussion of visual discrimination learning after HPC damage).

Interestingly, rats with HPC damage spent significantly more sessions below criterion than control rats during the process of gradually increasing the brightness of cues that surround the central cue (Figure 9). One possible explanation for the deficit by HPC rats could be that cue novelty interferes with accurate discrimination. However, HPC rats were able to discriminate successfully with other types of cue novelty, for example during cue size change and central cue replacement. Thus the deficit seen during surround cue fade-in is likely not to be due to cue novelty *per se*. Some other aspect of the fade-in process, possibly distraction of spatial attention to uninformative portions of the monitors may be responsible for the deficit that is observed.

It should be noted that some extra-hippocampal damage was apparent as a result of the lesion technique (Figure 7). This damage was minimal but included thinning of cortex and some damage to adjacent structures. It is therefore impossible to conclusively state that the deficits displayed by HPC damaged rats are a result of damage to the HPC alone. It is however a more parsimonious explanation that the deficits suffered by rats with HPC damage are a result of damage to this particular structure.

We can conclude that complete neurotoxic lesions of the HPC produce at least transient deficits in a visual discrimination task in which cue overlap is a feature and the magnitude of the deficit does not appear to increase with increasing cue ambiguity. These

findings offer some support to theories that suggest that the HPC acts as a pattern separator (O'Reilly and McClelland, 1994).

CHAPTER THREE

Experiment Two: Pattern Separation In Rats With Lesions Of The Dentate Gyrus

The results from Experiment 1 indicate that complete neurotoxic lesions of the HPC produce at least transient deficits in a visually based discrimination task that uses overlapping stimuli. There is theoretical and computational evidence that suggests pattern separation may not be a function of the entire HPC (McClelland, McNaughton, and O'Reilly, 1995). To further examine the relationship between HPC regions and the deficit in a pattern separation task an experiment was designed to measure the effects of lesions limited to the dentate gyrus (DG) on pattern separation ability. The DG has been proposed as an ideal subfield within the HPC to separate overlapping inputs (McClelland and Goddard, 1996; Norman and O'Reilly, 2003).

Norman and O'Reilly (2003) argue the sparseness of encoding within the DG gives rise to the ability of the HPC to pattern separate. This idea is similar to McClelland and Goddard's (1996) proposal that the pathway from entorhinal cortex (EC) to area CA3 (via the DG) is responsible for the separation of overlapping inputs arising in the neocortex. Any interruption of DG function should therefore diminish the ability to successfully pattern separate.

As of yet very few lesion studies have been conducted to test this assumption at the behavioural level. Gilbert, Kesner and Lee (2001) provide evidence that the DG is involved in mediating pattern separation in the spatial domain. Using the same experimental paradigm as employed by Gilbert, Kesner and DeCoteau (1998, see Chapter One), Gilbert, Kesner and Lee (2001) report that DG lesions impair pattern separation ability. DG animals were impaired at differentiating reward locations when the spatial

separation between the objects covering the food wells was small. Limiting pattern separation to the spatial domain still carries with it the same confound that has been previously addressed (Chapter One). Lesions of the DG have been shown to cause deficits in other tasks within the spatial domain (Xavier, Oliveira-Filho and Santos, 1999; Jeltsch, Bertrand, Lazarus and Cassel, 2001). Thus, in hopes of dissociating pattern separation ability from the spatial domain we tested animals with lesions limited to the DG in the same task used to assess pattern separation ability in HPC damaged rats. Given the theoretical and limited behavioural analysis we expect to see similar deficits in rats with lesions of the DG.

Methods

Subjects

Eleven male, experimentally naïve, Long Evans Black Hooded rats were used for this experiment. All housing and testing conditions were identical to the previous experiment. Testing was performed in accordance with CCAC and NIH guidelines.

Apparatus

As in Experiment 1, the VWT (Figure 2) was employed for all behavioural training and testing sessions.

Pre-Surgical Training

All pre-training procedures were identical to those used in Experiment 1. Initial discriminanda were identical to those employed previously (Figure 3A). As in Experiment 1, rats were trained to a criterion of 90% correct per session for a period of five sessions before any surgical procedures were initiated.

Surgical Procedure

General surgical procedures were similar to those outlined in Experiment 1. Methodology differed slightly in respect to lesion production. As in Experiment 1 all surgical procedures took place under aseptic conditions within 24 hours of the last training session.

Dentate Gyrus Lesions

All rats undergoing surgery received 2 mg/kg CDZ ip approximately fifteen minutes prior to induction. Induction was performed using a 4% concentration of isoflurane. Rats were maintained at a concentration of 2% isoflurane for the remainder of the surgical procedure. DG lesions were made by microinjecting colchicine (1.5 mg/ml) at three bilateral sites. Bregma was employed as a reference to determine location of the injection sites. Sites for injection were as follows, 3.3, 4.8 and 5.8 mm posterior to Bregma, \pm 1.5, 3.2, 5 mm lateral to Bregma. Depths were 3.7, 4.2 and 7.5 mm ventral to Bregma respectively. Injection time was four minutes per site at a rate of 0.125 μ l/min with the cannulae remaining in place for an additional four minutes post-injection to allow any remaining colchicine to diffuse away from the tip. Due to the lack of seizures, post surgical care involved only the administration of Buprinex® (buprenorphine HCl, 0.05 mg/kg). Rats were allowed to recover for a period of ten days in their home cage before testing commenced.

Shams

All Sham surgeries were conducted in the same manner as those in Experiment 1. Sham animals were allowed to recover for ten days in their home cage before behavioural testing began.

Post-Surgical Training

The post-surgical training procedure was identical to that as in Experiment One. Criterion measures were kept identical to those outlined previously.

Pattern Separation

The pattern separation paradigm was run in exactly the same manner as previously described in the design of Experiment 1 (see Figure 6 for an example of the pattern separation task). All criterion measures were kept constant from the previous experiment.

Histology

Histological procedures were identical to those described in Experiment 1. As previously discussed, brain tissue was analyzed for lesion location and completeness. Colchicine lesions produced extensive damage to the DG. Damage to extra-hippocampal structures was minimal. Some damage to adjacent HPC subfields other than the DG was evident (Figure13).

Results

Four Sham rats and seven DG rats completed Experiment 2. As in Experiment 1 an alpha level of 0.05 was employed for all statistical analysis, all means are reported as plus/minus SEM.

Post-Surgical Retention

ANOVA revealed no significant difference between Sham and DG rats in the number of sessions required to return to pre-surgical levels of discrimination performance, $F(1,9) = 3.013$, $p = 0.117$. DG rats required a mean of 2.71 (SEM \pm 1.04)

sessions to return to pre-surgical levels of performance with Sham rats taking 0.25 (SEM \pm 0.25) sessions to achieve criterion.

Post-Surgical Training

Cue size reduction performance did not differ significantly between the two groups, $F(1,9) = 0.266$, $p = 0.619$ with Sham and DG groups taking 3.25 (SEM \pm 2.36) and 2.14 (SEM \pm 1.04) sessions to resolve the cue reduction paradigm respectively. DG rats also did not differ significantly from Shams in the number of sessions spent below criterion during the surround fade-in process, $F(1,9) = 0.511$, $p = 0.493$. Mean number of sessions spent below criterion was 6.5 (SEM \pm 1.32) for Shams and 8.43 (SEM \pm 1.86) for DG rats. No significant difference was noted between groups during the central cue change, $F(1,9) = 1.573$, $p = 0.241$, with both Sham and DG groups requiring a mean of less than a single session to resolve the discrimination (0.5, SEM \pm 0.29, and 0.14 SEM \pm 0.14, respectively).

Pattern Separation

Statistical analysis of Pattern Separation One revealed no significant difference between the two groups, $F(1,9) = 0.113$, $p = 0.744$, with Sham rats taking 0.75 (SEM \pm 0.48) and DG rats taking 0.57 (SEM \pm 0.30) sessions to achieve criterion (Figure 14). No significant difference between groups was found during Pattern Separation Two, $F(1,9) = 0.299$, $p = 0.602$. Sham animals took a mean of 0.5 (SEM \pm 0.5) sessions to achieve criterion with DG animals taking on average 2 (SEM \pm 0.97) sessions to return to criterion performance (Figure 15). Statistical analysis of Pattern Separation Three yielded no significant differences between Sham and DG rats, $F(1,9) = 0.281$, $p = 0.615$, (Figure 16) with Sham and DG animals taking 4 (SEM \pm 2.61) and 6 (SEM \pm 1.27) sessions to

resolve the discrimination respectively. No animals were able to resolve Pattern Separation Four.

Discussion

Due to the findings in Experiment 1, it was hypothesized that similar deficits during the visual pattern separation task would be apparent in rats with lesions limited to the DG. This prediction is supported by theoretical assessment of DG function (McClelland and Goddard, 1996; Norman and O'Reilly, 2003).

Intact animals performed in a similar manner as to those in Experiment 1. With the introduction of a single redundant cue in Pattern Separation One, their behaviour was relatively unaffected. Pattern Separation Two saw little change in discrimination performance by intact rats, although the introduction of a third redundant cue in Pattern Separation Three produces an obvious increase in the number of trials to return to criterion. Importantly, rats with DG lesions were unimpaired in relation to control rats during the each of the pattern separation phases. Damage produced by colchicine injections was extensive and similar to the damage reported in several other studies assessing DG function (Xavier, Oliveira-Filho and Santos, 1999; Jeltsch, Bertrand, Lazarus and Cassel, 2001). These investigators all report impairments in behavioural tasks thought to be DG dependent. Thus, it seems unlikely that the damage that was produced in the current experiment was insufficient to disrupt DG function. Upon initial investigation, this finding disconfirms the hypothesis that the DG is the essential portion of the HPC system in which overlapping representations are separated.

Statistical analysis revealed no significant differences between DG and Sham groups in regards to post-surgical retention testing. This is surprising given the marked

difference in means (2.71 versus 0.25 sessions to return to criterion for DG and Sham animals respectively). The high variance found in the DG group may in part be responsible for this finding. Given this high variance no concrete claims relating to this behavioural finding can be made.

It seems we are initially forced to conclude that pattern separation ability is not dependent upon the DG. However, this conclusion may not be warranted. Given the effects of complete HPC lesions on rats in the pattern separation paradigm (Experiment 1) together with the theoretical (McClelland, McNaughton and O'Reilly, 1995) and computational research (French, 1999) selecting the DG as an ideal candidate for pattern separation a closer examination may be justified (see Table 1 for a comparison of deficits between HPC and DG damaged animals).

Given the impairments in Experiment 1 we can assume that the HPC plays an important role in pattern separation ability. The findings from Experiment 2 suggest that the ability to pattern separate may depend upon subfields other than the DG (CA3 or CA1). An alternative hypothesis is that rats performing the visual pattern separation task may not actually be pattern separating. It is possible to resolve this visual discrimination paradigm through the process of pattern completion. Simply put, pattern completion is the ability to recall a complete representation from a partial input. If both pattern completion and separation are processes that arise in the HPC, one would expect to see deficits in this task given a complete lesion paradigm. These are the results obtained in Experiment 1. Removal of the DG alone does not eliminate the proposed circuitry responsible for pattern completion (specifically CA 3 and CA 1), thus leaving discrimination ability of overlapping stimuli relatively intact.

A third alternative is that the deficits observed in previous experiments (specifically Experiment 1) are not a result of cue overlap (and thus not a result of a failure to pattern separate or complete). A possible explanation is that task difficulty exerts a negative effect on visual discrimination ability that is heightened in rats with lesions of the HPC. Thus, the deficits observed during the pattern separation paradigm may actually be an artifact of increasing task difficulty as opposed to a failure to pattern separate or complete. There is limited support within the scientific literature that suggests that the effects of task difficulty are magnified in rats with lesions of the HPC (Beylin et al., 2001).

CHAPTER FOUR

Experiment Three: Task Difficulty And Selective Lesions Of The Hippocampus

Given the results from the initial experiment and the prediction made in regards to the second, a third experiment was conducted to determine if rats with lesions of HPC or DG are impaired relative to Shams in a task that constantly increases in difficulty.

The results from Experiment 1 suggest that rats with complete lesions of the HPC are impaired in the VWT even when cue overlap is minimal. This impairment is likely due to the initial inability of rats with complete neurotoxic HPC lesions to pattern separate. An alternative explanation is that rats with lesions of the HPC are affected to a greater extent by task difficulty when compared to intact sham animals. It may not be the ambiguous nature of the cue *per se* that accounts for the deficit in discrimination performance during tasks that require pattern separation.

In their 2001 paper, Beylin et al. report that the HPC may become engaged simply as a result of increasing task difficulty. Beylin et al. (2001) note that when compared to a delay conditioning paradigm, trace conditioning takes many more trials to learn. They argue this is representative of the inherently difficult nature of trace conditioning. Their results suggest that animals with HPC lesions are impaired in the acquisition of a trace conditioning paradigm. In addition, when the delay conditioning paradigm is made more difficult, rats with HPC lesions experience significantly greater impairments than intact animals. Damage to the HPC impairs the acquisition of both trace and difficult delay paradigms in relation to intact animals. This evidence suggests that the HPC may become engaged as a function of the difficulty of the task itself.

In contrast to the results found by Beylin et al. (2001), Agster, Fortin and Eichenbaum (2002) report that rats with selective lesions of the HPC do not differ from controls in difficult tasks that place heavy demands on memory. In a study assessing paired associate learning, Bunsey and Eichenbaum (1996) find no difference in acquisition rate between intact rats and those with HPC lesions. In a similar task Dudchenko, Wood and Eichenbaum (2000) find no difference between lesion rats and shams in a task requiring them to recognize a list of up to 25 distinct odours. The information conveyed from these articles suggests that rats with lesions limited to the HPC can still acquire difficult information at a rate and magnitude similar to intact animals.

The relevant HPC lesion literature is clearly divergent as to whether or not task difficulty may account for some of the behavioural deficits that have been observed in HPC lesion rats. To test the possibility that task difficulty may exert a negative effect in the visual pattern separation paradigm (Experiment 1 and 2) that is exaggerated by lesions limited to the HPC, an experiment was run that required rats to resolve increasingly difficult visual discriminations.

Methods

Subjects

The subjects for this experiment were 18 experimentally naïve male, Long Evans Black Hooded rats. All subjects were between 90 and 100 hundred days of age at the onset of this experiment. Housing and testing conditions were the same as previously discussed in Experiments 1 and 2, all testing and surgical procedures followed CCAC and NIH guidelines.

Apparatus

The VWT (Figure 2) was employed for all behavioural training and testing. The apparatus was the same as described in the previous experiments.

Pre-Surgical Training

All the procedures used for pre-training in Experiment 3 were identical to those previously described in Experiments 1 and 2. The stimuli used for pre-training during Experiment 3 were the same as employed previously (Figure 3A).

Surgical Procedure

Surgical procedures involved NMDA induced HPC lesions (n = 6), colchicine lesions of the DG (n = 6) and Sham surgeries (n = 6). All surgeries were conducted using the same methodology previously described. All surgical procedures were conducted under aseptic conditions within 24 hours of the last behavioural session. Post-surgical recovery periods were identical to those as listed in Experiments 1 and 2.

Fade-Out Paradigm

After recovery from surgery, all rats were reintroduced to the original cues in the VWT (Figure 3A). Upon achieving the criterion of 90% correct for three consecutive sessions, the original cues were faded out over a series of steps in order to increase the overall difficulty of the task (Figure 17). Each step involved reducing the contrast between the stimuli and the background by 10% (initial cues at 100% contrast, final cue contrast at 10%, for a total of nine steps). A given rat experienced three sessions (10 trials per session, for a total of 30 trials) at each stage of contrast. Mean % correct was determined at each stage. If mean % correct was at or above 90% for three sessions, the rat was moved on to the next stage of fade-out. A rat was considered to have failed to

discriminate if performance dropped below 90% for two consecutive levels of contrast (for example, an animal that dropped below criterion at 60% contrast and then 50% contrast was considered to have failed to discriminate at a contrast of 60%). When a given animal returned to above criterion performance the first dropout was considered an artifact (thus an animal that failed at 60% but discriminated at 50% would not be removed). After a failure to discriminate, rats were re-run at a contrast of 100% to ensure the deficits seen were due to difficulty and not a general inability to reconcile the discriminanda. Criterion was the same as employed for the fade-out paradigm (mean of 90% correct over three consecutive sessions).

Histology

All animals were perfused using the same protocol as previously discussed in Experiments 1 and 2. Tissue was processed with Cresyl Violet and lesion size and location was determined, lesions were similar to those produced in Experiments 1 and 2 (Figures 7 and 13, respectively).

Results

Seventeen rats completed the task difficulty experiment. Five HPC animals, 6 DG animals and 6 Sham animals were included in statistical analysis. A single HPC animal failed to resolve the post-surgical discrimination and was subsequently removed from the study. All statistical analysis was conducted using an alpha level of 0.05.

Post-Surgical Retention

A one-way ANOVA showed a significant difference between groups (Sham, HPC and DG) in the number of sessions to return to pre-surgical performance (Figure 18), $F(2,14) = 5.859$, $p = 0.014$. Further post-hoc analysis (Fisher's LSD) revealed DG rats took

significantly more sessions to resolve post-surgical retention when compared to Shams, $p = 0.004$. Group means for number of sessions required to return to criterion were as follows: Sham 0.33 (SEM \pm 0.33), HPC 2.8 (SEM \pm 0.73), DG 5 (SEM \pm 1.46).

Fade-Out Paradigm

ANOVA revealed no differences between HPC, DG or Sham rats in regards to discrimination ability during the Fade-Out Paradigm, $F(2,14) = 0.588$, $p = 0.569$ (Figure 19). Sham animals failed to discriminate at a mean of 25 % contrast (SEM \pm 4.28). HPC rats lost discrimination ability at a mean of 34 % contrast (SEM \pm 6.78), with DG rats losing discrimination ability at a mean contrast of 30 % (SEM \pm 6.3). Due to the lack of significance no *post hoc* analysis was performed. All rats performed above criterion during re-exposure to the initial stimuli (contrast at 100 %), displaying an intact ability to discriminate.

Discussion

The results from Experiment 3 suggest that task difficulty does not differentially affect rats with lesions limited to the HPC or DG. We can therefore conclude that the deficits displayed by HPC damaged rats in Experiment 1 were due to some other factor than increasing task difficulty. A pattern separation deficit is an alternate explanation for the effects we observe in HPC damaged rats in Experiment 1.

The results reported in Experiment 3 are in agreement with several studies that have assessed the interaction between task difficulty and HPC damage (Bunsey and Eichenbaum, 1996; Dudchenko, Wood and Eichenbaum, 2000; Agster, Fortin and Eichenbaum, 2002). This task involved a gradual increase in the level of task difficulty, which effectively simulates the pattern separation paradigm that was employed. The

findings reported here strengthen the argument that the HPC is required for tasks that involve ambiguity or cue overlap.

These results differ from those reported by Beylin et al. (2001), but due to agreement with several other studies assessing task difficulty (as mentioned above) it is likely that the nature of the deficit discovered by Beylin et al. (2001) is limited to the specific task they employ. This assessment, combined with the results from other research suggests that the lack of an interaction between task difficulty and a disruption of HPC function is a generalized phenomenon.

An interesting impairment in regards to the retention of initial discrimination cues after HPC or DG damage was observed. Statistical analysis reveals an impairment in memory for visual stimuli within the DG group when compared to Sham animals, HPC and Sham animals did not differ significantly in regards to retention performance. This is surprising given the smaller lesion size in the DG group when compared to complete HPC lesions. This finding seems to negate suggestions that retrograde amnesia correlates positively with lesion size (Nadel and Moscovitch, 1997). The results from Experiment 2 found no significant difference between DG and Sham animals in retention for visual stimuli (despite markedly different mean scores). These between study transient deficits warrant further investigation.

CHAPTER FIVE

General Discussion

Experiment One

The results from Experiment 1 demonstrate that the HPC facilitates discrimination ability in a task that involves differentiating between visual stimuli with overlapping elements. Rats with lesions limited to the HPC experienced at least transient deficits in our pattern separation paradigm. These findings add support to theories that posit the HPC as an important structure for pattern separation ability (Marr, 1971; McClelland, McNaughton and O'Reilly, 1995; Rolls, 1996). These results are congruent with the findings put forth by Agster, Fortin and Eichenbaum (2002), in their assessment of HPC damaged rats inability to distinguish between events that are ambiguous in nature.

Interestingly, rats with HPC lesions were eventually able to resolve the pattern separation paradigm at all levels with the exception of Pattern Separation Four (as was the case with Sham animals). This same phenomenon is noted by Agster, Fortin and Eichenbaum (2002) who report that rats with radiofrequency lesions of the HPC are able to relearn a task in which sequence disambiguation was required.

The results from Experiment 1 suggest that when an intact HPC system exists, it is the site at which pattern separation takes place. When HPC function is disrupted, an initial failure to pattern separate occurs. These pattern separation deficits are likely due to the inability of other systems to initially pattern separate in the absence of the HPC.

The interference observed due to HPC specific damage while initially severe is not the 'catastrophic interference' suggested by Marr (1971), or French (1999). Rats with lesions of the HPC were eventually able to successfully discriminate between stimuli that

shared as much as 60% of their individual cues in common (Pattern Separation Three, Figure 6C). The milder form of overall interference that is evident may in part be due to the extensive pre-training that is required to prepare rats to perform the pattern separation paradigm. This is supported by the tendency for HPC damaged rats to demonstrate less of an impairment as the pattern separation task progresses. Agster, Fortin and Eichenbaum (2002) report a similar finding in rats with radiofrequency lesions limited to the HPC. They observe that HPC damaged rats are able to discriminate between ambiguous events after extensive postoperative training. A possible explanation for this occurrence is that HPC damaged rats exposed to the pattern separation task may be demonstrating the learning set phenomenon initially suggested by Harlow (1949). There is evidence for intact learning set ability in rats with damage limited to the HPC (Eichenbaum, Fagan and Cohen, 1986; Epp, Keith, Prusky, Douglas and Sutherland, 2004).

A significant reduction in pre-training should serve to increase the severity of the pattern separation deficit demonstrated by HPC damaged animals. Alternatively, increasing the overlap during the initial presentation of our pattern separation paradigm (for example presenting two redundant cues initially as opposed to one) should also serve to intensify the deficits experienced by HPC damaged rats.

During the surround cue fade-in process (Figure 4) HPC rats spent significantly more sessions below criterion than corresponding Sham animals (Figure 8). This impairment is a curious deficit and warrants some brief discussion. Several novel discrimination cues were presented (for example cue size reduction, Figure 3B and central cue change, Figure 5) during the post-surgical training period. HPC damaged rats were able to deal successfully with both cue size reduction and central cue replacement

and were unimpaired in comparison to Sham rats. Thus, we must assume the deficits displayed by HPC damaged animals during the surround fade-in process are the result of something other than the novelty of the discrimination. Two possibilities may explain this phenomenon.

It is well documented that HPC damage produces spatial deficits in the rodent (Sutherland, Kolb and Whishaw, 1982; Clark, Broadbent and Squire, 2005). Both the cue size reduction and central cue replacement involve changes to the discriminanda that occur at the same spatial location as the previous discrimination cue. Prior to pattern separation, the surround cue fade-in is the only learning set in which changes to the discrimination stimuli occur at a location other than the center of the computer monitor. The surround cue fade-in introduces a novel spatial dimension to the discrimination paradigm. HPC damaged rats in our visual discrimination task may experience deficits as a result of the introduction of this spatial novelty.

A second possibility is that HPC damage produces deficits in the acquisition of stimuli with increased complexity. The cue fade-in process is unique in that a simple cue is made more complex by the gradual introduction of additional information. This phenomenon has not yet been investigated and requires further examination.

The results from Experiment 1 provide convincing evidence that the HPC is responsible for pattern separation in tasks that involve common elements. This initial assessment of impaired pattern separation ability in rats with neurotoxic lesions of the HPC prompted an examination of the ability of rats with colchicine lesions limited to the DG to perform the same visual pattern separation task.

Experiment Two

The results of Experiment 1 suggest the HPC is the system within the brain responsible for separating events that share a degree of overlap. Within the HPC itself, the DG has been hypothesized as the critical structure for the separation of overlapping inputs (McClelland and Goddard, 1996). This hypothesis arises due to the sparse encoding that occurs within the HPC and specifically within the DG (Barnes, McNaughton, Mizumori, Leonard and Lin, 1990). Sparse encoding allows incoming inputs that share common features to be represented with minimal overlap.

Surprisingly, rats with lesions limited to the DG displayed no behavioural deficits during the entire pattern separation paradigm. Histological analysis revealed significant reduction in DG granule cell thickness and density as well considerable loss of DG volume (Figure 13). These results are consistent with reports of colchicine-induced deficits in tasks thought to be dependent upon intact DG function (Xavier, Oliveira-Filho and Santos, 1999; Jeltsch, Bertrand, Lazarus and Cassel, 2001). At first, this finding seems to refute any claims that DG function is critical for encoding situations in which overlap is a feature (for example, McClelland and Goddard, 1996; Rolls, 1996; Norman and O'Reilly, 2003).

This finding, in combination with the results from Experiment 1, suggests one of three possibilities. The first is that pattern separation is dependent upon the entire HPC. In this case, we might expect to see graded deficits, with pattern separation ability becoming worse as lesion size increases. No such graded deficits are apparent in animals with almost complete destruction of the DG.

A second possibility is that pattern separation is not a function of the DG, but instead relies upon other HPC subfields (CA3 or CA1). There are theoretical and experimental reasons to deny this possibility. The DG is the initial site at which overlapping inputs from the neocortex (via EC) are projected. Additionally, the sparse nature of encoding makes the DG ideal for pattern separation tasks. Thus, many of the theoretical approaches to pattern separation believe the DG to be the site at which the separation of overlapping inputs occurs. Experimental support for this is limited and somewhat indirect, but several papers have reported the DG as the site for pattern separation (Gilbert, Kesner and Lee, 2001; Lee, Yoganarasimha, Rao and Knierim, 2004; Leutgeb, Leutgeb, Treves, Moser and Moser, 2004).

A final possibility is that a resolution to the pattern separation tasks exists that does not require intact pattern separation ability, yet still requires an intact HPC. The effects of task difficulty can be ruled out (see Experiment 3). There are two processes within the HPC that benefit from sparseness of encoding, pattern separation and pattern completion. Pattern separation, as mentioned previously, is commonly thought to be DG dependent. The ability to pattern complete is typically associated with the CA fields. Pattern completion permits successful recall of a previously encoded representation given only a partial input. Sutherland, McDonald, Hill and Rudy (1989) have shown that rats with extensive damage to the HPC are impaired in tasks that require pattern completion. In a nonmatching-to-sample task rats with HPC lesions were impaired relative to controls when only partial cues were presented. Briefly, nonmatch-to-sample involves two explicit phases of testing (a sample phase and a choice phase). In the sample phase, rats are presented with a single cue. The choice phase requires rats to discriminate between the

previously encountered cue (sample phase) and a novel cue. In the nonmatch-to-sample paradigm the rat is rewarded for choosing the novel cue. Prior to surgery, Sutherland, McDonald, Hill and Rudy (1989) trained rats in a nonmatch-to-sample paradigm involving configural cues, each composed of two elements (one odour based and one visually based: for example, black-patchouly, white-almond, and striped-aftershave). When one modality was presented in the sample phase (for example, black) and a different presented in the choice phase (for example, patchouly vs. almond) rats with HPC were impaired relative to shams. Thus, with 50% of cues available rats with HPC damage demonstrate impairments in pattern completion ability relative to intact animals. This finding provides support for both configural association theory and pattern completion as a function of the HPC.

One can see that the redundant cues present in the pattern separation paradigm degrade the original stimuli such that only a partial representation of the original pattern exists. Thus, with a redundant central cue only four of the five original cues can be used to successfully discriminate between the two computer monitors. We now have a situation in which a partial input is present. Given the fact that the CA fields are left relatively intact, pattern completion ability should be undisturbed. Unfortunately at the behavioural level, it is impossible to differentiate between pattern separation and pattern completion. This is an unfortunate consequence of our discrimination task and limits our claims to the ability to successfully deal with overlapping stimuli.

Experiment Three

The behavioural paradigm assessing the effects of increasing task difficulty on discrimination ability revealed no significant differences between HPC, DG or Sham rats. On this basis we can conclude that the discrimination problems displayed by HPC damaged rats during Experiment 1 are not likely due to an increase in task difficulty. This finding supports our claim that HPC damaged rats are impaired in visual discriminations that contain a degree of overlap because of a failure to pattern separate/complete successfully.

The results from Experiment 3 are congruent with several studies that have assessed the ability of rats with HPC damage to learn and remember difficult tasks (Bunsey and Eichenbaum, 1996; Dudchenko, Wood and Eichenbaum, 2000; Agster, Fortin and Eichenbaum, 2002). These studies report findings from tasks dependent upon other modalities (specifically odour), suggesting this phenomenon is not limited to visual discriminations. The null effect of task difficulty on the behaviour of HPC damaged rats is therefore likely to be a generalizable phenomenon.

These results however, are disparate from those obtained by Beylin et al. (2001) in their auditory-based trace conditioning paradigm. Their finding may be due to something unique about auditory conditioning, although the generalization of these current findings to other domains such as odour (Agster, Fortin and Eichenbaum, 2000) weakens this suggestion. Instead, the conflicting reports surrounding task difficulty may be a consequence of how task difficulty is introduced.

The experimental paradigm that was employed involved a gradual introduction of task difficulty. A series of steps were required to move from the least to most difficult

visual discrimination (see Figure 19 for examples). This gradual increase in task difficulty is reasonable given the gradual nature of our pattern separation paradigm.

Beylin et al. (2001) assess the possible deleterious effects of task difficulty with the introduction of a single difficult episode. This methodology results in deficits in rats with lesions of the HPC. The paradigm used in the current study differs in regards to the methodology employed to introduce task difficulty (gradual versus a single rapid increase). It is possible that the gradual introduction of task difficulty allows a HPC damaged rat to successfully deal with the more difficult visual discrimination. This suggests that the remaining intact neocortical system may be able to successfully adapt to difficulty given a gradual enough introduction. This possibility is reminiscent of the slow versus fast learning systems posited by McClelland, McNaughton and O'Reilly (1995, see also O'Reilly and Rudy, 2000). The two task difficulty experiments may in fact be assessing the abilities of two different learning systems. The HPC dependent system which McClelland, McNaughton and O'Reilly (1995) argue employs a rapid learning rate, possibly allowing an intact rat to successfully deal with a rapid change in task difficulty (as in Beylin et al., 2001), and a slower neocortical system requiring a slow introduction of increasing task difficulty (our gradual increase in visual discrimination difficulty). This separation of learning rates has been demonstrated in some human studies assessing the ability of patients with selective HPC damage in acquisition of new semantic information (Holdstock, Mayes, Isaac, Gong and Roberts, 2002), although task difficulty has yet to be specifically studied in a human population.

Thus, given a slow enough introduction of task difficulty a lack of a HPC should not be deleterious to discrimination performance. Given repeated exposures and a gradual

introduction, the slow learning rate evident in the neocortex should be able to effectively encode difficult tasks. Thus, if the discrimination task employed to assess task difficulty in the current study involved a more rapid introduction of difficulty (for example dropping from 100% contrast to 40% contrast) we might expect to see significant impairments in HPC damaged rats.

The cue size reduction provides some additional insight into the exact interaction of difficulty and HPC lesions. During cue size reduction (see Figure 3B) there is an inherent, sudden increase in the difficulty of the discrimination. The exact magnitude of this increase in difficulty is hard to assess. Nonetheless, rats with damage of the HPC did not differ significantly from Sham animals in their ability to resolve this sudden increase in difficulty. Thus, before any behavioural deficits might be observed, a larger increase in difficulty may be needed. This requires further investigation before any concrete claims of the interaction between task difficulty and HPC lesions can be made. For now any claims surrounding increased task difficulty in a visual discrimination task are limited to situations involving gradual increases in difficulty.

General Conclusions

Given the experimental findings it seems that the HPC is important in situations in which cue overlap or ambiguity is a feature. One of the functions of the HPC may be to increase or highlight the differences between two or more inputs that share common features (pattern separation). Unfortunately, at the level of behaviour it is impossible to discriminate between the processes of pattern separation and pattern completion. Both processes have been suggested as functions of the HPC (McClelland, McNaughton and O'Reilly, 1995) and evidence from research assessing pattern completion (Sutherland,

McDonald, Hill and Rudy, 1989) as well as pattern separation (Gilbert, Kesner and DeCoteau, 1998) concurs with theoretical conjecture.

There is evidence from other studies that suggests the DG is the site of pattern separation within the HPC (Lee, Yoganarasimha, Rao and Knierim, 2004). Evidence for CA field pattern completion has also been reported (Leutgeb, Leutgeb, Treves, Moser and Moser, 2004). In this study such distinctions cannot be made. Despite this shortcoming, there is evidence that these processes (pattern separation and completion) are evident in the behaviour of animals. There is also evidence that the behavioural deficits that are apparent are due not to task difficulty but rather due to an inability to deal with feature ambiguity. These findings partially support the idea of a role for the HPC in situations involving feature ambiguity or cue overlap. Data from human research suggests that HPC damage produces profound amnesia for certain forms of memory (Milner, Squire and Kandel, 1998). There is also experimental evidence that reports increased susceptibility to interference in human amnesiacs (Rosenbaum et al., in press). This study highlights the possibility that some of these memory deficits may result from a failure to distinguish between memories that share a degree of commonality. Future experimentation in the domain of visual discrimination may help to further illuminate pattern separation in the HPC.

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APPENDIX ONE

Future Experiments

A significant difference between HPC damaged rats and Shams during Pattern Separation One was evident, an effect that disappeared during Pattern Separation Two and Three. An initial exposure to a higher degree of overlap should be helpful in determining the extent of pattern separation deficits in rats with lesions of the HPC. An initial presentation involving a higher degree of overlap should produce increased behavioural deficits. Thus, an initial exposure to Pattern Separation Two or Three (as opposed to Pattern Separation One) should help to further illuminate the effects of cue ambiguity in rats with HPC damage.

Adrenalectomy (ADX) has been demonstrated to produce damage that is specific to the granule cell layer of the DG (MacLennan, Smith and Darlington, 1998). Lesions produced by ADX are similar to those caused by intracranial microinjection of colchicine. ADX induced granule cell layer lesions offer at least two advantages to those produced by colchicine injection. Damage is extremely specific, and surrounding HPC subfields are essentially unaffected. ADX induced lesions also remove the possible confound associated with overlying cortical damage due to injection tracks and possible diffusion of colchicine away from the DG. Rats with lesions of the DG due to ADX should provide a good model to investigate pattern separation ability. Despite the absence of any significant effects in Experiment 2 assessing DG damage, the possibility that any intact DG may be contributing to pattern separation ability cannot be completely ruled out. It has been previously reported that ADX produces complete ablation of the DG granule cell layer (Spanswick, Epp, Keith, Muzylouski, Melvin and Sutherland, 2004).

Thus, ADX rats will allow us to conclusively determine whether our task is truly DG dependent.

Given the null results from Experiment Two, we are forced to conclude that rats may be pattern completing in order to resolve our discrimination paradigm. If the CA subfields are the location of pattern completion (Leutgeb, Leutgeb, Treves, Moser and Moser, 2004), any lesion in the area should serve to reduce pattern completion ability. Thus, a lesion study focusing specifically on the CA fields within the HPC should provide us with some insight into the process required to resolve our discrimination paradigm. If no deficit is observed, we can conclude that rats are likely to be pattern separating. These results would suggest that the paradigm that was employed can be resolved either by pattern separation (intact DG) or pattern completion (intact CA fields). However, if a behavioural deficit is discovered we must conclude that the task is CA field dependent and these rats are likely to be resolving the discrimination via pattern completion.

Due to the methodology employed, any claims in regards to task difficulty are limited to situations in which difficulty is introduced gradually. When introduced gradually, task difficulty does not exert a negative effect that is heightened in rats with HPC damage in our visual discrimination task. These results differ from Beylin et al. (2001) who report that the introduction of a single difficult task exerts a negative effect on the behaviour of HPC damaged rats. To clarify the effects of task difficulty, an experiment comparing a graded and sudden introduction of task difficulty in our visual discrimination task would prove useful.

APPENDIX TWO

Experiment Two: Additional Data

Several findings in Experiment 2 (Chapter Three) were not graphed for sake of brevity. These results are presented here to allow for qualitative comparison to performance of Sham and HPC animals in Experiments 1 and 3.

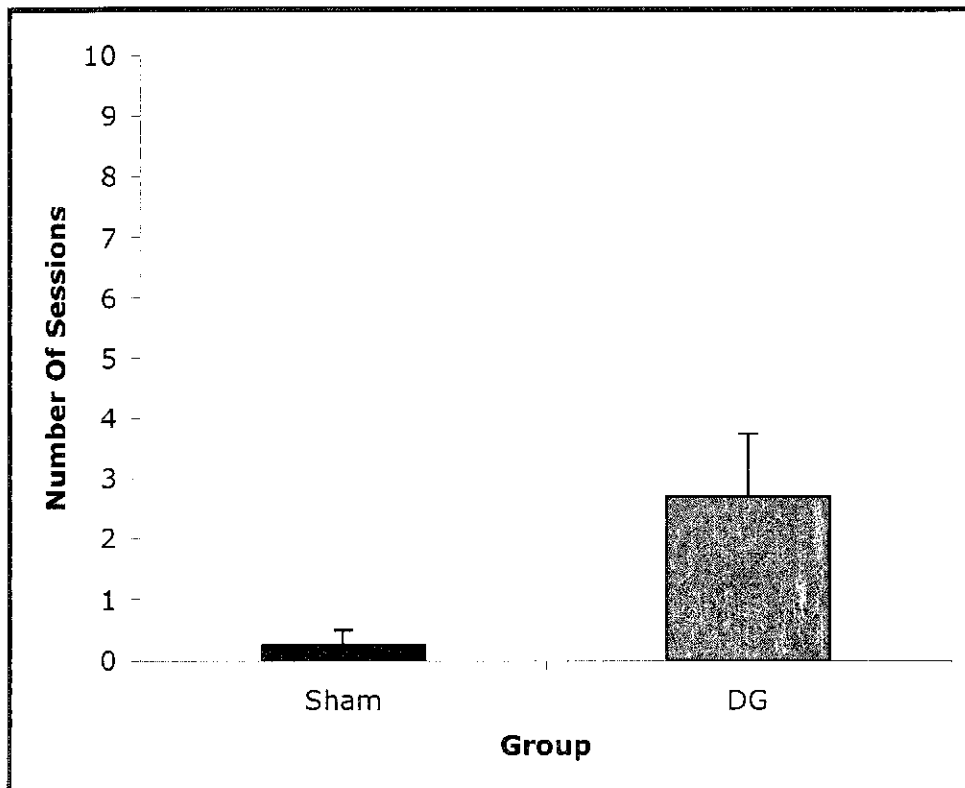


Figure A. Mean number of sessions to return to criterion post-surgery (+ SEM). No significant difference was found between DG ($n = 7$) and Sham animals ($n = 4$).

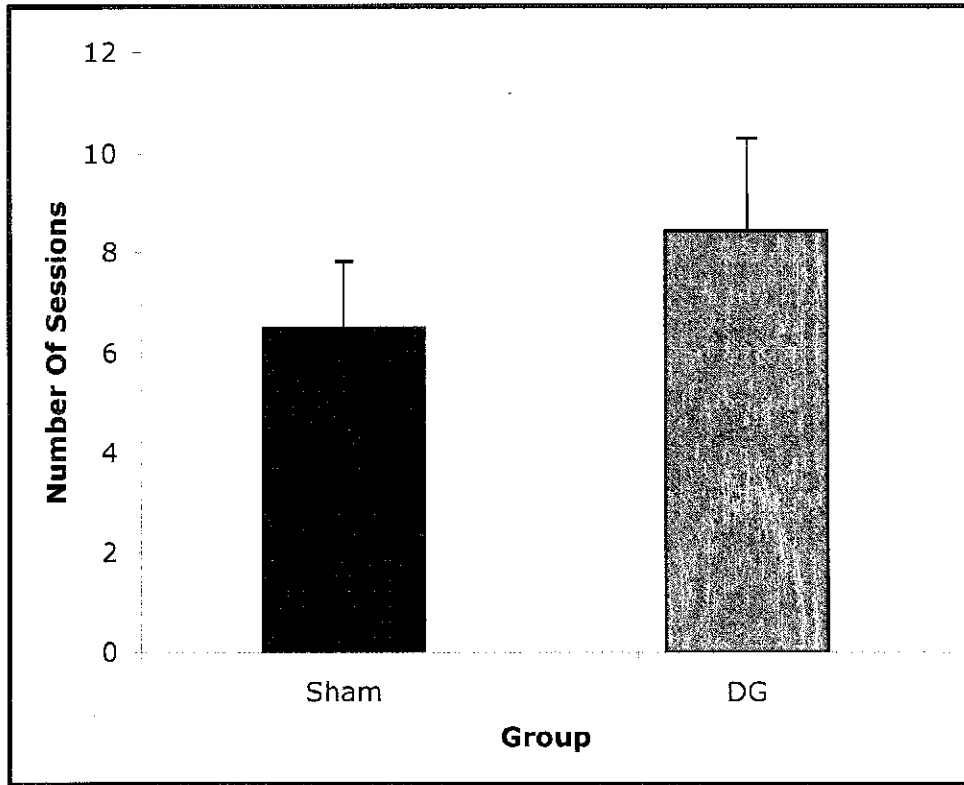


Figure B. Mean number of sessions spent below criterion during surround fade-in (+ SEM). DG rats ($n = 7$) and Sham rats ($n = 4$) did not significantly differ in the number of sessions spent below criterion.

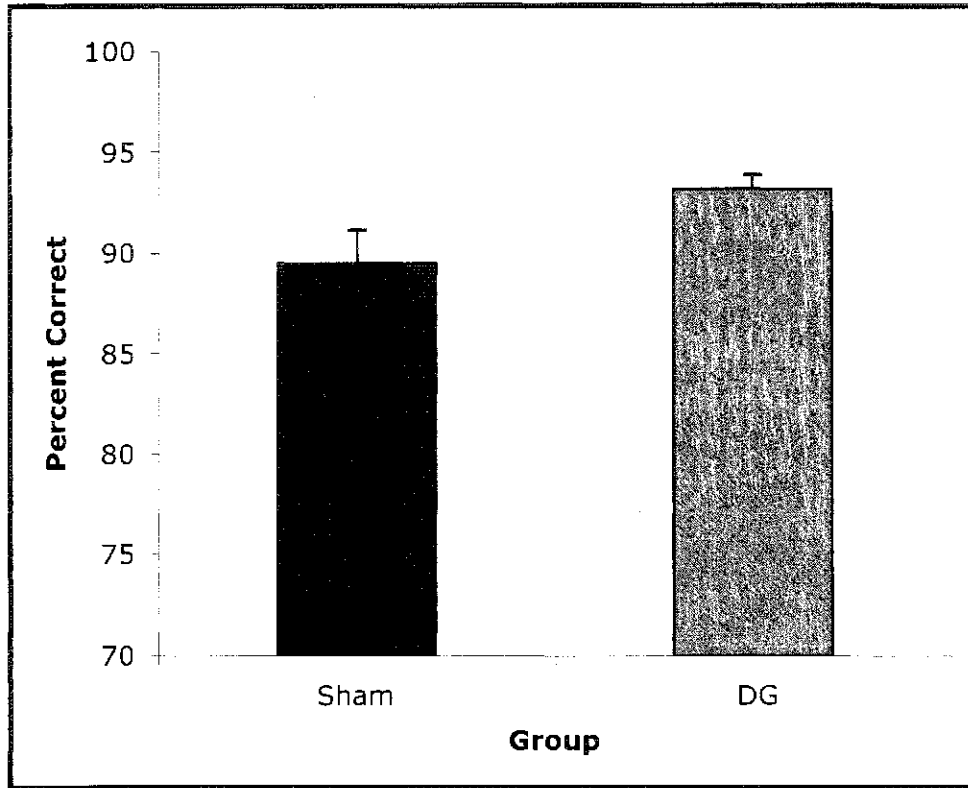


Figure C. Mean performance (+ SEM) of DG (n = 7) and Sham rats (n = 4) over the first 5 sessions of Pattern Separation One. No between group significant differences were noted.

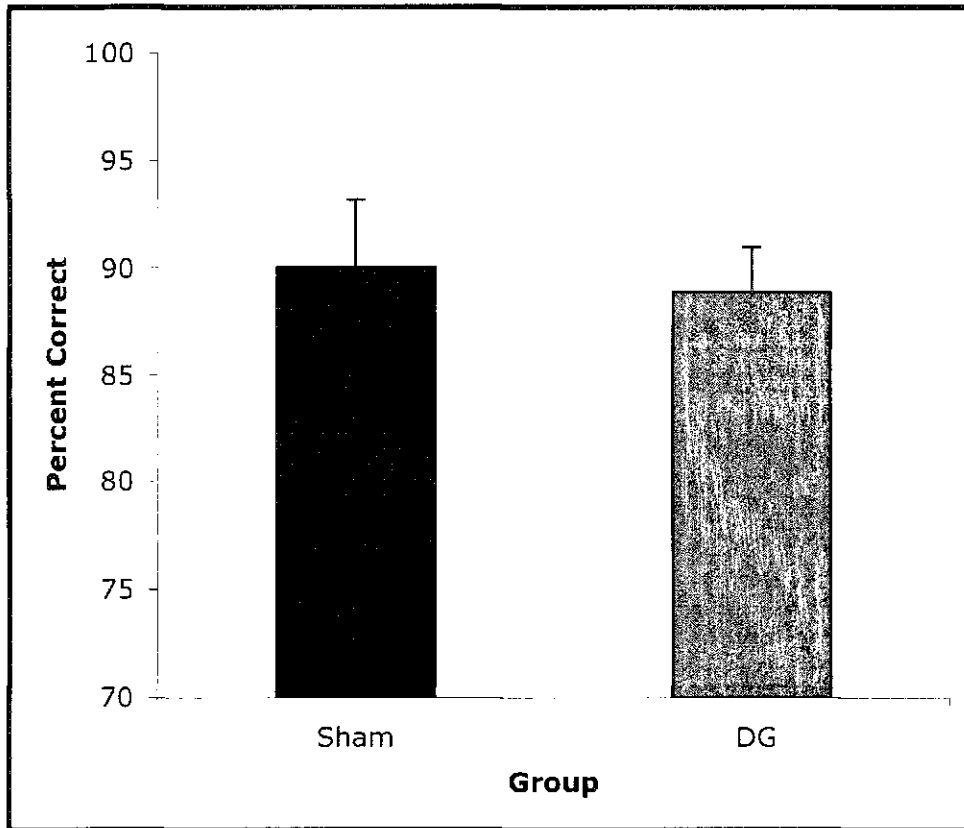


Figure D. Mean performance (+ SEM) of DG (n = 7) and Sham rats (n = 4) over the first 5 sessions of Pattern Separation Two. No between group significant differences were noted.

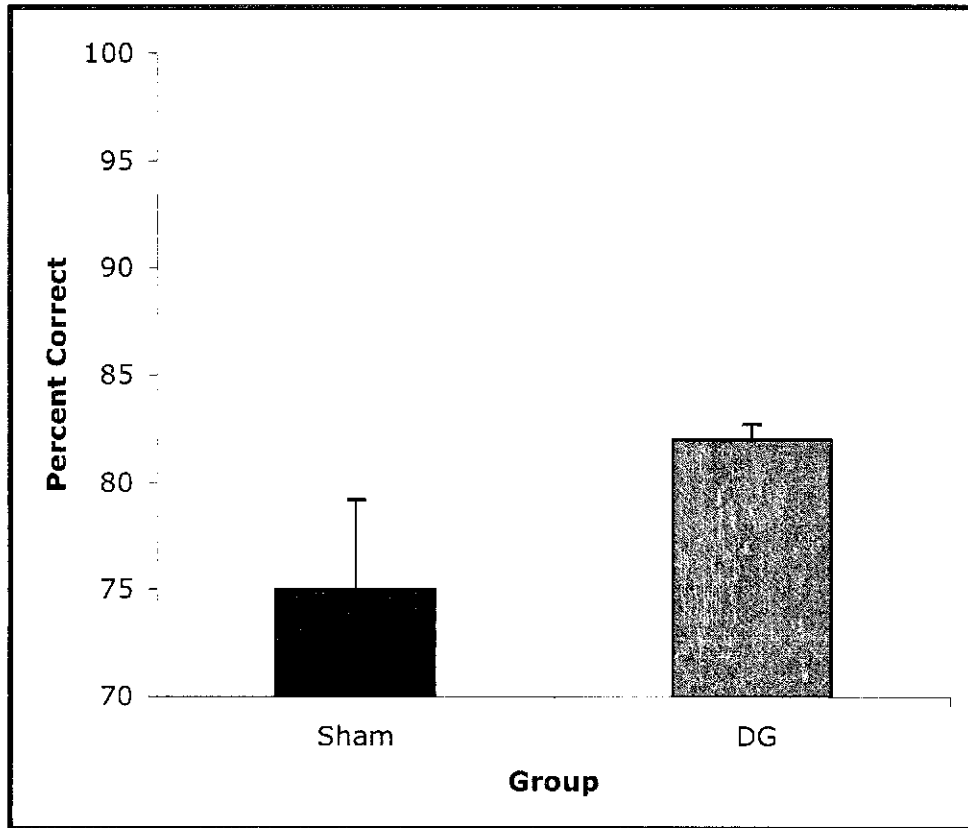


Figure E. Mean performance (+ SEM) of DG (n = 7) and Sham rats (n = 4) over the first 5 sessions of Pattern Separation Three. No between group significant differences were noted.

APPENDIX THREE

Correlational Data

To further clarify the nature of deficits suffered by rats with lesions of the HPC, correlations between lesion size and performance were calculated. Correlations were only calculated for those situations in which a deficit was found between HPC, DG or Sham animals.

Experiment One

Lesion size was quantified on a seven-point scale; complete ablation of the HPC was scored as a seven, with no damage scored as a one. Ratings on the seven-point scale were correlated with behavioural measures taken at specific stages of the experimental timeline. Statistical analysis revealed no significant correlation between lesion size and post-surgical retention of the original discrimination stimuli, $r(4) = 0.52$, $p = 0.48$. No significant correlation was found between lesion size and number of sessions spent below criterion during the fade-in process, $r(4) = 0.36$, $p = 0.64$. Analysis of Pattern Separation One revealed a significant correlation between lesion size and number of sessions to attain criterion $r(4) = -0.96$, $p = 0.04$. There was a trend for lesion size and number of sessions to return to criterion to correlate in Pattern Separation Two, $r(4) = -0.90$, $p = 0.10$. This trend continued during Pattern Separation Three, $r(4) = -0.90$, $p = 0.10$. These results suggest that a smaller lesion may impair performance to a greater extent than a more complete lesion of the HPC.

Experiment Two

Rats with lesions limited to the DG did not differ from Sham animals at any stage of training or testing. Thus, no correlations were performed assessing lesion size and behavioural performance.

Experiment Three

A significant difference was found between DG and Sham animals in regards to number of sessions to return to criterion during post-surgical retention in Experiment 3. This significant difference prompted a statistical analysis of lesion size and its relationship to post-surgical retention. Lesion size was quantified in the same manner as Experiment 1. Statistical analysis revealed no significant correlation between lesion size and post-surgical retention, $r(5) = 0.19$, $p = 0.76$.

	Post-surgical Retention	Cue Size Reduction	Surround Cue Fade-In	Central Cue Replace
<u>HPC</u>	*	-	*	-
<u>DG</u>	-	-	-	-

	Pattern Separation One	Pattern Separation Two	Pattern Separation Three	Pattern Separation Four
<u>HPC</u>	*	*	-	-
<u>DG</u>	-	-	-	-

Table One. Performance of HPC and DG animals in each phase of behavioural training relative to sham animals. * indicates a deficit.

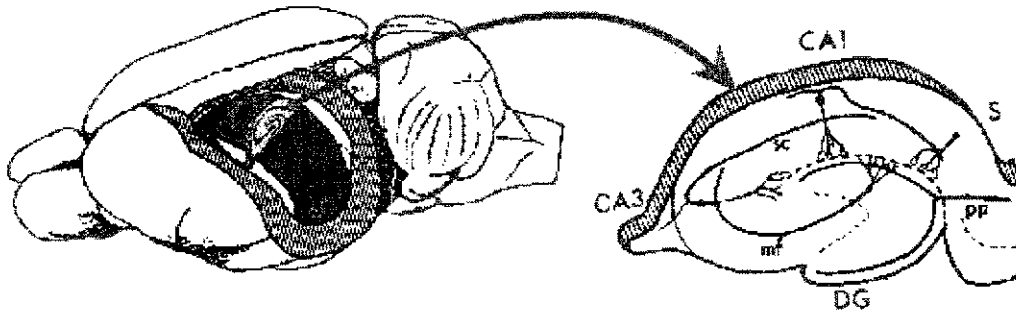


Figure 1. The location of the hippocampus within the rat brain (highlighted in blue). An expanded view displays the major subfields and neural pathways within the hippocampus itself. pp = perforant pathway, DG = Dentate Gyrus, mf = mossy fibers, sc = schaeffer collaterals, S = Subiculum
(Adapted from <http://www.psychol.ucl.ac.uk/kate.jeffery/lab/research.htm>)

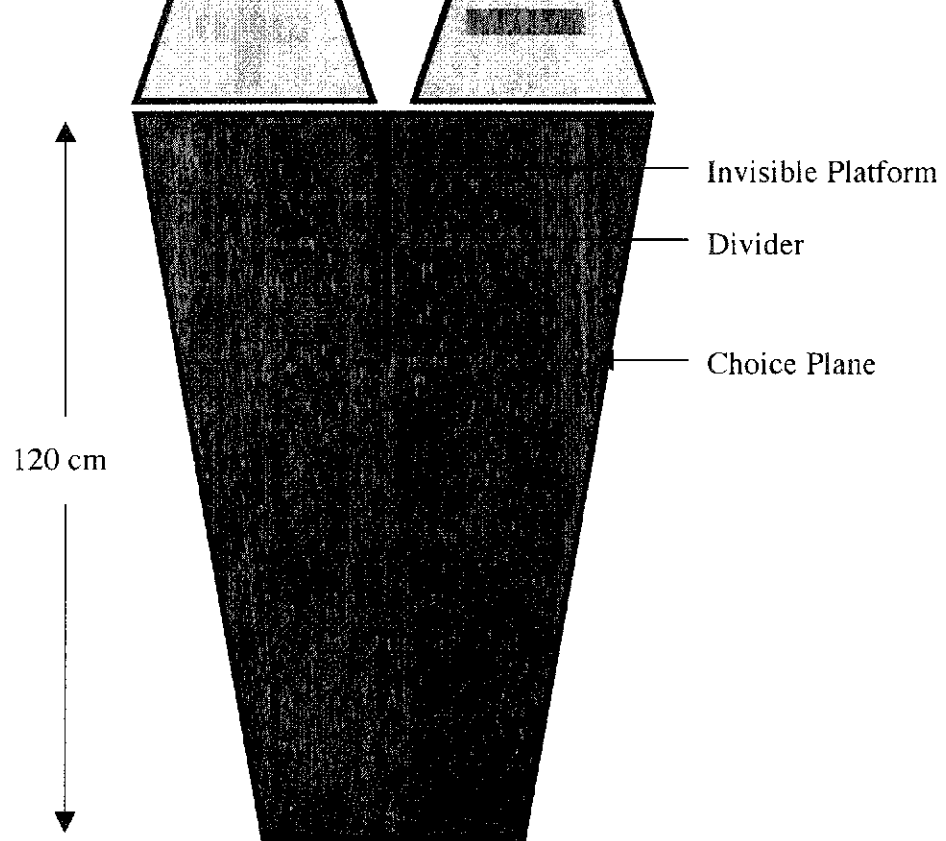


Figure 2. The Visual Water Task (VWT). Rats are placed in the pool facing away from the computer monitors. The positive stimulus is presented above the invisible platform allowing the animal to escape from the water.

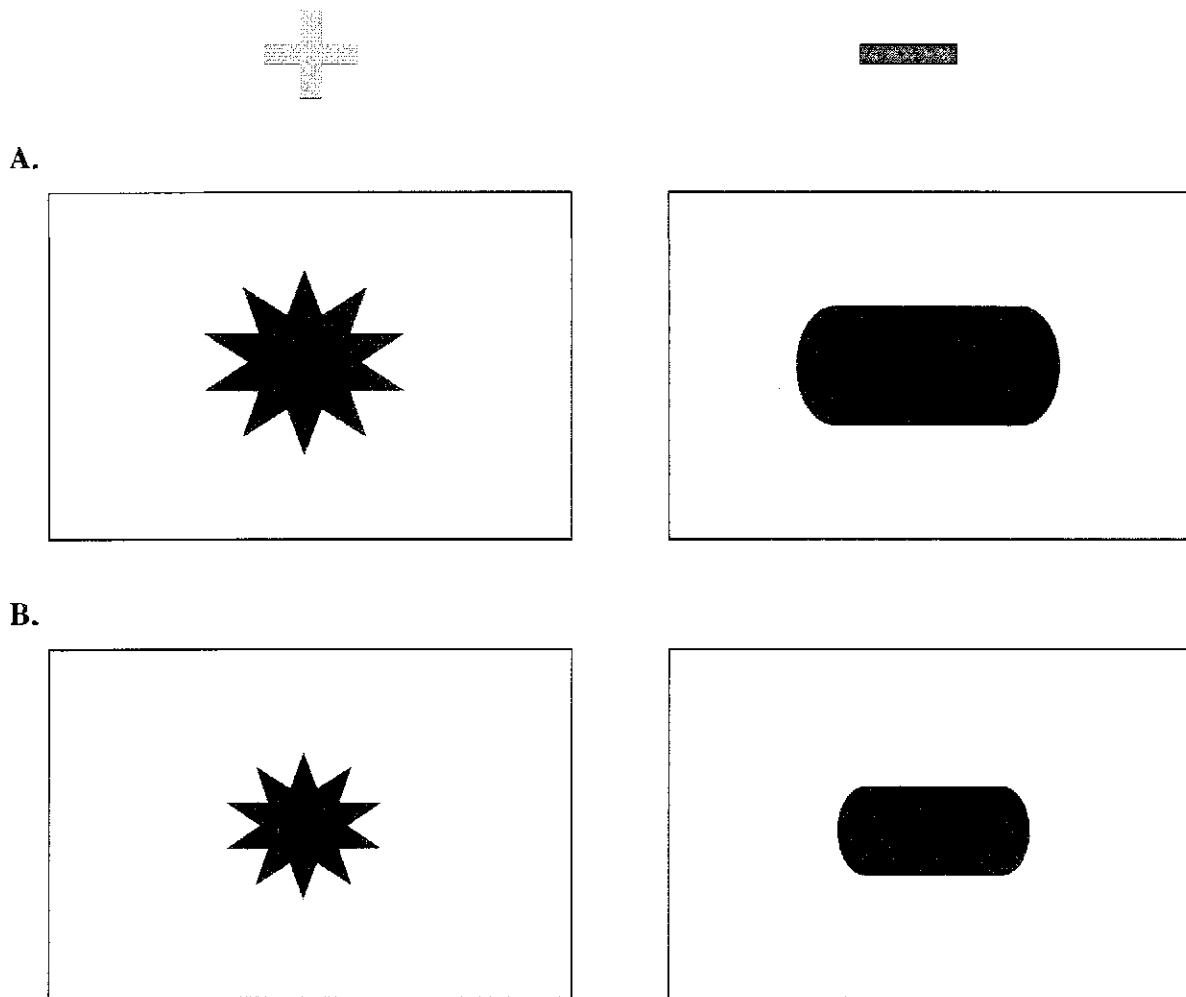
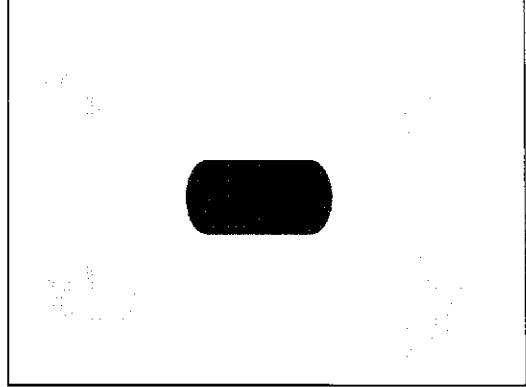
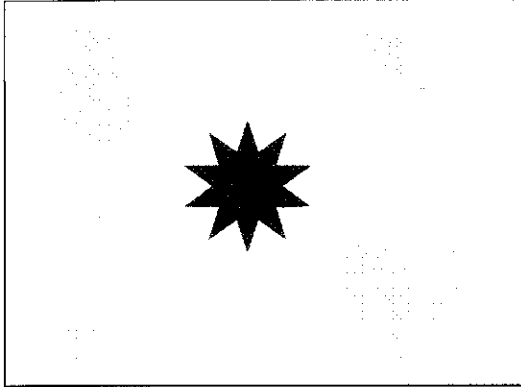


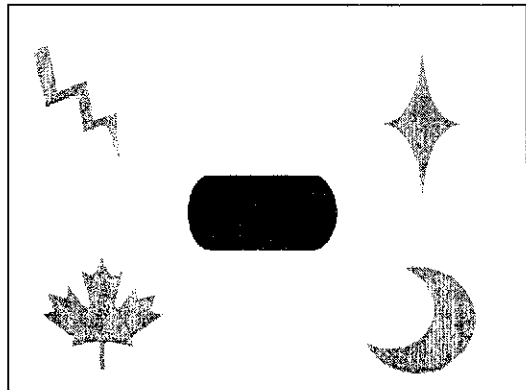
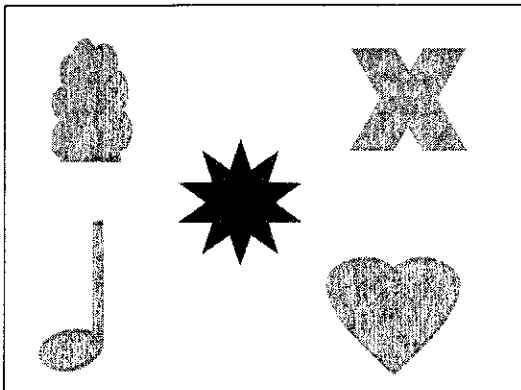
Figure 3. Initial discrimination cues used in the VWT. **A)** Positive and negative stimuli used for pre-surgical training and post-surgical retention testing in the VWT. **B)** Positive and negative stimuli after cue size reduction to allow for surround fade-in.



A. FADE-IN ONE



B. FADE-IN THREE



C. FADE-IN FIVE

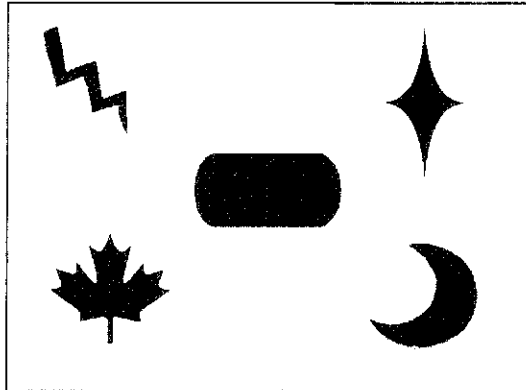
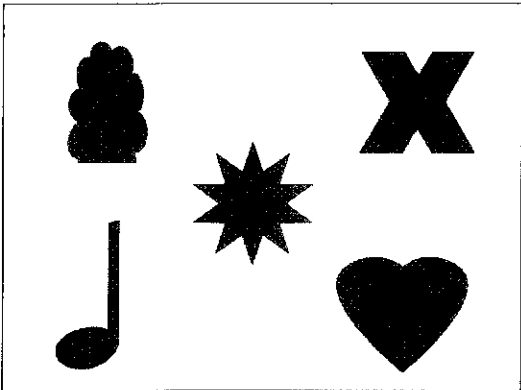


Figure 4. Examples of the surround cue fade-in process. **A)** Fade-In One, surround at 20% contrast. **B)** Fade-In Three, surround at 60% contrast. **C)** Fade-In Five, surround at 100% contrast

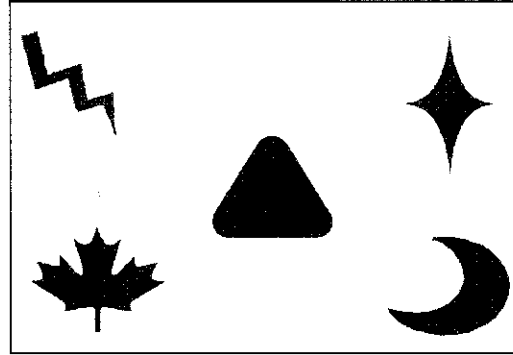
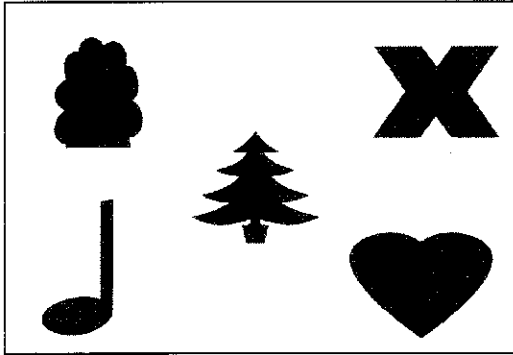
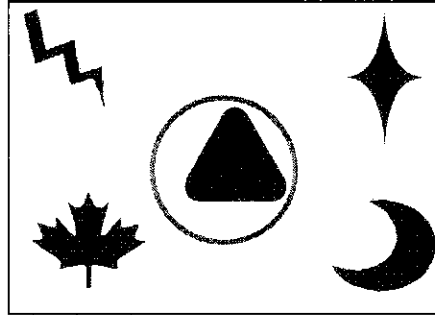
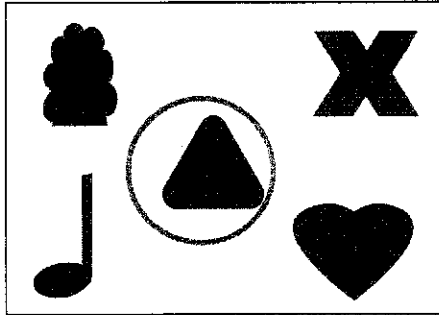
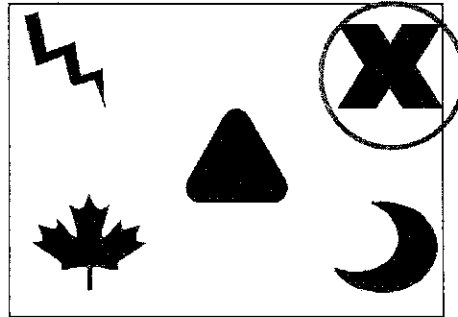
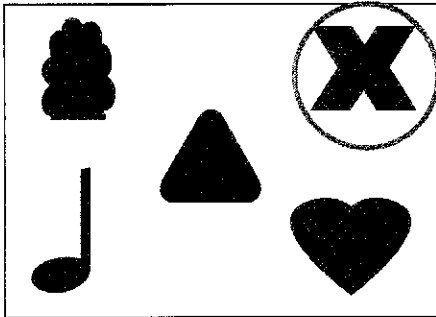


Figure 5. Stimuli as they appear after central cue replacement. Each monitor now displays 5 distinct cues.

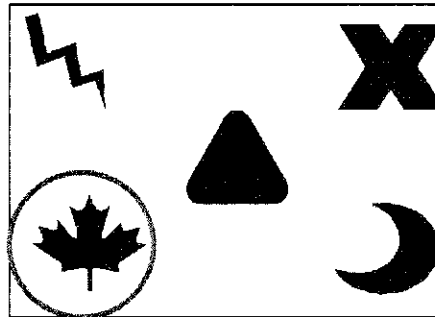
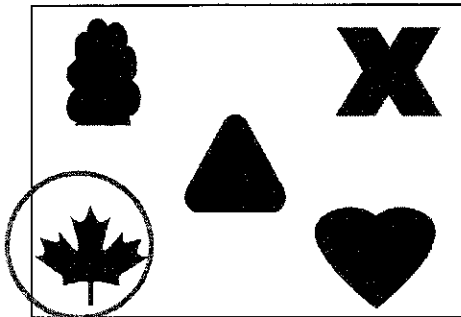
A. PATTERN SEPARATION ONE



B. PATTERN SEPARATION TWO



C. PATTERN SEPARATION THREE



D. PATTERN SEPARATION FOUR

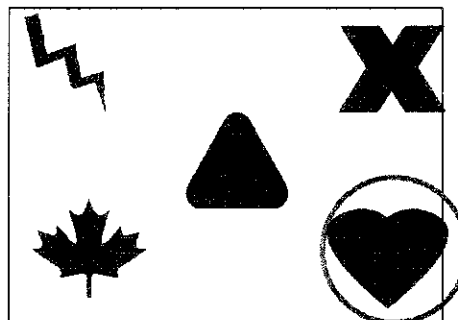
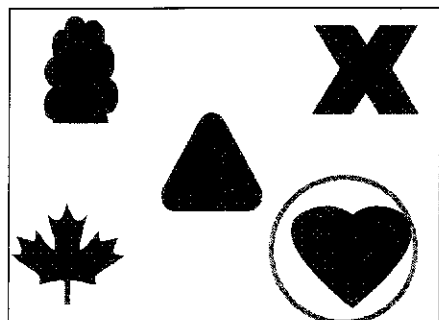


Figure 6. Visual Cues Used For Pattern Separation. A-D, Pattern Separation One through Four respectively. Cue redundancy is increased at each stage of the pattern separation process. Red circles highlight the cue made redundant in each stage of pattern separation.

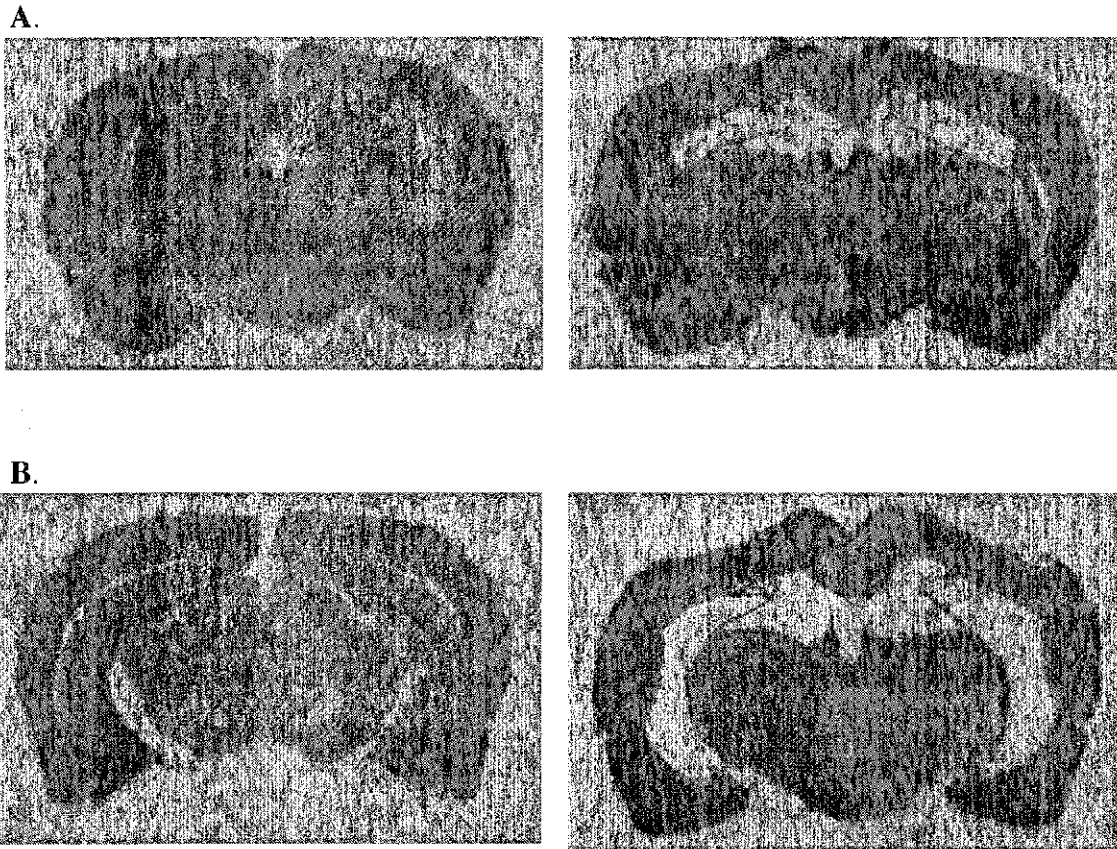


Figure 7. A large HPC lesion. Representative sections taken from a Sham (left) and HPC lesion animal (right). **A)** Anterior sections display complete destruction of the HPC with minimal damage to extra-hippocampal areas. **B)** Posterior sections exhibit similar damage.

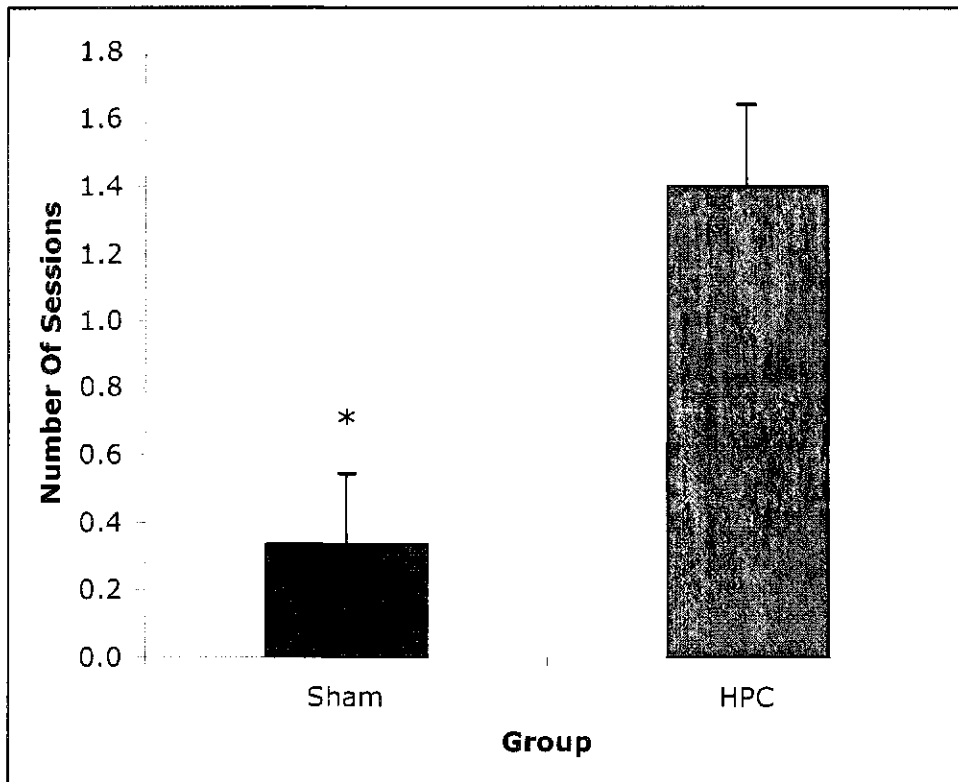


Figure 8. Mean number of sessions to return to criterion post-surgery (+SEM). HPC animals (n = 5) took significantly more sessions to return to pre-operative performance when compared with Sham rats (n = 6). * significant difference.

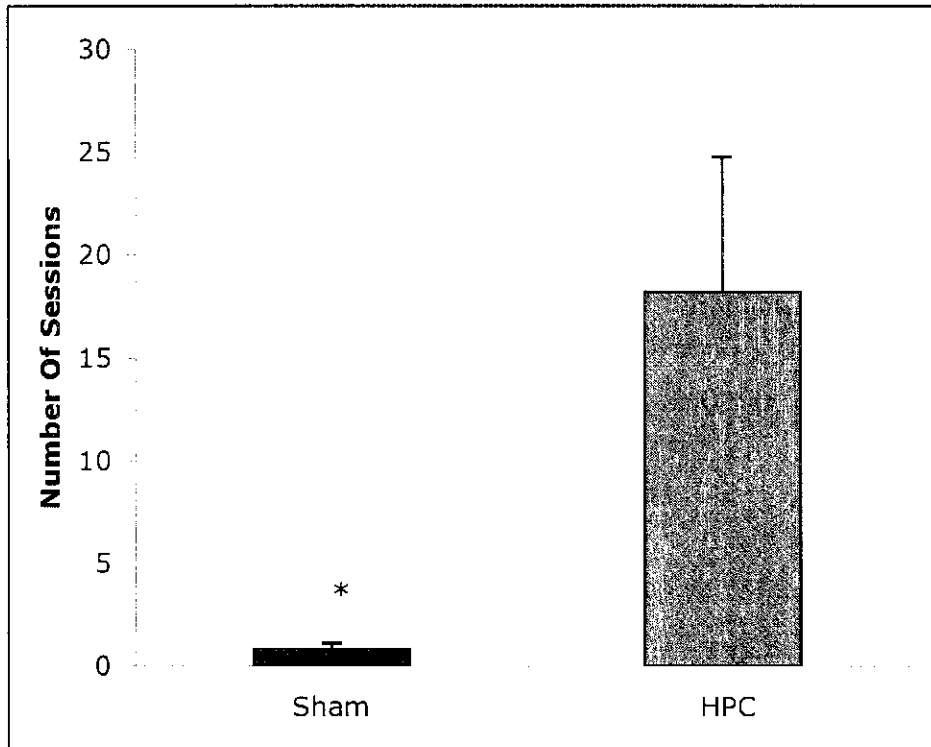
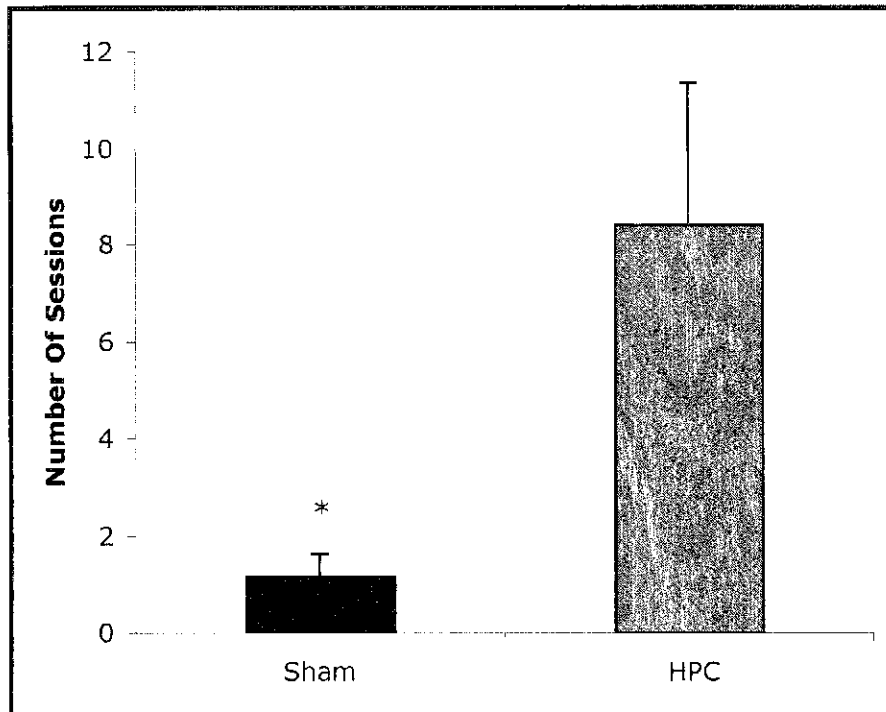


Figure 9. Mean number of training sessions spent below criterion during surround fade-in (+ SEM). HPC rats (n = 5) spent significantly more sessions below criterion than Sham animals (n = 6). * significant difference.

A.



B.

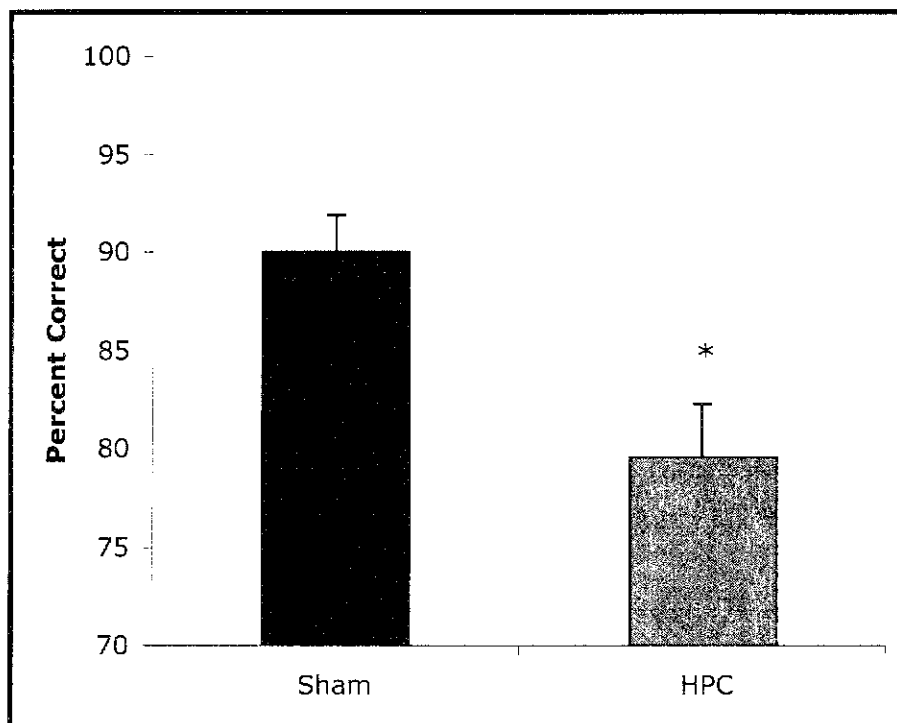
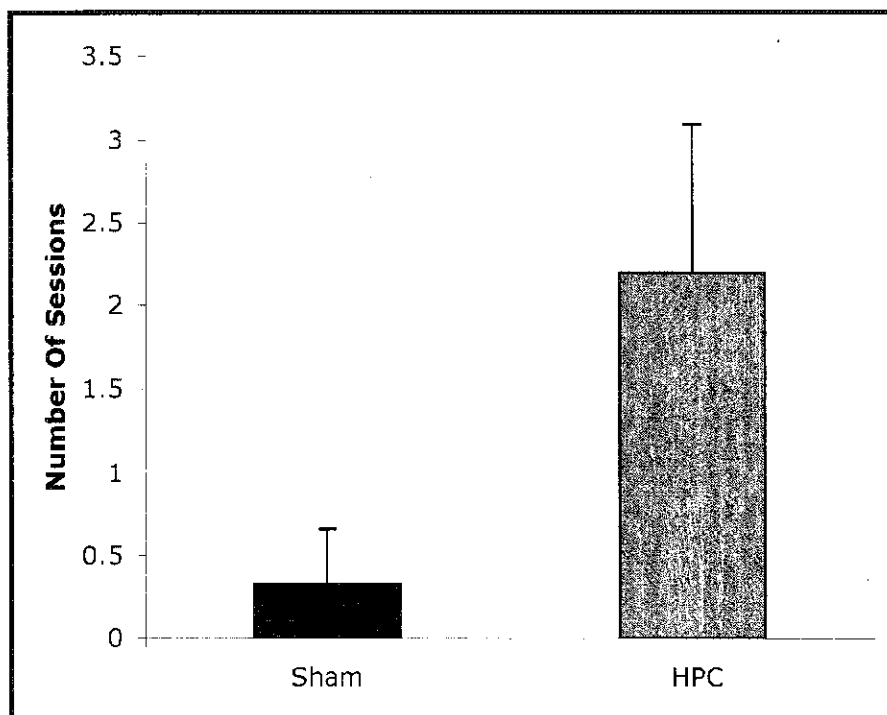


Figure 10. Performance of HPC (n = 5) and Sham rats (n = 6) during Pattern Separation One. **A)** Mean number of sessions to return to criterion (+ SEM). **B)** Mean performance (+ SEM) over the first 5 sessions of Pattern Separation One. * significant difference.

A.



B.

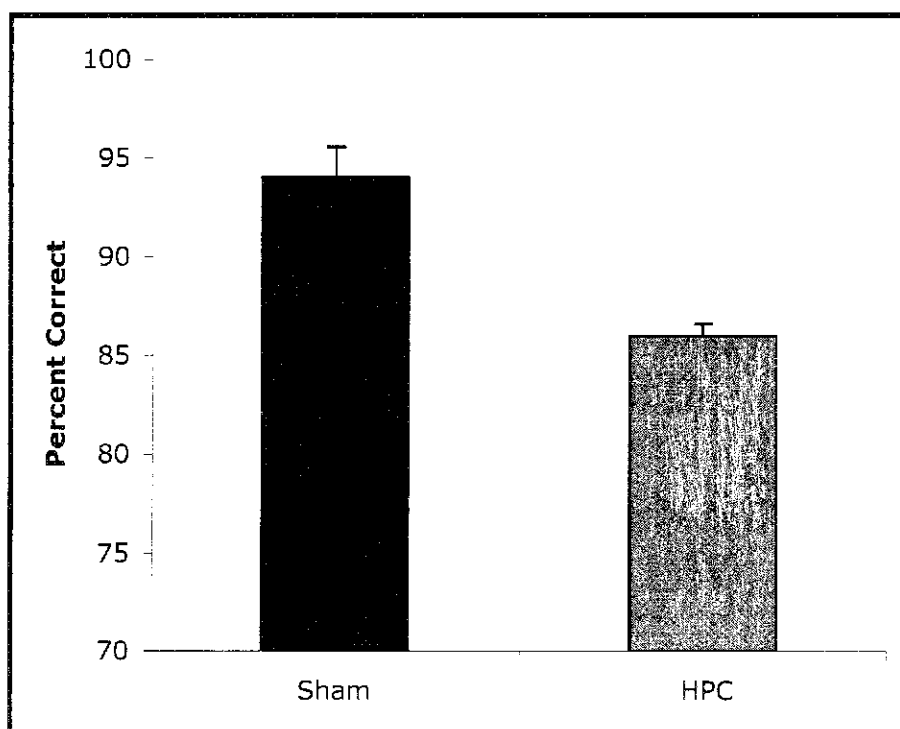


Figure 11. Performance of HPC (n = 5) and Sham rats (n =6) during Pattern Separation Two. **A)** Mean number of sessions to return to criterion (+SEM). **B)** Mean performance (+SEM) over the first 5 sessions of Pattern Separation Two.

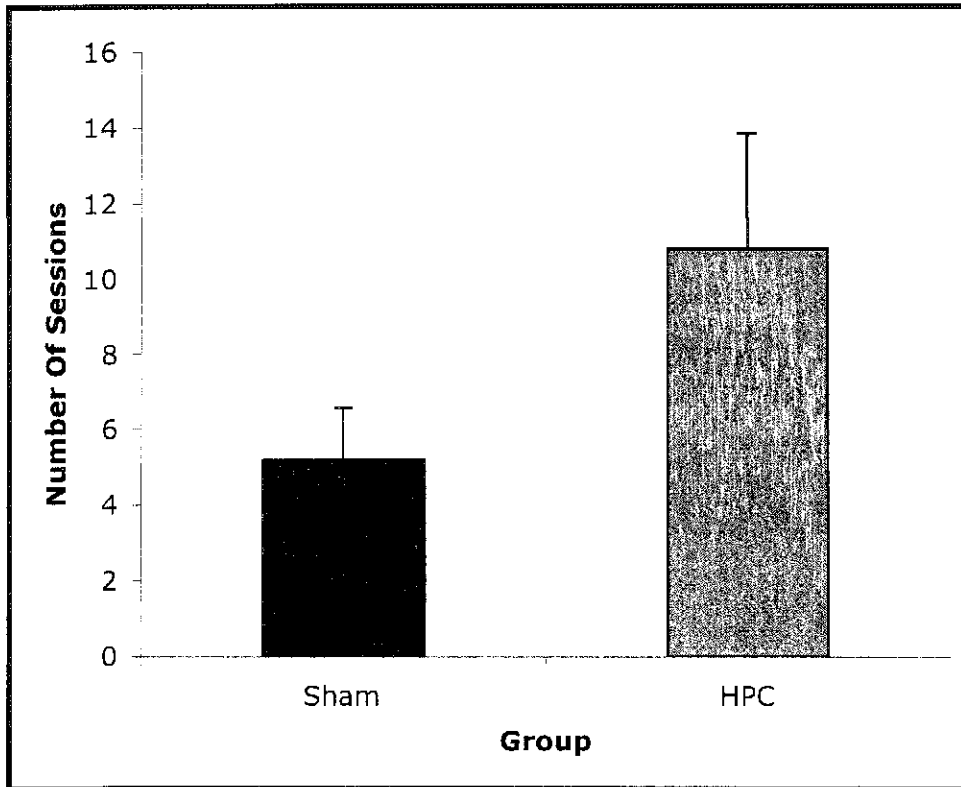
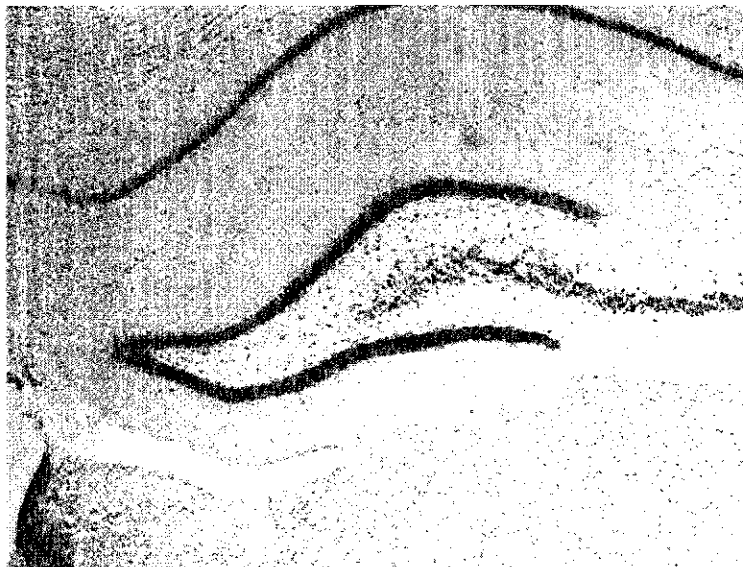


Figure 12. Mean number of sessions to return to criterion with 3 redundant cues in pattern separation three. No differences were found between HPC or Sham rats, $p = 0.11$

A. Sham



B. Colchicine Induced DG lesion

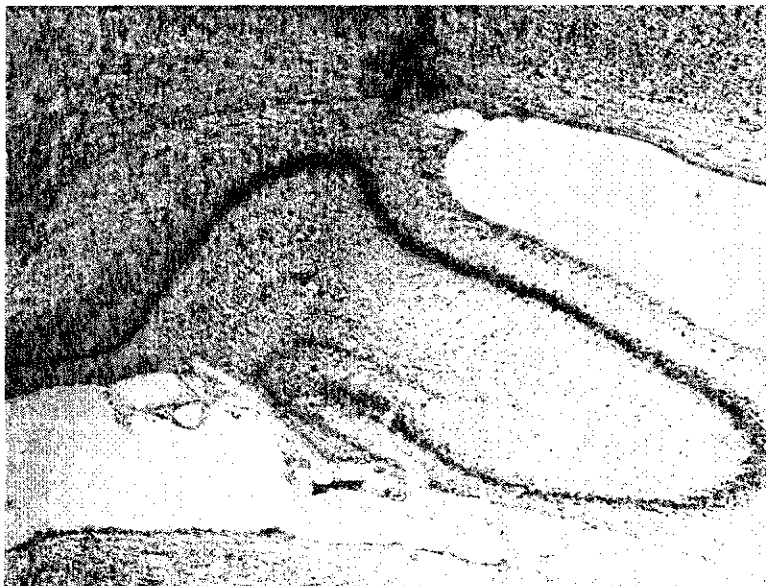


Figure 13. Representative sections taken from a Sham (A) and a DG lesion animal (B). Dentate thickness, size and density are substantially lower in the Colchicine injected animal.

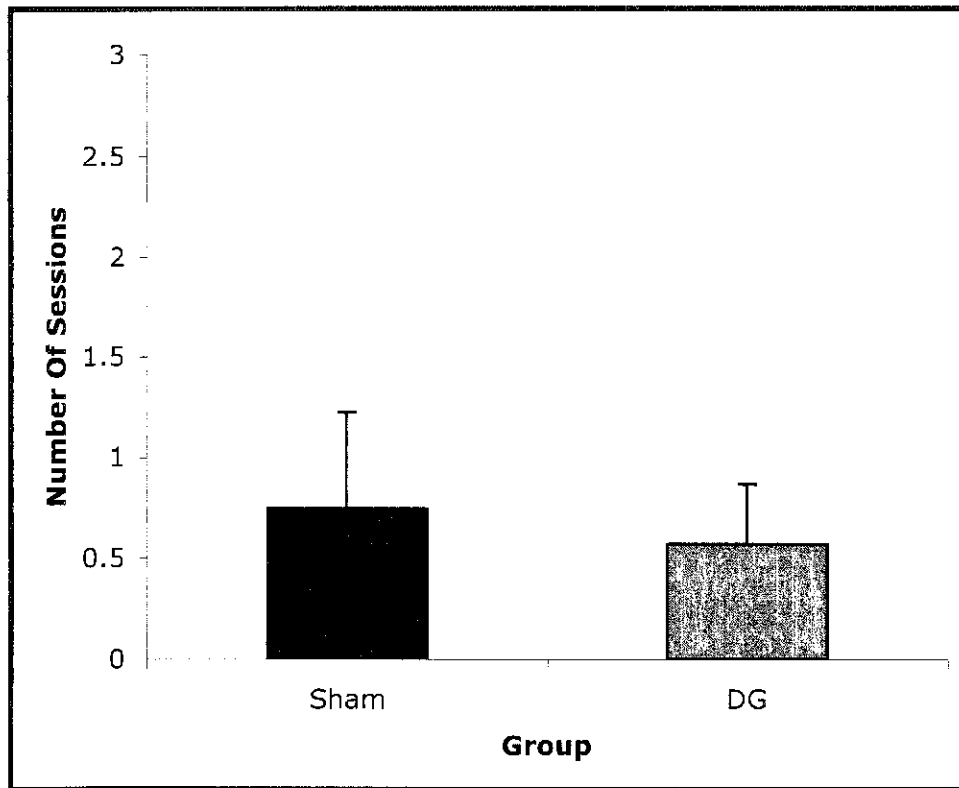


Figure 14. Mean number of sessions to return to criterion during Pattern Separation One (+ SEM). Sham (n = 4) and DG (n = 7) rats did not differ significantly.

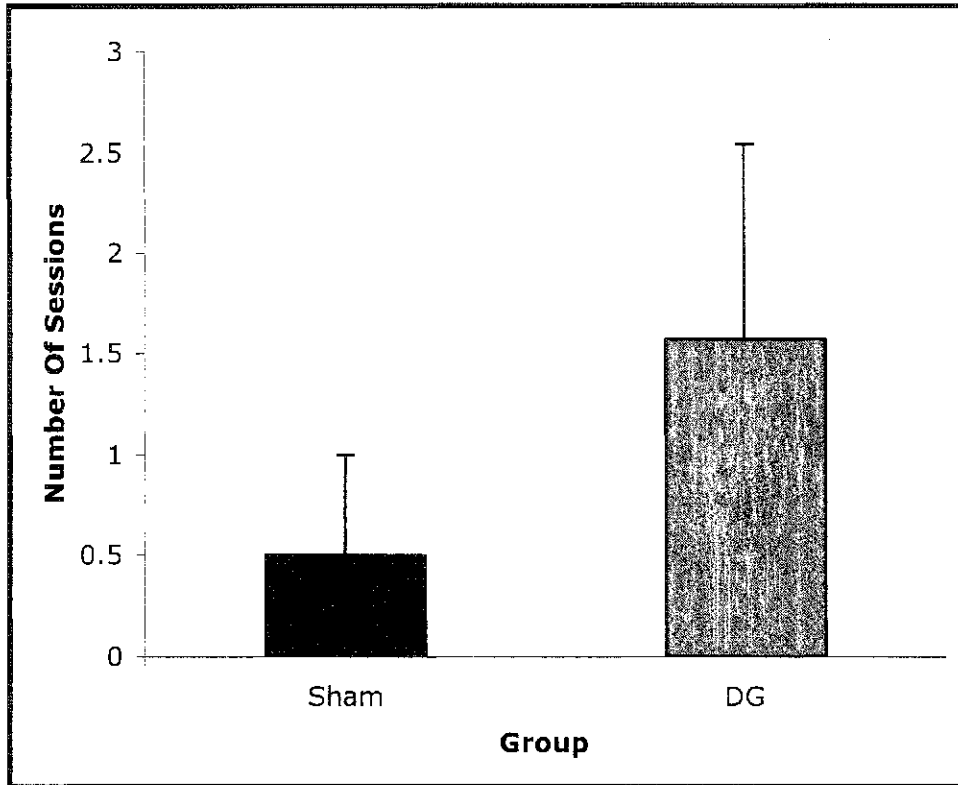


Figure 15. Mean number of sessions to return to criterion during Pattern Separation Two (+ SEM). Sham (n = 4) and DG (n = 7) rats did not differ significantly.

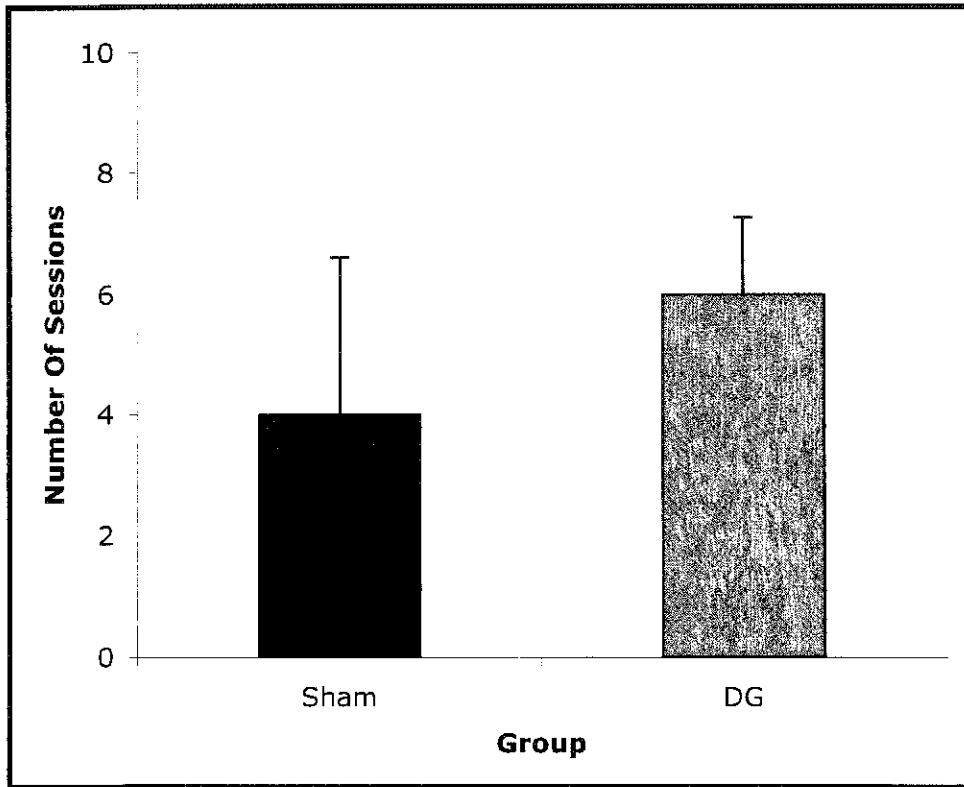
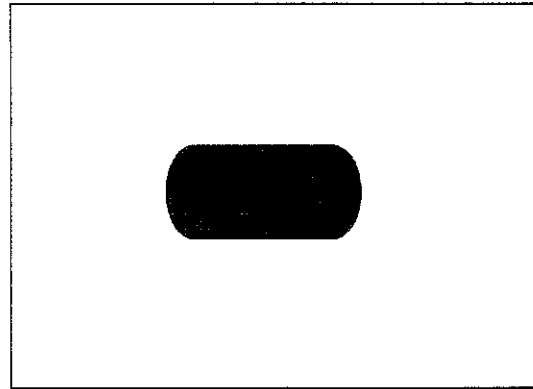
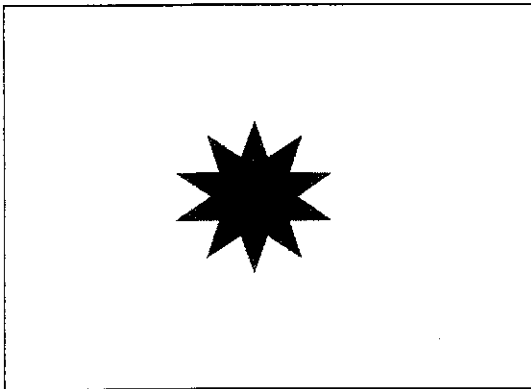


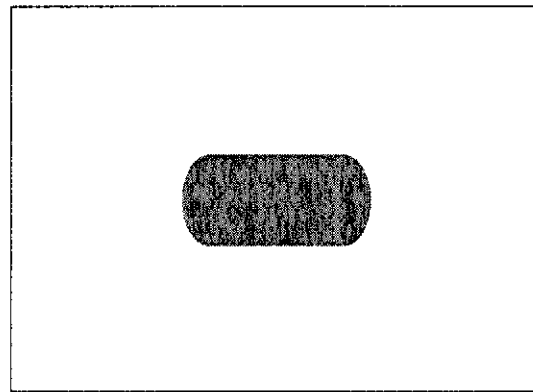
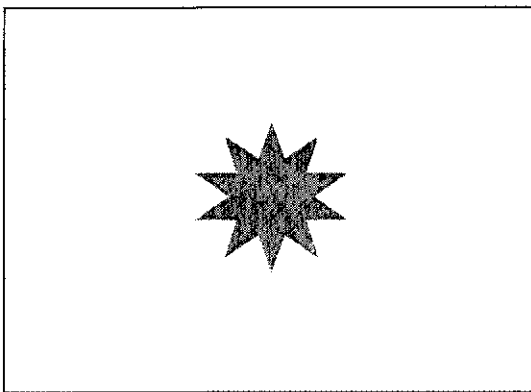
Figure 16. Mean number of sessions to return to criterion during Pattern Separation Three (+ SEM). No significant difference was found between Sham (n = 4) and DG (n = 7) rats.



A. FADE-OUT ONE



B. FADE-OUT FIVE



C. FADE-OUT NINE

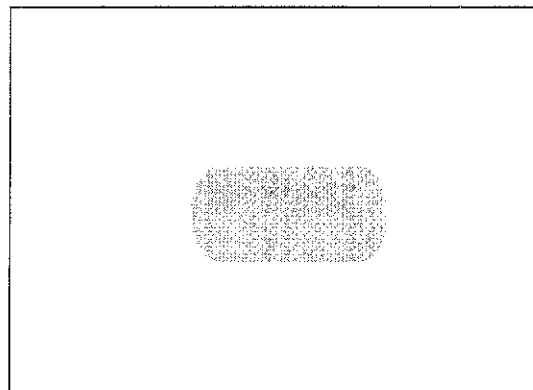
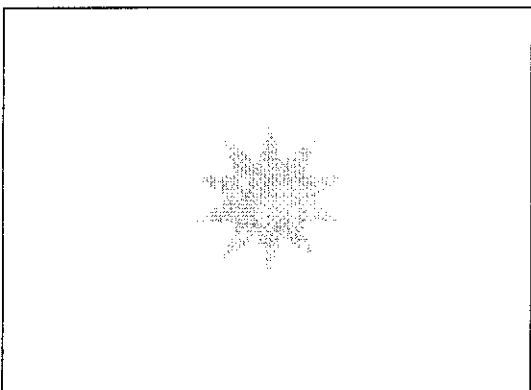


Figure 17. Examples of the cue fade out paradigm. **A)** Original Cues at 100% contrast relative to background. **B)** Cues at 60% contrast. **C)** Cues at 20% contrast

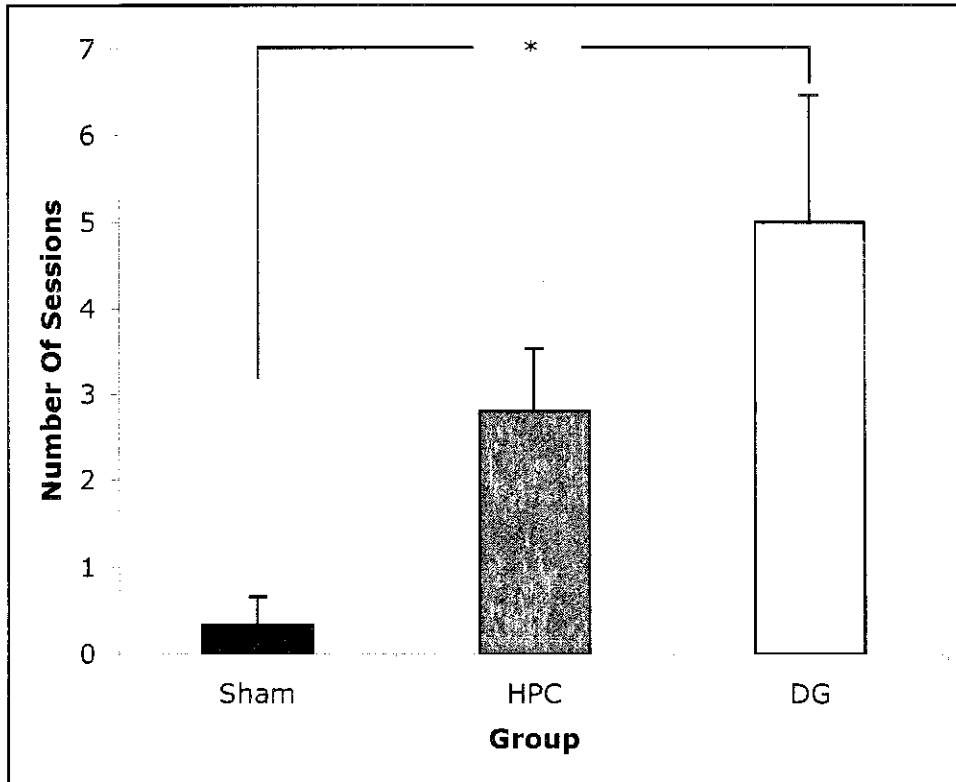


Figure 18. Mean number of trials to return to criterion after Sham (n = 6), HPC (n = 5) or DG (n = 6) lesions (+SEM). Rats with lesions limited to the DG took significantly more trials to return to pre-surgical level of performance when compared with Sham animals. * significantly different.

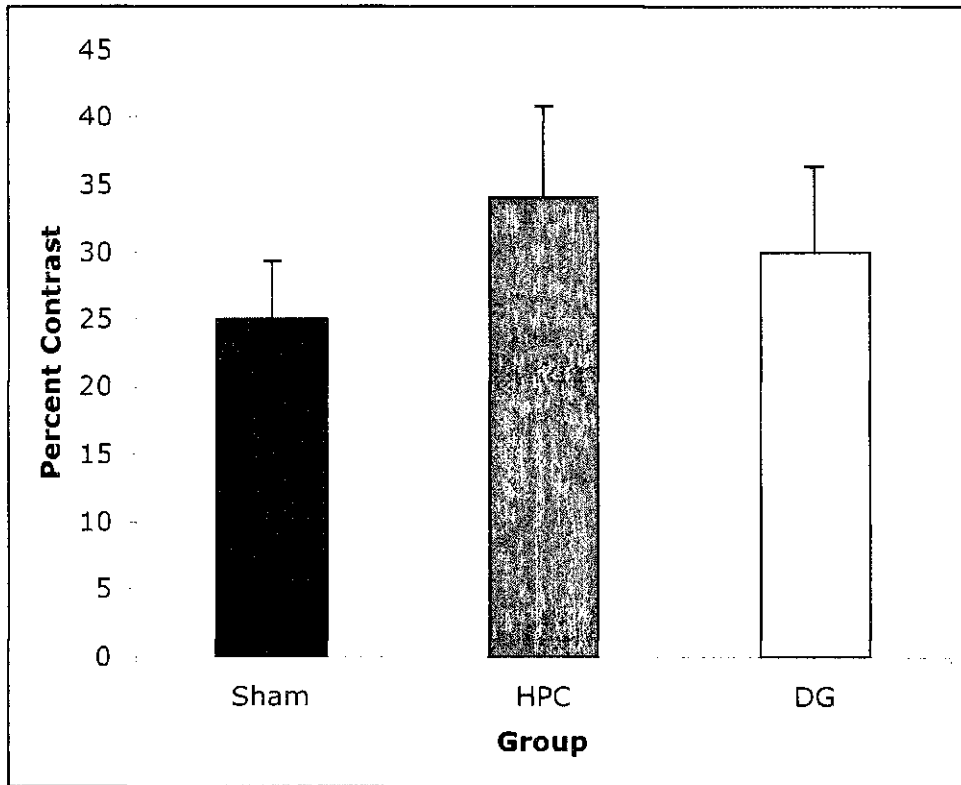


Figure 19. Mean contrast level at which rats fail to discriminate between stimuli in the VWT (+SEM). Sham (n = 6), HPC (n = 5) and DG rats (n = 6) did not differ in their ability to discriminate.