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The Roles of the Dorsal and Ventral Hippocampus in Fear and Memory of a Shock Probe Experience

by

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Abstract

This thesis examined the effects of temporary inactivation of the dorsal or ventral hippocampus on unconditioned and conditioned fear, using the shock-probe test. Rats received either dorsal or ventral hippocampal infusions of Lidocaine, muscimol or saline, *before* or *after* exposure to an electrified shock-probe. A retention test in the same apparatus was given 24 hr later, at which time the hippocampus was no longer inactivated, and the probe was disconnected from the shock-source. We found that ventral hippocampal inactivation impaired fear behaviour during acquisition, and dorsal hippocampal inactivation impaired fear behaviour during retention. We conclude that the: 1) ventral hippocampus mediates unconditioned fear behaviour, 2) the dorsal hippocampus mediates fear memory and 3) afferent input from brain structures located outside of the hippocampus are not responsible for the differential effects of dorsal and ventral hippocampal inactivation on fear and memory.

I would like to thank Elif Engin for her assistance with these experiments; Dr. Fred Colbourne, Dr. Chris Sturdy, and Dr. Ian Winship for their contribution as thesis committee members. Finally, I would especially like to thank Dr. Dallas Treit for his effort, guidance and encouragement in supervising this thesis.

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Chapter 1.

Introduction

Historical views of hippocampal function

In a seminal experiment, Kluver and Bucy (1937; 1939) observed several abnormalities in monkeys, subsequent to bilateral excision of the temporal lobes. The lesions included large portions of the hippocampus, amygdala, and overlying cortex. Among the abnormalities displayed by these monkeys were 1) visual agnosia, or "psychic blindness" (inability to visually recognize common objects, 2) "compulsory" oral investigation of objects, both inedible and edible 3) "inappropriate" sexual behaviour (e.g., initiating sexual behaviour toward the same sex, or inanimate objects), and 4) "taming," or a dramatic loss of fear (e.g., while monkeys would normally avoid humans or attack them if they approached, after surgery, the monkeys would approach, follow, and quietly interact with humans, as well as other animals, even predators such as large snakes; Kluver and Bucy, 1937; 1939). In subsequent studies Kluver and Bucy showed that this behavioral "syndrome" was not due to lesions of visual system pathways to and from the temporal lobes, or to temporal lobe connections with the frontal lobes, and could not be replicated with lesions of the frontal, occipital or parietal cortices (Kluver and Bucy, 1939). Importantly, they reported that this syndrome did *not* occur after unilateral temporal lobe excision, or unless the hippocampus itself was bilaterally lesioned.

At about the same period of time, James Papez (1937) proposed a neural theory of emotion in which the hippocampus played a central part. He proposed

that the hippocampus, through its connections with the cingulate cortex and the hypothalamus, composed a circuit he called the 'stream of feeling.' MacLean (1949) elaborated on this "Papez Circuit," adding the amygdala, septum, and prefrontal cortex, but maintained that the hippocampus itself was the major interface between the subcortical, "visceral," non-verbal, emotional brain, (i.e., the "limbic system"), and the neocortical, exteroceptive, "word" brain. The integration of afferents coming from these internal and external sources formed the basis of emotional experience. MacLean further specified that the pyramidal cells of the hippocampus directly translate the sub-symbolic 'codes' of visceral sensations into the conscious, symbolic "codes" of language [e.g., pain-fear], much the same way as the keys of a piano code different musical sounds, which together can form an infinite variety of musical forms (MacLean, 1949).

While MacLean's general "limbic system" account of the emotional brain has persisted (despite a few, significant critiques; e.g., LeDoux, 1996), the center piece of his "emotional brain," the hippocampus, is no longer viewed in the same way. The view of hippocampal function that is dominant now was inspired by a single, famous, neurological patient, named H.M. Brenda Milner and others began studying H.M.'s memory capacities after his medial temporal lobes were excised in a final attempt to treat his intractable epilepsy (Milner, 1972). Although the surgery was successful in reducing H.M.'s seizures, Milner, after a series of careful observations and testing, found that the operation had also produced a severe, anterograde, memory deficit in which H.M could no longer form new "declarative" (conscious) memories. For example, H.M. was unable to remember

people, situations, tasks, or events encountered after his surgery, despite frequent re-exposure to them (e.g., he would not recognize a person he had met on many occasions after his surgery, such as Milner herself). At the same time, H.M.'s ability to acquire "procedural memories" (e.g., learning how to trace a star shape reflected in a mirror) was left intact, even though before each daily training session, he could not recall having practised the task before, or what the name of the task was, or who the person administering the task was—none of these conscious or "declarative" memories about the task were ever acquired (Milner, 1965).

Some have suggested that H.M.'s memory impairments resulted from a specific impairment in the *consolidation* of new memories, and that the hippocampus mediates this function. In other words, the hippocampus transfers (consolidates) the short-term memory of a novel stimulus or situation into a long-term representation, which is stored elsewhere in the brain (perhaps in the neocortex; Squire et al., 2004). An alternative theory is that the hippocampus itself may permanently store long-term memories by creating multiple copies ('traces') of the original memory when that memory is reactivated over time (known as Multiple Trace Theory, Nadel and Moscovitch, 1997). Nadel and Moscovitch also postulated that these multiple memory 'traces' are created within diverse locations in the hippocampus. Furthermore, older memories have more multiple traces, and therefore are more resistant to hippocampal damage. Newer memories have fewer 'traces,' making them more susceptible to hippocampal

damage. In essence, the theory combines both consolidation and long term storage into one hippocampal process.

Although Nadel and Moscovitch's model might explain why H.M. retained memories of events taking place many years before his surgery (some of his hippocampus remained intact), and why new events are less well represented in H.M.'s hippocampus (most of his hippocampus was missing) the theory does not explain H.M.s *complete* lack of new declarative memories, or his unimpaired acquisition of new procedural memories.

While declarative or "explicit" memory in humans reflects a general memory process, other, more specialized memory processes may also be housed in the hippocampus. One example is "spatial" memory, which has been repeatedly shown to be hippocampal-dependent. For example, in the "Morris water maze" (Morris, 1984) rats must find a platform located under the surface of an opaque pool of water. In order to remember the location of the platform, once found, rats must rely on spatial cues or landmarks situated around the outside of maze. After a few trials, non-lesioned rats can find the platform during a non-cued retention test, showing they have acquired a spatial memory of the location. However, hippocampal-lesioned rats during the same, non-cued retention test cannot find the platform, suggesting they have no memory of its location (e.g., Morris et al., 1990). Subsequent studies using a variety of spatial memory tasks all demonstrate that successful performance during a retention trial is critically dependent on the integrity of hippocampus (e.g., the T-maze test; Bannerman et al., 1999).

O'Keefe and Nadel (1978) proposed a complementary "Cognitive-Map" theory to explain how the hippocampus mediates spatial memory. The theory proposes that 'place-cells' in the hippocampus create a spatial representation (i.e., cognitive map) of the environment. When an organism is in a particular location within a spatial environment, specific 'place-cells' associated with that location are activated in the hippocampus. The function of these 'place-cells' allows an organism to orient itself to a particular location in a spatial environment in relation to other locations in that environment (i.e., allocentric spatial orientation). Several studies have lent support to this theory (see Best et al., 2001).

Anxiety and the Hippocampus

While these findings clearly establish a role for the hippocampus in higher cognitive functions such as memory, they do not exclude its role in other fundamental processes such as emotion (e.g., MacLean, 1949). For example, Gray (1982), and subsequently McNaughton and Corr (2004) developed a theory of hippocampal function more akin to Paul MacLean's earlier formulations, but one that was also cognisant of modern data and theory. In its essence, their theory suggests that the hippocampus (as well as the septum), by actively inhibiting competing stimuli, allow the organism to focus on relevant stimuli and thereby enhance adaptive behavior. This septo-hippocampal 'behavioural inhibition system' or BIS is especially engaged by stimuli, whether innate or learned, that pose threats to the organism, and which engender fear or anxiety. Accordingly, lesions of the hippocampus or septum should also result in anxiety reduction, or anxiolysis.

The core evidence that Gray et al., marshalled in support of this aspect of their theory is correlational: i.e., the fact that septal-hippocampal lesions and anxiolytic drugs both produce similar effects in a variety of classical learning paradigms (e.g., one-way active avoidance; Gray and McNaughton, 1983). More direct support for the theory comes from demonstrations of anxiolytic-like effects of septal and hippocampal lesions in well-validated animal models of anxiety (Menard and Treit, 1996; Pesold and Treit, 1992; Treit and Menard, 1997; Treit and Pesold, 1990; Treit et al., 1993). Studies have also shown that pharmacological inactivation of the septum or hippocampus by microinfused $GABA_A$ agonists or sodium channel blockers also produces anxiolysis in these animal models (Pesold and Treit, 1996; Treit and Menard, 2000; Engin and Treit, 2007; McEown and Treit, 2009).

Summary of the Hippocampal Anatomy

The hippocampal formation consists of six sub-regions that include the dentate gyrus (DG), hippocampus proper (CA1, CA2 and CA3), subiculum-presubiculum, parasubiculum and entorhinal cortex (EC). The hippocampal formation is a 'c' shaped portion of the brain that forms a septotemporal axis: i.e., extending from the septal nuclei to the temporal lobe. Only the CA1, CA2, CA3 and DG are found at the most rostral ("septal") regions of the hippocampal formation, while the subiculum-presubiculum and parasubiculum appear more caudally. The entorhinal cortex is located in the most caudal section of the structure. The dorsal and ventral regions of the hippocampus are designated by transecting the hippocampus along the horizontal longitudinal axis. The upper and

lower sections relative to the horizontal longitudinal axis are referred to as the dorsal and ventral hippocampus respectively (see Moser and Moser, 1998).

The hippocampus receives both cortical and sub-cortical inputs from various regions of the brain. Cortical inputs are thought to mediate some cognitive and memory functions whereas the sub-cortical inputs may mediate more fundamental behavioral functions. The majority of axonal connections within the hippocampus are unidirectional; in other words, each sub-region of the hippocampus receives unidirectional input from another sub-region (e.g., between CA3 and CA1). However, each sub-region also performs functions which are independent of this input.

Functional specialization within the hippocampus

Several animal studies provide evidence that the roles of the dorsal and ventral hippocampus in memory and fear may differ. It has been suggested that the dorsal hippocampus predominately mediates memory (particularly spatial memory) and the ventral hippocampus mediates anxiety or fear (see Bannerman et al., 2004 for a review). Lesioning or inactivating the dorsal hippocampus results in impairments in spatial memory (e.g., Eijkenboom and Van Der Staay, 1999; McHugh et al., 2008), whereas lesioning or inactivating the ventral hippocampus impairs a variety of unconditioned fear reactions (i.e., anxiety; Pentowski et al., 2006). However, there are many instances in which this putative relationship has not been supported (for a review see Engin and Treit, 2007). Furthermore, most of the studies in this area examine the effects of hippocampal disruption on either fear or memory processes, but not both. This complicates the

direct assessment of functional specialization within the dorsal and ventral hippocampus.

Accordingly, my purpose in undertaking this thesis is to further explore the putative roles of the dorsal and ventral hippocampus in memory and fear by using a paradigm in which both of these processes can be measured.

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Chapter 2

The Role of the Dorsal and Ventral Hippocampus in Fear and Memory of a Shock-Probe

Introduction

The shock probe burying test is an experimental animal model of anxiety in which subjects are shocked by making contact with a stationary, electrified probe attached to one of the walls of a Plexiglas chamber (Pinel and Treit, 1978; Treit and Pinel, 2005). After this contact-induced shock, subjects typically engage in burying behaviour (pushing bedding material toward and/or over the probe), while simultaneously avoiding the probe. Both burying behaviour and probe avoidance are indicative of anxiety or fear in this test, since both are suppressed by anxiolytic drugs (e.g., Treit, 1990). Encoding of this aversive event is thought to be partly mediated by the hippocampus (Lehmann et al., 2005; Lehmann et al., 2006). Lesioning the hippocampus is thought to impair the rats' ability to form an association between contextual stimuli in the burying chamber and the aversive shock, as measured by subsequent burying behaviour and latency to contact a non-electrified probe during re-exposure to the testing environment (Lehmann et al., 2005).

The hippocampus plays important roles in both mnemonic and emotional functions (e.g., Bannerman et al., 2004; Degroot and Treit, 2004; Degroot and Nomikos, 2005; Engin and Treit, 2007; McNaughton, 1997). The hippocampus has long been implicated in the consolidation of "explicit" or "declarative" memory (e.g., Corkin, 2002; Zola-Morgan and Squire, 1993), based on the

anterograde memory deficits seen in human patients after bilateral lesions of the hippocampus (e.g., H.M; Milner 1972). Lesion studies in rats have suggested that the dorsal hippocampus is especially important in spatial memory processes (Morris et al., 1982; O'Keefe and Nadel, 1978; White and Gaskin, 2006), and in contextual fear conditioning (e.g., Bannerman et al., 2004; Lehmann et al., 2005; Oler et al., 2005). Lesions of the ventral hippocampus of rats, by comparison, block a variety of unconditioned fear reactions, including "freezing" behaviour to cat odour or electric foot-shock; avoidance of conspecifics in the social interaction test; open-arm avoidance in the elevated plus maze test; novel-food avoidance in an unfamiliar environment; light-avoidance in a two compartment, light-dark box; and defecation in the open field test (Bannerman et al., 1999; Bannerman et al., 2002; Bannerman et al., 2003; Hock and Bunsey, 1998; McHugh et al., 2004; Kjelstrup et al., 2002; Pentowski et al., 2006). Based on these findings, it has been suggested that the roles of the ventral and dorsal hippocampus in fear and memory may differ (Bannerman et al., 2004).

Tests that measure both fear and memory would seem to be particularly useful for assessing the effects of dorsal and ventral hippocampal lesions. In the shock-probe burying test, for example, both fear and memory can be monitored by first observing a rat's immediate (unconditioned) reactions to shock from the electrified probe ("acquisition") and then, 24hr later, by observing the rat's (conditioned) reactions to an identical, non-electrified probe ("retention"). Using this paradigm, Lehmann et al. (2005) found that hippocampal-lesioned rats had shorter latencies to contact a non-electrified probe than sham-lesioned controls

during a retention test 24 hrs after shock exposure. Hippocampal-lesioned rats also buried the probe significantly less than sham lesioned controls during the retention test. Together, these findings support the general hypothesis that the hippocampus is involved in both the behavioural reactions to, and the memory of, aversive events.

The lesions in the Lehman et al. (2005) study, however, included both the dorsal and the ventral aspects of the hippocampus. Thus, the individual roles of the dorsal and ventral hippocampus in the acquisition and retention of an aversive event were not revealed. In addition, the lesions themselves were permanent, and made before behavioural testing. Therefore, it is difficult to separate the effects of hippocampal lesions on unconditioned reactions to the probe from conditioned reactions to the probe during subsequent exposure. A remedy for this problem is provided by intracerebral microinfusion of sodium channel blockers such as tetrodotoxin (TTX) or lidocaine, which temporarily inactivate neuronal signalling in the infused area (Fozzard et al., 2005). With this technique, the effects of hippocampal inactivation during acquisition can be separated from effects seen during retention 24 hr later, when the hippocampus is no longer inactivated.

Previous studies of the acute effects of reversible TTX-inactivation of the dorsal hippocampus on unconditioned fear reactions showed that shock-probe avoidance was impaired. Similar inactivation of the ventral hippocampus impaired shock-probe burying (e.g., Degroot and Treit, 2004). In combination, these findings suggest that the shock-probe test might be a useful tool for disambiguating the effects of dorsal and ventral hippocampal inactivation during

both acquisition and later retention, when memory of the probe-shock is required *and* when the hippocampus is functionally intact.

Thus, the specific purpose of the present study was to examine the effects of reversible inactivation of either the dorsal or ventral hippocampus on 1) *unconditioned* fear behaviour during the first exposure to the electrified probe (acquisition), and 2) *conditioned* fear behaviour during a second exposure 24hr later, to an identical, non-electrified probe (retention). Lidocaine inactivation occurred just before acquisition (Experiment 1) or immediately after acquisition (Experiment 2).

Lidocaine infusions were given immediately after acquisition in Experiment 2 to eliminate the possibility that pre-acquisition lidocaine in Experiment 1 had produced a motivational deficit (e.g., fear reduction), and thereby impaired the expression of fear during the retention test, rather than impairing the memory of a fearful experience. Such a possibility would be supported if retention performance was impaired in Experiment 1 but not in Experiment 2. If lidocaine infusions impaired retention in both experiments, however, then the diminished retention in Experiment 1 would more likely reflect a mnemonic effect of hippocampal inactivation during acquisition.

In summary, if the ventral hippocampus is primarily involved in the expression of unconditioned fear reactions, then ventral hippocampal inactivation occurring just prior to acquisition (Experiment 1) should impair defensive behaviour toward the probe during the acquisition test, but not during the retention test. Conversely, if the dorsal hippocampus is primarily responsible for

encoding the shock-probe experience, then its temporary inactivation during acquisition should impair defensive behaviour directed toward the probe during the retention test. Furthermore, this memory deficit should occur regardless of whether inactivation of the dorsal hippocampus occurred just before (Experiment 1) or just after the acquisition session (Experiment 2). Ventral hippocampal inactivation given before or after acquisition should have little effect on defensive behaviour during retention. The current experiments were designed to test each of these predictions.

Results

EXPERIMENT ONE*:* EFFECTS OF PRE-ACQUISITION INACTIVATION

Subjects and Histology

Data from four rats, two with an obstructed cannula and two that could not be shocked, were discarded. Data from five additional animals were discarded because of misplaced cannulae. Included cannulae placements are shown in Fig. 2-1. In addition, the data from three rats ("outliers") were discarded from the analysis of time spent in the shock-probe half of the chamber because their scores were more then three standard deviations from the mean. The behavioral data were assessed with ANOVA (α=0.05), followed by a priori pair-wise comparisons (t-tests, $\alpha = 0.05$, one-tailed), or a posteriori post hoc comparisons (LSD tests, $\alpha = 0.05$). ANOVA of the saline infused animals in both control groups (i.e., shock-experienced control and shock-naïve control groups) was not significant (i.e., $p > .05$), and therefore these control groups were collapsed across infusion site (i.e., dorsal and ventral) for all comparisons.

Acquisition I

There was a significant overall difference in the duration of burying during acquisition ($F(3, 48) = 3.83$, $p < .01$; see Fig. 2-2). As expected, shock-experienced, saline-infused control rats buried the probe significantly more than shock-naïve saline-infused control rats ($t(19) = -4.30$, $p < .001$). More importantly, temporary inactivation of the ventral hippocampus significantly suppressed burying behavior compared to shock-experienced, saline-infused controls ($t(27) = -2.94$, $p < .01$). In contrast, temporary inactivation of the dorsal hippocampus did not significantly suppress burying behavior compared to shock-experienced saline-infused controls ($t(30) = -1.02$, $p = .15$). A direct comparison of the burying behaviour of ventral and dorsal hippocampal-inactivated animals differed significantly in the predicted direction (ventral hippocampus < dorsal hippocampus; $t(29) = 1.70$, $p < .05$). Mean shock reactivity did not differ between the three shocked groups ($F(2, 43) = 2.56$, $p = .08$; see Table 1), suggesting that the suppression of burying behavior by ventral hippocampal inactivation was not confounded by side-effects on shock sensitivity (e.g., reduced pain sensitivity). Although there was a significant overall difference in still time ($F(3, 48) = 4.43$, $p < .001$; see Fig. 2-3), post hoc HSD tests showed that dorsal hippocampal-inactivated and ventral hippocampal-inactivated rats did not differ significantly from the shocked control group ($p = .80$ and $p = .16$ respectively). These results suggest that the effects of ventral-hippocampal inactivation on probe-burying were not due to differences in general activity.

Overall group differences in the number of shocks received during acquisition just failed to reach significance. ($F(2, 43) = 2.94$, $p = .06$; see Table 1). There was a tendency, however, for dorsal hippocampus-inactivated rats to receive more shocks than the saline-infused controls ($t(30) = 1.26$, $p = .10$; see Table 1).

In summary, the results of the acquisition test showed that temporary inactivation of the ventral hippocampus (but not the dorsal hippocampus) significantly decreased shock-probe burying, a measure of unconditioned fear in the shock-probe burying test.

Retention I

Significant overall differences were observed in rats' burying behaviour during the retention test ($F(3, 48) = 3.94$, $p < .01$; see Fig. 2-4). As expected, shock-naïve rats buried the probe significantly less than shock-experienced controls ($t(19) = -4.02$, $p < .001$). Importantly, rats that had received temporary inactivation of the dorsal hippocampus during acquisition buried the probe significantly less than did the shock-experienced saline-infused controls ($t(30) = -2.19$, $p < .05$). However, rats that had received temporary ventral hippocampal inactivation during acquisition also buried the probe significantly less than shock-experienced saline-infused controls ($t(27) = -1.82$, $p < .05$), and direct comparison of the dorsal hippocampus and ventral hippocampus-inactivated rats failed to show a significant difference in the duration of their burying behaviour during the retention test ($t(29) = -.27$, $p = .39$).

There was, however, a consistent and significant between-groups difference in the amount of time that rats spent in the half of the chamber containing the shock-probe during the retention test ($F(3, 48) = 9.18$, $p < .001$; see Fig. 2-5). Predictably, shock-naïve rats spent significantly more time in the shock-probe end than did shock-experienced controls ($t(19) = 5.60$, $p < .001$). Also consistent with our expectations, dorsal hippocampal-inactivated rats spent significantly more time in the probe-half of the chamber than shock-experienced controls ($t(30) = 2.95$, $p <. 01$), suggesting they had not encoded or retained a memory of their initial shock-probe experience. In contrast, ventral hippocampal-inactivated rats, as predicted, did not spend any more time in the shock-probe half of the chamber than shock-experienced controls ($t(27) = .34$, $p = .36$), suggesting that they had remembered the shock-probe experience. Furthermore, direct comparison showed that the dorsal hippocampal- inactivated rats spent significantly more time in the shock-probe half of the chamber than the ventral hippocampal-inactivated rats ($t(29) = 1.96$, $p < .05$). Overall, this measure of retention suggested that temporary inactivation of the dorsal hippocampus during acquisition had interfered with rats' memory of the shock-probe experience, whereas similar inactivation of the ventral hippocampus had not.

EXPERIMENT 2*:* EFFECTS OF POST-ACQUISITION INACTIVATION

Subjects and Histology

Data from seven rats, six with an obstructed cannula and one that could not be shocked, were discarded. Data from eight additional animals were subsequently discarded because of misplaced cannulae. Accurate placements are shown in Fig.

2-6. In addition, the data from two rats ("outliers") were discarded from the analysis of time spent in the shock-probe half of the chamber because their scores were more then seven standard deviations from the mean. ANOVA of the saline-infused animals in both control groups (i.e., shock-experienced control and shock-naïve control groups) was not significant (i.e., $p > .05$), and therefore these control groups were collapsed across infusion site (i.e., dorsal and ventral) for all comparisons.

Acquisition II

Post-acquisition lidocaine infusions could not have had an effect on burying behaviour that occurred during acquisition and thus the shocked groups did not differ significantly among themselves on this measure ($p > .05$ between all shock- exposed groups). However, as expected, shocked rats buried more than non-shocked rats ($F(3,33) = 10.21$, $p < .001$; see Table 1). Probe contacts ($F(2, 26) = .71$, $p = .50$; see Table 1), shock reactivity ($F(2, 26) = 1.88$, $p < .17$; see Table 1), and still time ($F(3, 33) = .30$, $p = .82$; see Table 1) did not differ significantly between the groups.

Retention II

Unexpectedly, there were no group differences in burying behaviour during the retention test ($F(3, 33) = 1.35$, $p < .27$; see Fig. 2-7), although planned comparisons again showed shock-experienced controls buried the probe significantly more than shock-naïve controls ($t(13) = 2.10$, $p < .05$), indicating retention of their initial shock-probe experience.

On the other hand, there was a significant between-groups difference in the amount of time that rats spent in the shock-probe- half of the chamber ($F(3, 31) = 19.67$, $p < .001$; see Fig. 2-8). Shock-experienced rats spent significantly less time in the probe end of the chamber than shock-naive rats, suggesting they had remembered being shocked during acquisition ($t(13) = -11.03$, $p < .001$). More importantly, dorsal hippocampal-inactivated rats spent significantly *more* time in the shock-probe half of the chamber than shock-experienced controls ($t(13) = 2.71$, $p < .01$), suggesting they had not remembered their previous shock-probe experience. Ventral hippocampal-inactivated rats, in contrast, were not significantly different from shock-experienced controls ($t(11) = .99$, $p = .17$), suggesting that their memory was intact. The difference in time spent in the probe end of the chamber between dorsal hippocampal-inactivated and ventral hippocampal-inactivated rats failed to reach significance ($t(18) = 1.45$, $p = .08$). Nevertheless, this measure of retention suggested that temporary inactivation of the dorsal hippocampus just after acquisition, as it had done just before acquisition (Experiment 1), significantly decreased rats' memory of the shock-probe experience, whereas similar inactivation of the ventral hippocampus had not.

Discussion

The results of this study provide support for a functional dissociation between the dorsal hippocampus and ventral hippocampus, namely: 1) the dorsal hippocampus, among its other mnemonic functions, is responsible for encoding a memory of a discrete, fearful experience, while 2) the ventral hippocampus, along

with its other behavioural functions, is primarily responsible for the expression of untrained fear reactions to a discrete object. These conclusions are supported by a number of the current findings.

First, temporary inactivation of the ventral hippocampus impaired unconditioned burying behaviour during the first exposure to the shock-probe ('acquisition' session). By comparison, inactivation of the dorsal hippocampus during the first exposure to the shock-probe did not impair unconditioned burying behaviour. These results alone indicate that the ventral hippocampus, and not the dorsal hippocampus, is more involved in the expression of unconditioned fear responses.

Second, inactivating the dorsal hippocampus impaired probe avoidance during the 'retention' session 24hr later, regardless of whether inactivation occurred during or just after acquisition. In contrast, inactivating the ventral hippocampus during or just after acquisition did not impair probe avoidance during the 'retention' session. These data by themselves suggest that the dorsal hippocampus is directly involved in the consolidation of fear memories.

Third, it is important to emphasize that inactivation of the dorsal hippocampus immediately after acquisition in Experiment 2 did not (and could not) suppress fear motivation, or affect other performance factors during acquisition. Therefore the impairment in probe avoidance these rats displayed during the retention test was not due to non-mnemonic effects of hippocampal inactivation during acquisition (e.g., decreased aversive motivation; see introduction). This particular result strongly supports our view that temporary

inactivation of the dorsal hippocampus during acquisition specifically impaired consolidation of the initial shock probe experience.

Be this as it may, however, the burying behaviour of dorsal hippocampal-inactivated rats during retention in Experiment 2 was not significantly impaired compared to shock-experienced, non-inactivated controls, which was unexpected. One possibility is that such impairment was obscured by a "floor" effect. Compared to the burying behavior of shock-experienced rats in Experiment 1 during retention, the burying behavior of shock-experienced rats in Experiment 2 during retention was substantially less (see Fig. 2-4 and 2-7 for comparison). Thus, detecting a statistical difference between this shock control group and the dorsal experimental group was much less likely in Experiment 2. Nevertheless, the memory impairment produced by dorsal hippocampal inactivation was clearly apparent in the probe avoidance behavior in both experiments, lessening the importance of this possible confound.

More problematic is the finding that temporary inactivation of the ventral hippocampus prior to acquisition in Experiment 1 did impair burying behaviour during retention compared to shocked controls. This suggests that the ventral hippocampus does play some role in the consolidation of fear memories, at least with respect to burying behavior. There seem to be two possibilities here. One is simply that ventral hippocampal inactivation during acquisition in Experiment 1 had dampened the fear motivation required for successfully encoding the shock-probe experience, and this *non-mnemonic* factor explains the impairment of burying during the retention test, not a memory deficit. In any case, ventrally

lesioned hippocampal rats avoided the probe end of the chamber more than dorsally inactivated hippocampal rats, and more than non-shocked controls in both Experiment 1 and Experiment 2. The latter findings buttress our general conclusion that the ventral group had retained a memory of the shock-probe experience whereas the dorsal group had not.

A more remote possibility is that the ventral hippocampus *is* involved in the learning and memory of certain conditioned fear responses (i.e., burying behaviour), but not others (i.e., probe avoidance), or that a memory system specialized for fear (e.g., amygdala; LeDoux, 2000) also has specific tags or compartments for active (e.g., defensive burying) versus passive defensive behaviors (e.g., freezing, passive avoidance), which affect later recall. Regardless of the viability of this degree of specialization, or the specific locations in the brain that might subserve it, it is possible that some scenario like this may ultimately inform the present results. The fact is, however, that permanent lesions or temporary inactivation of the ventral hippocampus with TTX have been reported to impair tone and/or contextual fear conditioning (e.g., Bast et al., 2001; Richmond 1999; Zhang et al., 2001). These data, although limited, suggest that the ventral hippocampus may indeed be involved in more than the expression of simple, unconditioned fear reactions. The extent of its involvement in consolidation, however, relative to the dorsal hippocampus, is not entirely clear, as is any result in the present study that unequivocally demonstrates that the ventral hippocampus is generally involved the consolidation of fear memories. On the contrary, our results during acquisition in Experiment 1 are more clearly in

line with previous studies showing that pre-test ventral hippocampal lesions reliably block a variety of *unconditioned* fear behaviours (e.g., Bannerman et al., 1999; Bannerman et al., 2002; Bannerman et al., 2003; Degroot and Treit, 2004; Hock and Bunsey, 1998; McHugh et al., 2004; Kjelstrup et al., 2002; Pentowski et al., 2006).

In this regard, it is important to emphasize that there are clear procedural differences between the shock-probe paradigm and pure Pavlovian fear conditioning. For example, after receiving a certain number of probe-shocks, a rat's avoidance of the probe may be more reflective of an instrumental behaviour, in which the consequences associated with probe-contact could reinforce probe-avoidance (Maren, 2003; Lehmann et al., 2005). A simple association between the probe (CS) and the spatially contiguous shock (UCS) is probably also formed (Pinel and Treit, 1978), but unlike standard Pavlovian fear conditioning, in which the occurrence of the shock UCS invariably follows the tone CS, the occurrence of the UCS in the shock-probe paradigm depends entirely on the rat's "voluntary" behaviour. In addition to these differences, there are reasons to believe that shock-probe burying and shock-probe avoidance during the retention test involve unique neural substrates in addition to, or in parallel with, those required for simple Pavlovian fear conditioning (Cohen and Castro-Alamancos, 2007). In this light, it may not be surprising that some differences exist between our results and the results obtained using pure Pavlovian fear conditioning. While it is possible that the ventral hippocampus encodes simple Pavlovian associations between a CS and a UCS (e.g., tone and shock), in our view the ventral hippocampus is not required

for encoding associations such as those between voluntary behaviours and their consequences (e.g., avoidance of a probe previously associated with electric shock). Thus, the interplay between classical and instrumental conditioning in the shock-probe paradigm may be an important factor in explaining some of the effects of ventral and dorsal hippocampal lesions.

Another issue is which aspects of the shock-probe "experience" are encoded during acquisition. For example, Lehmann et al. (2005) found that the anterograde memory impairments produced by hippocampal lesions were not evident in the shock-probe procedure if the test environment experienced during acquisition was different from the test environment during retention. They interpreted these results in terms of contextual fear conditioning: i.e., sometime during or shortly after acquisition, the hippocampus encoded contextual information surrounding the shock-probe, not just the probe itself. Because animals in our study were exposed to the same experimental context during acquisition and retention, we were not able to disambiguate the roles of the dorsal and ventral hippocampus in contextual versus cue fear conditioning. Future experiments using the shock-probe test will be directed toward resolution of this issue.

Finally, the relatively large separation between the dorsal and ventral hippocampal targets makes it unlikely that drug diffusion to non-target areas played a significant role in our results. Sodium channel blockade, however, in spite of its experimental advantages as a technique for temporary neural inactivation (see introduction), could have silenced not only neural activity within

the dorsal and ventral hippocampus, but also axonal signals flowing between and through these two structures. In theory then, it is possible that inhibition of 1) neuronal signalling between the dorsal and ventral hippocampus, and/or 2) the recruitment of outside structures such as the septum or amygdala, which are also interconnected and involved in anxiety, could have contributed to the present results. Future experiments in which local inhibition is produced by microinfusion of $GABA_A$ agonists (e.g., muscimol) will help resolve these issues.

In summary, our results support two main conclusions: 1) the dorsal hippocampus is primarily responsible for encoding *conditioned* fear behaviour in the shock probe test (e.g., avoidance of a non-electrified shock-probe present during retention), and 2) the ventral hippocampus is primarily responsible for the expression of *unconditioned* fear behaviour elicited by a variety of aversive stimuli, including an electrified shock-probe. Thus, our results contribute to an emerging literature suggesting that the dorsal and ventral hippocampus play distinct roles in memory and fear.

Experimental Procedures

Subjects

One hundred twenty male Sprauge-Dawley rats (Ellerslie, Edmonton, Alberta, Canada) were used. Each animal weighed between approximately 150-250 grams upon arrival. Food and water were available ad libitum. Animals were individually housed in polycarbonate cages and kept on a light/dark cycle (12:12 hour; lights on at 0700 hours). Behavioural testing occurred during the light portion of the cycle.

Surgery

All surgeries conformed to the Society for Neuroscience Guidelines, CCAC guidelines and to local animal care protocol # 116204. Just prior to surgery, all subjects were injected with: 1) an analgesic (Rimadyl; 0.1cc, s.c.) to mitigate potential post-operative pain; 2) atropine sulfate (0.1mg/0.2ml, i.p.) to reduce any potential respiratory complications encountered during surgery; and 3) saline to avoid dehydration (3cc, s.c., once before surgery). Subjects were anesthetised with Isoflourane gas (4% concentration in O_2 gas) and maintained at a 2% concentration throughout the duration of the surgery. Subjects were bilaterally implanted with either 22-gauge, 5mm guide cannulae into the dorsal hippocampus (n = 60; -4.2 mm AP, 2.0 mm DV, +/- 4.1 mm lateral to the midline) or 22-gauge, 8mm guide cannulae into the ventral hippocampus (n = 60; -5.2 mm AP, 5.7 mm DV, +/- 5.6 mm lateral to midline). These anatomical coordinates were selected using a stereotaxic atlas (see Paxinos and Watson, 1986). Two days after surgery, all cannulae were checked for obstruction and hibitaine was applied to the surgical area.

Shock-probe Burying Test

Upon arrival, subjects were allowed three days to acclimatize to the colony room. After the acclimatization period all subjects were individually handled for 5 minutes per day over a four day period. Surgeries were then performed with behavioural testing occurring six days post-surgery. Two days post surgery, each subject was habituated to the test apparatus for a total of fifteen minutes per day over a period of 4 days. The test apparatus consisted of a 40 × 30 × 40 cm

Plexiglas chamber, with 5 cm of bedding material (wood chips) evenly spread over the chamber floor. An electrified Plexiglas shock-probe (6.5 cm long 0.5 cm in diameter) was helically wrapped with 2 copper wires and inserted through a hole in one of the walls of the chamber, 2 cm above the bedding material. The probe was electrified using a 2 pole precision animal shocker (Model H13-15, Colbourne Instruments) set at 2mA. The shock-probe was removed during habituation.

Infusion procedure.

Subjects were randomly assigned to either a control or experimental group. The control group received an intra-hippocampal infusion of physiological saline (infusion rate: 1µl/30 sec.; pH 7.4) and the experimental group received an intra-hippocampal infusion of 8% lidocaine hydrochloride (Sigma) dissolved in physiological saline (Infusion rate:1µl/30 sec). In the first experiment, infusions were administered five minutes prior to the acquisition session and in the second experiment immediately after the acquisition session. Infusions were administered with a 10 µl Hamilton microsyringe using an infusion pump (Harvard Apparatus 22). The cannulae were left in place for 30 seconds after drug administration to allow for diffusion. Past research indicates that an 8% concentration of lidocaine hydrochloride is able to produce a 20-25 minute blockade of sodium channels, which effectively inhibits all neural activity in the infusion area (e.g., Wall et al., 2004).

Acquisition I and II.

Subjects were tested in groups representing three conditions 1) lidocaine infusions into the ventral hippocampus (n = 32); 2) lidocaine infusions into the dorsal hippocampus (n = 33); and 3) vehicle (saline) infusions (n = 55). Half of the vehicle controls received dorsal hippocampal saline infusions and the remainder received ventral hippocampal saline infusions. For half of the subjects in the saline-infused control group the probe was electrified during acquisition (shocked controls) and for the remainder the shock-probe was not electrified (naïve controls). All subjects that received lidocaine infusions were exposed to an electrified probe during acquisition. Each subject was individually placed into the chamber facing away from the shock-probe. Testing began after the initial contact-induced probe-shock, and lasted 15 minutes. Subjects' data were discarded if they took longer than 15 minutes to initially contact the probe. The number of contact-induced shocks, burying time, still time (i.e., sitting or laying on the chamber floor, with normal respiration and orienting behavior), and mean shock reactivity was recorded (see Figure 2-9 for an overview of experimental procedures). Mean shock reactivity was evaluated using a four point scale (see Pesold and Treit 1992 for further details).

Retention I and II.

After a 24-hour delay, subjects were re-tested in the same environment in which acquisition occurred, except that subjects 1) did not receive a shock when they touched the probe, and 2) did not receive infusions of either lidocaine or vehicle. Duration of burying and the amount of time spent on the half of the chamber that contained the shock-probe was recorded (Lehmann et al., 2005).

Histology

After the completion of behavioural testing, subjects were deeply anesthetised using pentobarbital (Nembutal, 100 mg/kg, i.p) and subsequently perfused with a 10% formalin solution. Their brains were removed and placed in a specimen jar containing a 10% formalin solution. After forty-eight hours, brains were sectioned (60 μm), stained with thionin, and mounted on microscope slides. The locations of cannulae were confirmed microscopically. The behavioural data for subjects with either one or both cannulae outside of the target area (dorsal hippocampus or ventral hippocampus) were omitted from analysis.

Statistical Analyses

Analysis-of-variance (ANOVA) was used to analyze the behavioural data, with alpha set at p=0.05. Because of violations of the homogeneity of variance assumption, burying duration and still time were transformed to their base 10 logs prior to ANOVA. For comparisons where the direction of a behavioural difference was predicted a priori, effects were analyzed using one-tailed t-tests with a type I error rate of alpha = 0.05. Post hoc HSD tests were used for comparisons in which a posteriori differences were observed.

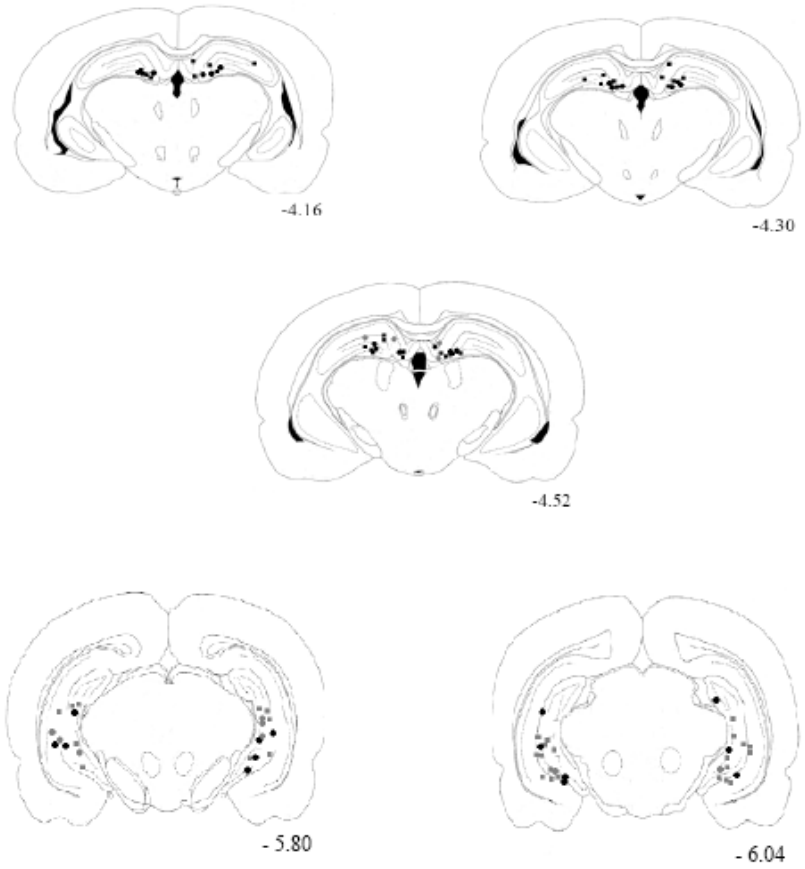

Figure 1

Fig. 2-1 - Illustrations of coronal sections of the rat brain adapted from Paxinos and Watson (1986) displaying the approximate location of dorsal and ventral hippocampal infusion sites in Experiment 1. The numbers indicate A-P coordinates relative to bregma. Symbols representing cannulae placement by group: 1) black square corresponds to lidocaine dorsal infused animals; 2) grey square corresponds to lidocaine ventral infused animals 3) black circle corresponds to shocked control vehicle infused animals; and 4) grey circle corresponds to shock-naïve, vehicle-infused control animals.

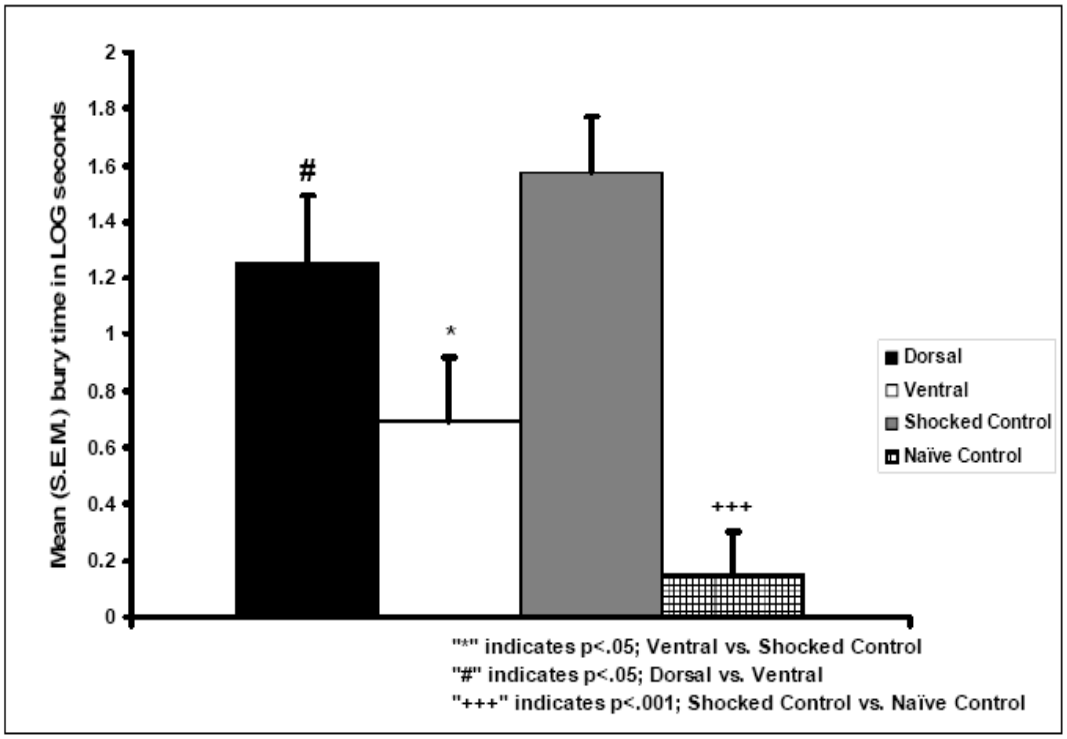

Figure 2

Fig. 2-2 - Mean (± S.E.M.) acquisition bury time (experiment one) of dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and shock-naïve control (vehicle) rats.

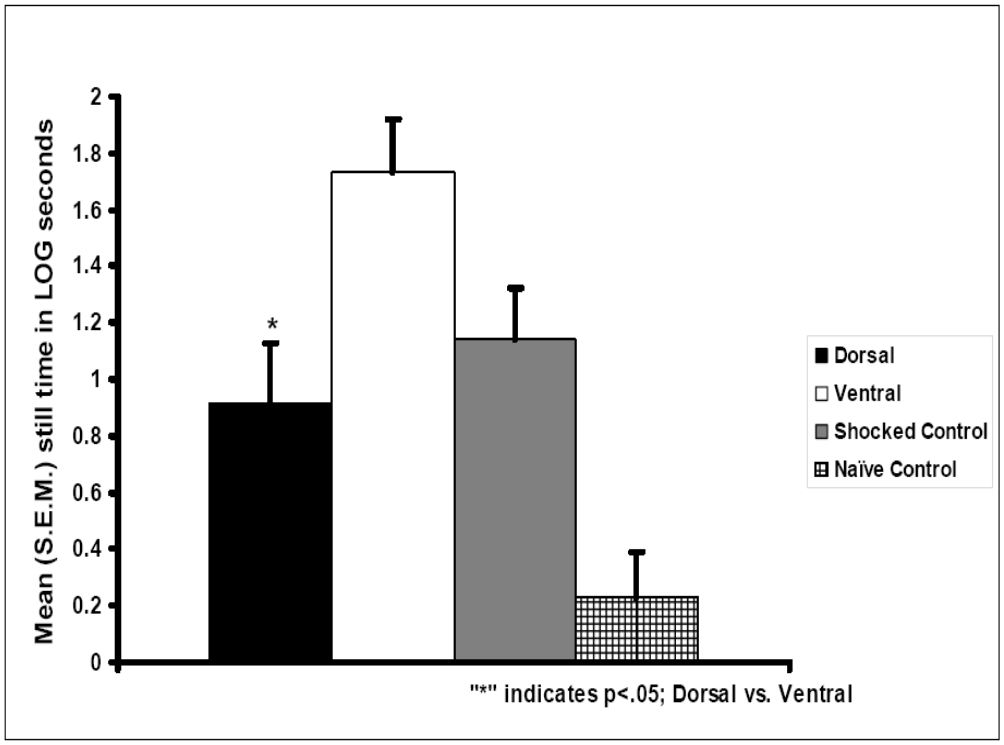

Figure 3

Fig. 2-3 - Mean (± S.E.M.) still time (experiment one) of dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and naïve control (vehicle) rats.

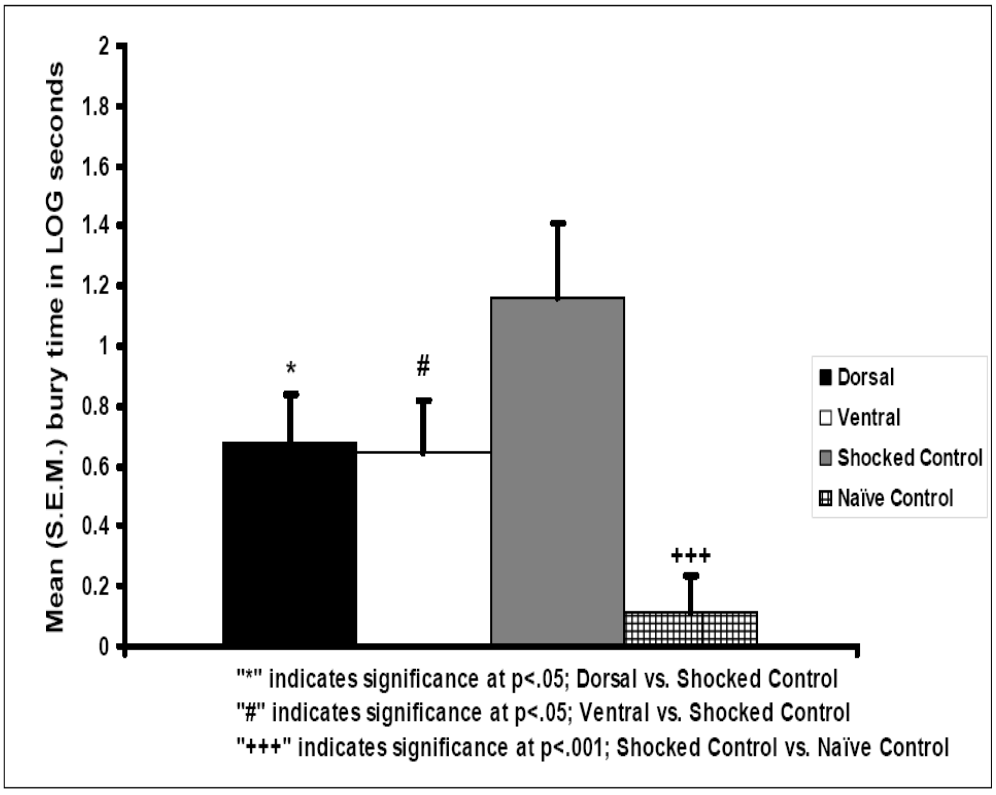

Figure 4

Fig. 2-4 - Mean (± S.E.M.) retention bury time (experiment one) between dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and naïve control (vehicle) rats.

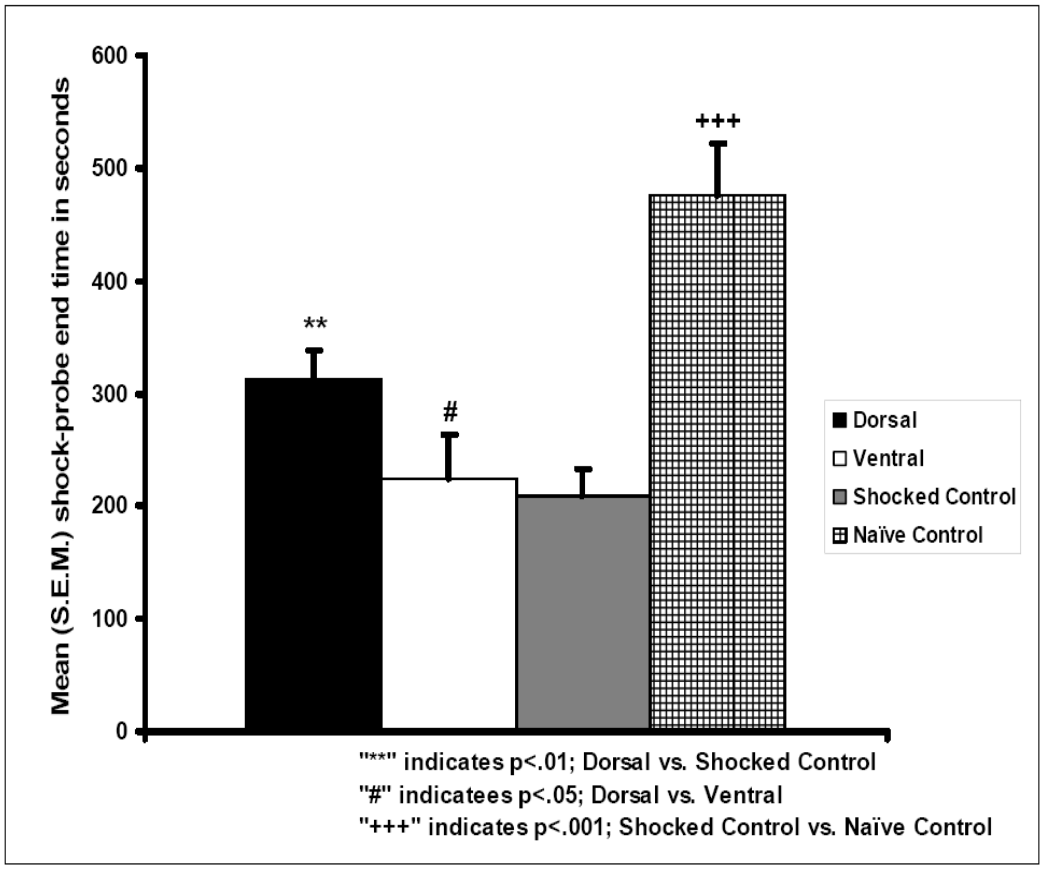

Figure 5

Fig. 2-5 - Mean (± S.E.M.) retention shock-probe end time (experiment one) between dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and naïve control (vehicle) rats.

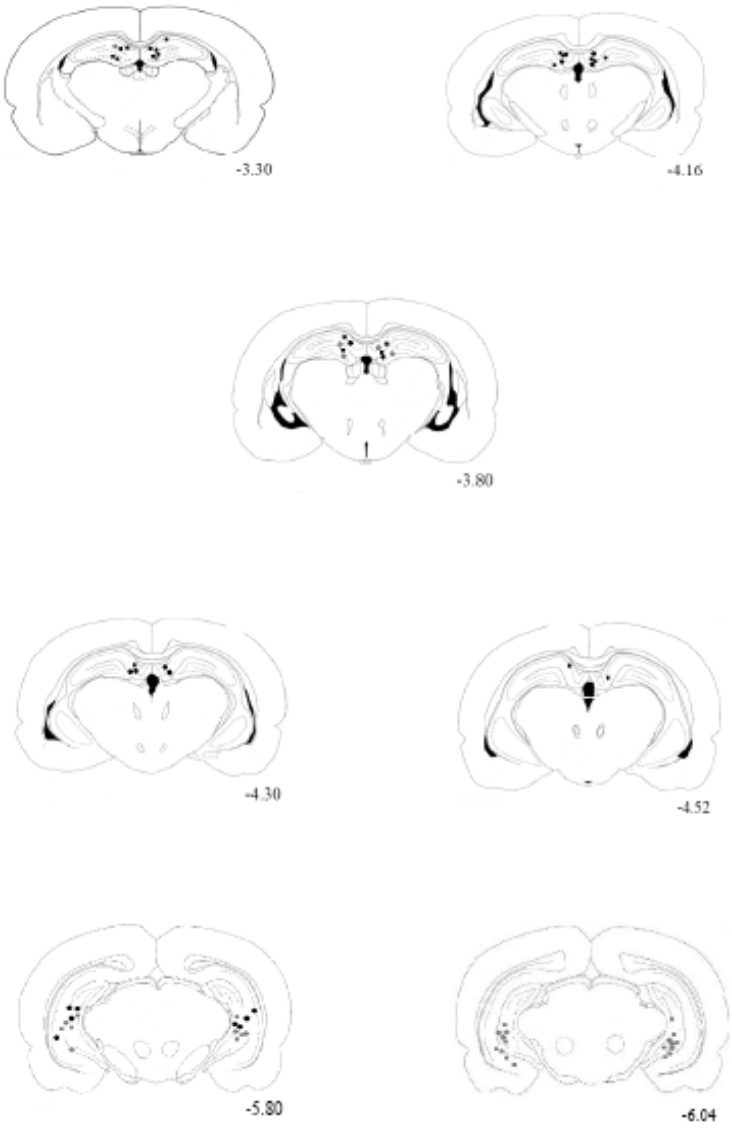

Figure 6

Fig. 2-6 - Illustrations of coronal sections of the rat brain adapted from Paxinos and Watson (1986) displaying the approximate location of dorsal hippocampal infusion sites in Experiment 2. The numbers indicate A-P coordinates relative to bregma. Symbols representing cannulae placement by group: 1) black square corresponds to lidocaine dorsal infused animals; 1) grey square corresponds to lidocaine ventral infused animals; 3) black circle corresponds to shocked control vehicle infused animals; and 4) grey circle corresponds to naïve control vehicle infused animals.

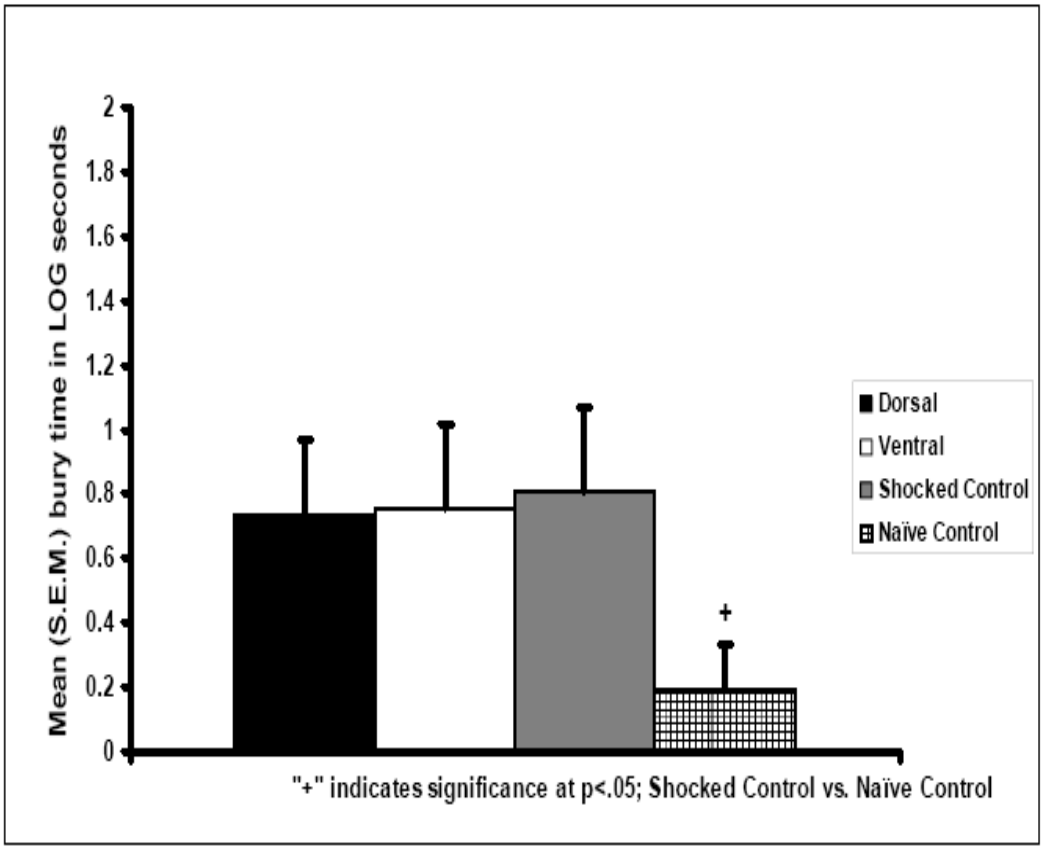

Figure 7

Fig. 2-7 - Mean (± S.E.M.) retention bury time (experiment two) between dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and naïve control (vehicle) rats.

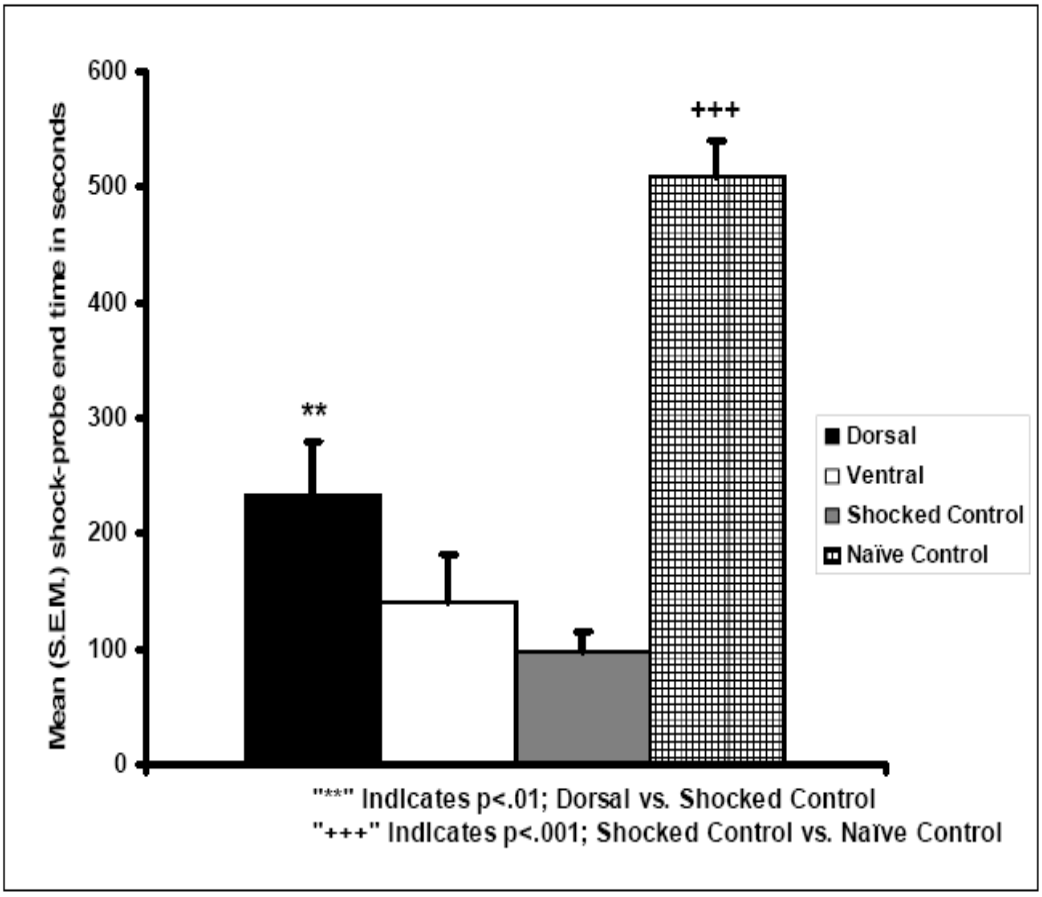

Figure 8

Fig. 2-8 - Mean (± S.E.M.) retention end time (experiment two) between dorsal hippocampus (lidocaine), ventral hippocampus (lidocaine), shocked control (vehicle) and naïve control (vehicle) rats.

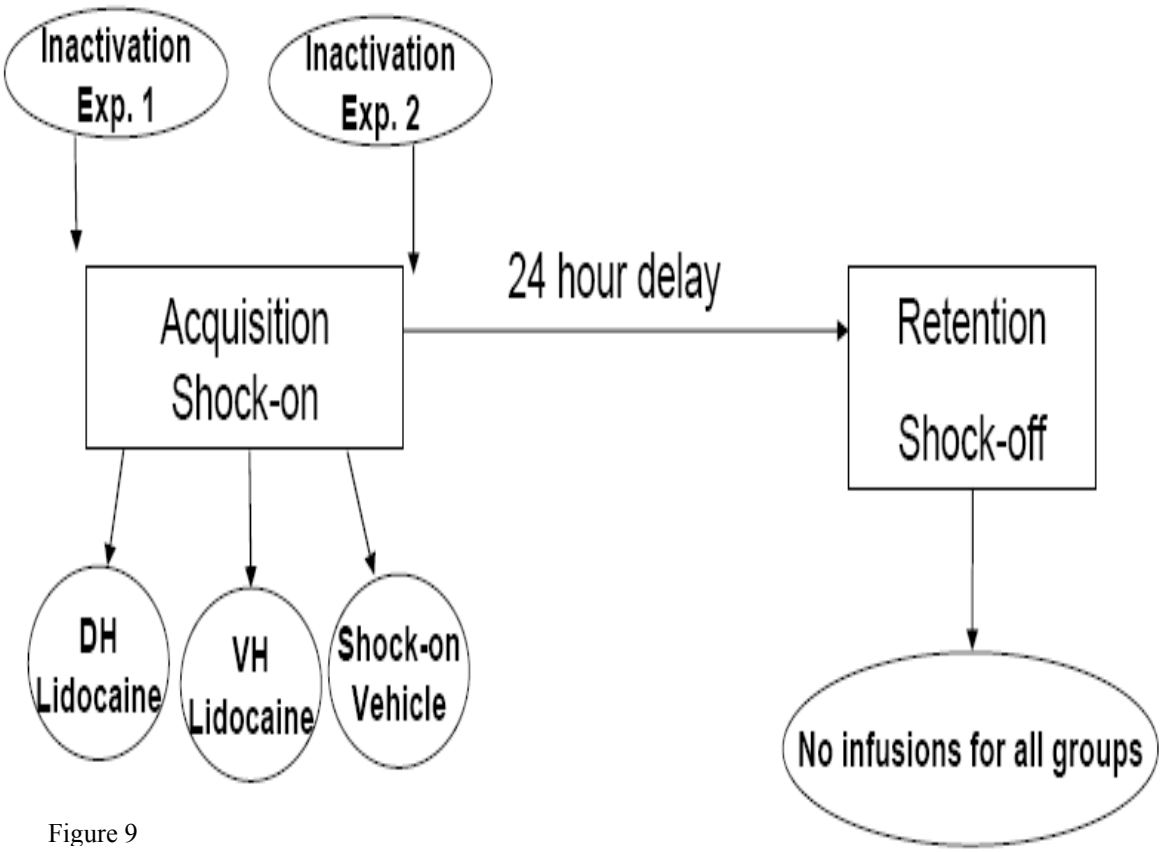

Figure 9

Figure 2-9 – Procedural overview of Experiment 1 and Experiment 2.

Experiment one	Dorsal hippocampus	Ventral hippocampus	Naïve controls	Shocked controls
Acquisition	(*n* = 17)	(*n* = 14)	NA	(*n* = 15)
Shock number	2.29 (.25)	2.71 (.24)	NA	1.87 (.22)
Shock reactivity	1.26 (.13)	1.34 (.11)	NA	1.66 (.15)
Experiment two	**Dorsal hippocampus**	**Ventral hippocampus**	**Naïve controls**	**Shocked controls**
Acquisition	(*n* =11)	(*n* = 9)	(*n* = 8)	(*n* = 9)
Still time (LOG)	.44 (.21)	.61 (.29)	.28 (.19)	.54 (.30)
Shock number	1.82 (.30)	2.33 (.30)	NA	2.11 (.35)
Shock reactivity	1.33 (.14)	1.73 (.16)	NA	1.51 (.15)
Bury Time (LOG)	1.42 (.22)	1.36 (.25)	.14 (.09)	1.65 (.19)

Table 1

Table 2-1 – Mean (± S.E.M.) acquisition: 1) number of received shocks and shock reactivity for experiment 1 and 2) still time, number of received shocks, shock reactivity, bury time for experiment 2.

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Chapter 3

Inactivation of the Dorsal or Ventral Hippocampus with Muscimol differentially affects Fear Memory

Introduction

Several findings suggest that the ventral and dorsal hippocampus seperately mediate fear and memory. For example, in rats, lesions of the dorsal hippocampus impair cognitive functions such as spatial memory [Morris et al., 1982; O'Keefe and Nadel, 1978; Gaskin and White, 2006], whereas lesions of ventral hippocampus impair unconditioned fear behaviours such as open-arm avoidance in the elevated plus-maze [e.g., Bannerman et al., 1999; Bannerman et al., 2002; Bannerman et al., 2003; Hock and Bunsey, 1998; McHugh et al., 2004; Kjelstrup et al., 2002].

One of the shortcomings of many of these studies, however, is that lesions of the hippocampus, for the most part, were permanent and produced before behavioural testing. Because permanent lesions endure throughout behavioral testing, separating their effects on unconditioned fear from their effects on fear memories is not entirely straightforward. In addition, these lesions can engender neural changes outside of the target area that can undermine the apparent site-specificity of the results. An alternative approach involves intracerebral microinfusion of sodium channel blockers such as tetrodotoxin (TTX) or lidocaine, which temporarily inactivate neuronal signalling in the infused area [Fozzard et al., 2005]. With this technique, the effects of hippocampal inactivation on unconditioned fear can be separated from its effects on fear

memories that are expressed at a later time, when the hippocampus is no longer inactivated.

Tests that measure both fear and memory would seem to be particularly useful for assessing the effects of hippocampal lesions. In the shock-probe burying test, for example, both fear and memory can be monitored by first observing a rat's immediate (unconditioned) reactions to shock from the electrified probe ("acquisition") and then, 24 hr later, by observing the rat's (conditioned) reactions to an identical, non-electrified probe ("retention"). Using this paradigm, Lehmann et al. [2005] found that hippocampal-lesioned rats had shorter latencies to contact a non-electrified probe than sham-lesioned controls during a retention test 24 h after shock exposure. Hippocampal-lesioned rats also buried the probe significantly less than sham lesioned controls during the retention test. Together, these findings support the general hypothesis that the hippocampus is involved in both the behavioural reactions to, and the memory of, aversive events.

Encouraged by these results, we recently showed that temporary inactivation of the dorsal hippocampus with lidocaine (a sodium channel blocker) also impaired rats' probe-avoidance 24hr after being shocked from the probe, without disrupting their intial unconditioned responses to the probe, whereas similar inactivation of the ventral hippocampus produced the opposite pattern of results, disrupting rats' unconditioned fear responses during their initial exposure to the shock-probe, without affecting their avoidance of the probe 24 hr later. [for details see McEown and Treit, 2009]. Thus, we were able to replicate the major

finding of Lehmann et al. [2005] while extending their results in a number of imporant ways. First, because our lesions were temporary, we could more easily separate lesion effects on unconditioned fear of the probe from those on memory of the shock-probe experience. Second, we showed that rats' failure to avoid the probe during the retention test was site-specific, occuring after dorsal but not after ventral hippocampal inactivation. Third, and in a broader context, our results lent important support for the putative dissociation between the roles of dorsal and ventral hippocampus in memory and fear, respectively [for a rewiew see Engin and Treit, 2007].

Needless to say, however, our results were not completely free of methodological shortcomings. For example, lidocaine, and any other sodium channel blocker, inactivates all axonal signalling within the target area, regardless of origin [see Fozzard et al., 2005 for a review of local anesthetic action]. Therefore, it is possible that some or all of our results were due to inactivation of axonal signals received from structures entirely outside of the hippocampus. For example, the septum and amygdala send axons to the hippocampus, and both of these outside structures are distinctly involved in fear processing [e.g., Corcoran et al., 2005; Degroot and Treit, 2005]. To mitigate the possible influence of extra-hippocampal signalling, we decided to replicate our previous findings using muscimol, a direct, $GABA_A$ receptor agonist that produces neuronal inhibition while leaving axonal conduction intack [e.g., Beaumont et al., 1978; Gavish and Snyder, 1980].

If the dorsal hippocampus mediates conditioned fear behaviour (i.e., fear memory) in the shock-probe burying test then post-acquisition dorsal hippocampus muscimol infusions should impair conditioned fear behaviour (i.e., probe avoidance) during retention. The opposite should occur with post-acquisition ventral hippocampus muscimol infusions: i.e., inactivation of the ventral hippocampus should not impair probe avoidance (i.e., fear memory) during retention.

This experiment was designed to test these predictions.

Experimental Methods

Subjects

Forty-five male Sprauge-Dawley rats (Ellerslie, Edmonton, Alberta, Canada) were used. Each animal weighed between approximately 200-250 grams upon arrival. Food and water were available ad libitum. Animals were individually housed in polycarbonate cages and kept on a 12:12 hour light/dark cycle (lights on at 0700 hours). Behavioural testing occurred during the light portion of the cycle. All surgeries conformed to the Society for Neuroscience Guidelines, CCAC guidelines and to Biosciences Animal Care and Use Committee care protocol # 116804. Just prior to surgery, all subjects were injected with: 1) an analgesic (Rimadyl; Pfiezer Canada; 0.1cc, s.c.) to alleviate potential post-operative pain; 2) atropine sulfate (Bimeda Animal Health Inc.; 0.1mg/0.2ml, i.p.) to reduce any potential respiratory complications encountered during surgery; and 3) saline to avoid dehydration (3cc, s.c., once before surgery). Subjects were anesthetised with Isoflurane gas (4% concentration in O_2 and N_2O

gas) and maintained at a 2% concentration throughout the duration of the surgery. Subjects were bilaterally implanted with 22-gauge, 7mm guide cannulae into the dorsal hippocampus (n = 22; -4.2 mm AP, 2.0 mm DV, +/- 4.1 mm lateral to the midline) or into the ventral hippocampus (n = 23; -5.2 mm AP, 5.7 mm DV, +/- 5.6 mm lateral to midline). These anatomical coordinates were selected using a stereotaxic atlas [Paxinos and Watson, 1986]. Two days after surgery, all cannulae were inspected for obstruction, and hibitane was applied to the surgical area.

Upon arrival, rats were allowed three days to acclimatize to the colony room. After the acclimatization period all rats were individually handled for 5 minutes per day over a four day period. Surgeries were then performed with behavioural testing occurring six days post-surgery. Two days post surgery, each rat was habituated to the test apparatus for a total of fifteen minutes per day over 4 days. The test apparatus consisted of a 40 × 30 × 40 cm Plexiglas chamber, with 5 cm of bedding material (wood chips) evenly spread over the chamber floor. An electrified Plexiglas shock-probe (6.5 cm long 0.5 cm in diameter) was helically wrapped with 2 copper wires and inserted through a hole in one of the walls of the chamber, 2 cm above the bedding material. The probe was electrified using a 2 pole precision animal shocker (Model H13-15, Colbourne Instruments) set at 2mA. The shock-probe was removed during habituation.

Infusion procedure

Rats were randomly assigned to either a control or experimental group. The control group received an intra-hippocampus infusion of physiological saline

(infusion rate: 1µl/1 min for 30 seconds.; pH 7.4) and the experimental group received an intra-hippocampus infusion of 1µg/1µl muscimol (Sigma) dissolved in physiological saline (Infusion rate: 1µl/1min for 30 seconds) resulting in a 0.5µl infusion per hemisphere as per Corcoran et al. [2005]. Infusions were administered immediately after the acquisition session for all subjects. Infusions were administered with a 10 µl Hamilton microsyringe using an infusion pump (Harvard Apparatus 22). The cannulae were left in place for 30 seconds after drug administration to allow for diffusion. Past research indicates that a 1µg/1µl concentration of muscimol is able to produce inactivation of neural tissue within 0.8 to 1.8mm from the infusion site and remains active in this tissue for up to 60 minutes post infusion [e.g., Corcoran et al., 2005].

Acquisition III

Rats were tested in groups representing three conditions 1) muscimol infusions into the ventral hippocampus (n = 12); 2) muscimol infusions into the dorsal hippocampus (n = 11); and 3) vehicle (saline) infusions (n = 22). Half of the vehicle controls received dorsal hippocampus saline infusions and the remainder received ventral hippocampus saline infusions. For half of the rats in the saline-infused control group the probe was electrified during acquisition (shocked controls) and for the remainder the shock-probe was not electrified (naïve controls). All rats that received muscimol infusions were exposed to an electrified probe during acquisition. Each subject was individually placed into the chamber facing away from the shock-probe. Testing began after the initial contact-induced probe-shock, and lasted 15 minutes. The number of contact-

induced shocks, still time (i.e., sitting or laying on the chamber floor, with normal respiration and orienting behaviour), and mean shock reactivity was recorded. Mean shock reactivity was evaluated using a four point scale [see Pesold and Treit, 1992 for further details].

Retention III

After a 24-hour delay, rats were re-tested in the same environment in which acquisition occurred, except that rats 1) did not receive a shock when they touched the probe, and 2) did not receive infusions of either muscimol or vehicle. The amount of time spent in the half of the chamber that contained the shock-probe was recorded [McEown and Treit, 2009].

Histology

After the completion of behavioural testing, rats were deeply anesthetised using pentobarbital (Nembutal, 100 mg/kg, i.p) and subsequently perfused with a 10% formalin solution. Their brains were removed and placed in specimen jars containing a 10% formalin solution. After forty-eight hours, brains were sectioned (60 μm), stained with thionin, and mounted on microscope slides. The locations of cannulae were confirmed microscopically. The behavioural data for rats with either one or both cannulae outside of the target area (dorsal hippocampus or ventral hippocampus) were omitted from analysis. Data from another rat was discarded because of an obstructed cannulae.

Statistical Analyses

The behavioral data were assessed with one-way ANOVA (α=0.05), followed by pair-wise comparisons (t-tests, $\alpha = 0.05$, two-tailed). ANOVA of the

saline infused animals in both control groups (i.e., shock-experienced control and shock-naïve control groups) was not significant (i.e., $p > .05$), and therefore these control groups were collapsed across infusion site (i.e., dorsal and ventral) for all comparisons. An a priori comparison of dorsal and ventral retention scores was conducted with a t test (1-tailed).

Results

Acquisition III

Not surprisingly none of the behaviours seen during the acquisition session were affected by muscimol, which was infused **just after** this session. During acquisition the number of probe contacts ($F(2, 33) = .78$, $p = .46$), shock reactivity ($F(2, 33) = .54$, $p = .58$) and still time ($F(3, 41) = .45$, $p = .71$) did not differ significantly among the groups.

Retention III

As predicted, there was a significant between-groups difference in the amount of time that rats spent in the shock-probe-half of the chamber during the retention test ($F(3, 41) = 61.40$, $p < .001$; see Fig. 3-1). Shock-experienced rats spent significantly less time in the probe end of the chamber than shock-naive rats, indicating they had remembered being shocked during acquisition ($t(20) = -12.72$, $p < .001$). More importantly, dorsal hippocampus-inactivated rats spent significantly *more* time in the shock-probe half of the chamber than shock-experienced controls ($t(22) = 2.02$, $p < .05$), indicating that they had not remembered being shocked during acquisition. Conversely, ventral hippocampus-inactivated rats were not significantly different from shock-experienced controls

($t(23) = .15$, $p = .87$), indicating that they retained a memory of the initial shock-probe experience. The difference in time spent in the probe end of the chamber between dorsal hippocampus-inactivated and ventral hippocampus-inactivated rats just failed to reach statistical significance ($t(21) = 1.38$, $p = .07$, 1-tailed).

Discussion

In summary, dorsal hippocampal inactivation immediately after acquisition impaired probe avoidance during retention; conversely, ventral hippocampal inactivation did not impair probe avoidance during retention. Because muscimol-induced inactivation of the hippocampus would spare axonal conduction from distant sites, it seems unlikely that disruption of septal or amygdalar afferents could explain our previous results. For the same reason, it seems somewhat unlikely that an indiscriminate perturbation of *intra*-hippocampal communication could explain our previous results, although muscimol could block the somatodendritic origin of some of these signals. It should also be noted that while our results might apply to instrumental associations in the shock-probe test, they might not apply to classically conditioned associations (e.g., between shock and tone; see [Bast et al., 2001; McEown and Treit, 2009]. On the other hand, because hippocampal inactivation occurred *after* the shock-probe acquisition session, drug effects on non-memorial processes such as fear motivation cannot explain the memory impairments seen during the retention session. Thus, the present study provides strong evidence that the dorsal and ventral regions of the hippocampus play distinct roles in fear and memory, adding to a growing literature on this subject.

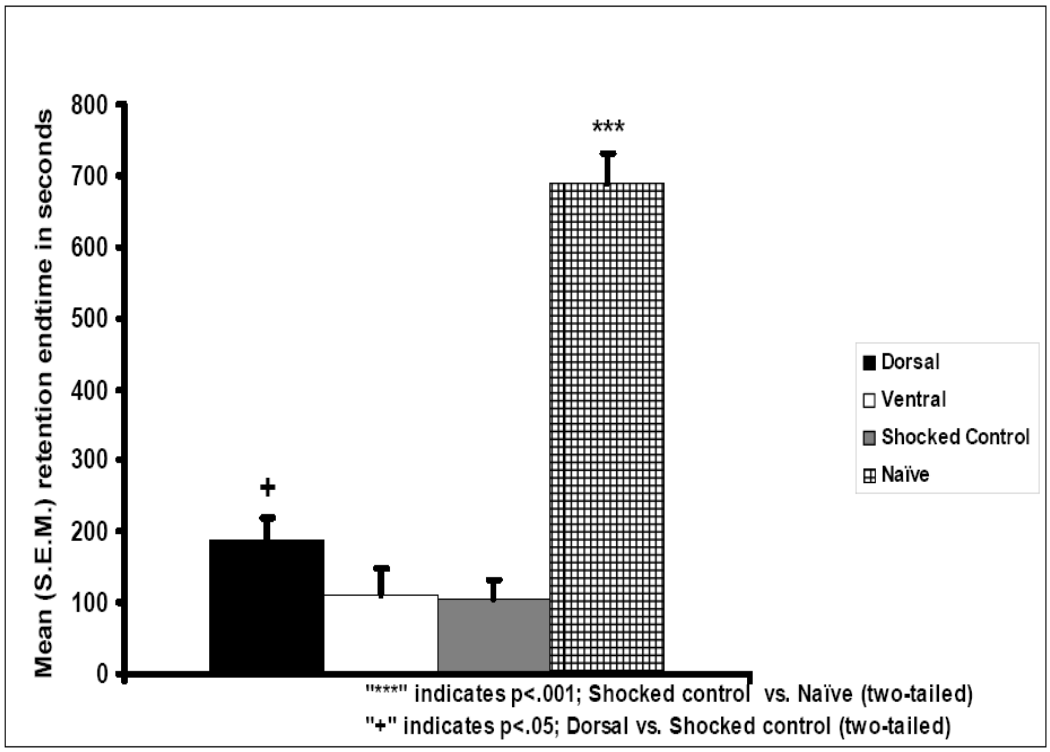

Figure 1

Fig. 3-1 - Mean (± S.E.M.) retention shock-probe end time between dorsal hippocampus (muscimol), ventral hippocampus (muscimol), shocked control (vehicle) and naïve control (vehicle) rats.

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Chapter 4

General Discussion

This thesis provides support for separate roles of the dorsal and ventral hippocampus in fear and memory of a shock-probe experience. In three experiments presented in this thesis, rats with temporary inactivation of the dorsal hippocampus during acquisition spent more time in the end of the chamber containing the shock-probe during retention than controls, thereby suggesting that their memory of the initial shock-probe experience was impaired. Conversely, ventral hippocampal inactivation during acquisition impaired burying behavior during acquisition, whereas dorsal hippocampal inactivation did not. Finally, the behavioral effects of lidocaine inactivation of dorsal and ventral hippocampus were probably mediated by local signal disruptions rather than from perturbations of afferent signals coming from structures outside of the hippocampus: muscimol, a selective agonist of post-synaptic GABAA receptors, produced the same pattern of behavioral effects as lidocaine when infused into the dorsal and ventral hippocampus.

Even though these experiments strongly support the initial hypotheses, several limitations of the data need discussion. First, not all of the measures of fear behavior in the three experiments changed in the expected direction in response to hippocampal inactivation. For example, expected changes in defensive burying did occur, but not in every experiment; similarly, changes in passive avoidance of the probe [i.e., end time] were usually significant, but not always. If these measures of fear had co-varied consistently across all

experimental conditions, and especially during the retention tests, the current results would have been strengthened considerably. A second issue is the outcome of direct comparisons between inactivation of the dorsal and ventral hippocampus. Had these directional comparisons been significant in all instances, our conclusions would have been more convincing. As it was, more often than not, evidence of functional dissociations between the dorsal and ventral hippocampus were supported with significant but less compelling differences between shock-saline control groups and shock-drug groups. Furthermore, by examining the histology figures (see Figures 2-1 and 2-6) we can conclude that these differences were not due to inactivation of a particular sub-region in the dorsal hippocampus versus inactivation of a particular sub-region in the ventral hippocampus as these placements were randomly dispersed throughout the hippocampus in each experiment. Finally, and perhaps most serious, was the finding in the second experiment that ventral hippocampal inactivation, contrary to prediction, significantly impaired memory of the probe during the retention test. While there may be alternative explanations of this finding (see discussion section, experiment 2), it is clearly inconsistent with the proposed functional dissociation between the dorsal and ventral hippocampus. Only future studies can resolve these empirical issues.

A potential alternative interpretation of the significant behavioral dissociations that did occur after ventral or dorsal hippocampus inactivation comes from the presence of ‘place cells’ in the dorsal hippocampus (see O’Keefe and Nadel, 1978). Inactivation of place cells in the dorsal hippocampus may have

impaired allocentric spatial navigation in the shock-probe chamber, and this, rather than specific memory impairment, may have disrupted rats' probe avoidance during the retention tests. However, place-cells are located all along the dorsal-ventral axis of the hippocampus (Kjelstrup et al., 2008). Therefore, the effects of ventral hippocampus inactivation should have mirrored the effects of dorsal hippocampal inactivation, which was not the case. Nevertheless, the effects of dorsal and ventral hippocampal inactivation on retention performance were similar in one instance, which adds some weight to the alternative hypothesis. In most instances, however, ventral hippocampal inactivation left probe avoidance during retention intact.

In conclusion, this thesis provides support for three main conclusions: 1) the dorsal hippocampus is responsible for mediating memory of a shock-probe experience; 2) the ventral hippocampus mediates unconditioned fear reactions to the shock-probe and 3) the observed dissociation in fear and memory processes was not mediated by functional disruption of structures located outside of the hippocampus. This thesis adds to a growing literature on the separate roles of the ventral and dorsal hippocampus in fear and memory.

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