Hippocampal control of behaviour

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Abstract

Converging lines of evidence point to executive functions of the hippocampus, such as contributions to decision-making and the inhibition of behaviour, quite outside its traditional roles in memory and spatial learning. These functions are normally associated with prefrontal and striatal structures such as the orbitofrontal cortex and the nucleus accumbens. This thesis seeks to expand our knowledge of non-traditional hippocampal function by studying functional differences across the dorsal-ventral axis. Research from our lab has indicated that the ventral half of the hippocampus (vHC) is responsible for some aspects of inhibitory control and decision-making in the context of time. However, the dorsal half (dHC) was not directly compared to the vHC in several of these tasks even though past experiments with spatial components did show dHC deficits. The objective of this thesis was to disentangle these findings by directly comparing the dHC and vHC in a series of non-spatial inhibitory control and decision-making tasks. Rats with cytotoxic hippocampal lesions were tested in touchscreen boxes on visual discrimination learning and delay discounting paradigms.

When tested on the discrimination learning and reversal task, dHC and vHC lesions impaired rats in their ability to acquire the stimulus-reward relationship and adapt to the changing rules during the reversal. Relative to the sham controls, both operated groups made many errors indicating perseverative behaviour. In the delay discounting task, rats with vHC lesions showed normal discounting behaviour when delays increased sequentially but adopted a low impulsivity delay-independent strategy when trial order was randomized. The dHC rats had difficulty discriminating the reward magnitudes thereby responding randomly. We conclude that although both divisions of the hippocampus contribute to aspects of cognitive-executive behaviour, the dHC is highly sensitive to reward.

Plusieurs lignes d'évidence dirigent aux fonctions exécutives de l'hippocampe, telles les contributions à la prise de décision et l'inhibition du comportement, dehors ses rôles traditionnels en mémoire et l'apprentissage spatial. Ces fonctions sont normalement associées aux structures préfrontales et de striatum tel le cortex orbitofrontal et le noyau accumbens. Cette thèse cherche à approfondir notre connaissance de fonctions hippocampiques en étudiant les différences par rapport à l'axe dorsoventral. Recherche de notre laboratoire a indiqué que la moitié ventrale de l'hippocampe (vHC) est responsable pour quelques aspects de contrôle d'inhibition et de prise de décision dans le contexte du temps. Par contre, la moitié dorsale (dHC) n'a pas été comparée au vHC par rapport à plusieurs de ces tâches quoique des essais avec des composantes spatiales aient démontré des déficits dans les sujets avec lésions dHC. L'objectif de cette thèse est de dénouer ces résultats en comparant directement le dHC et le vHC dans une série de tâches non spatiales de contrôle d'inhibition et de prise de décision. Les rats avec lésions hippocampiques cytotoxiques ont été analysés dans des boîtes avec écran tactile sur des paradigmes de la discrimination visuelle et de délai d'actualisation.

Une fois étudié pour l'apprentissage de la discrimination et de l'inversion, des lésions dHC et vHC ont affaibli l'habileté à acquérir la relation stimulus-récompense et d'adapter à l'inversion. Relatifs aux contrôles factices, les groupes ont fait plusieurs erreurs indiquant un comportement persévérative. Dans la tâche de délai d'actualisation, les rats avec lésions vHC ont démontré des habitudes normales quand les délais ont augmenté séquentiellement mais ont adopté une stratégie de basse impulsivité indépendante au délai quand l'ordre de l'essai était randomisée. Les rats avec lésions dHC avaient de difficulté à discerner les grandeurs de récompense et ont ainsi répondu aléatoirement. Nous concluons que quoique les deux divisions de l'hippocampe contribuent aux habitudes cognitivo-exécutif, le dHC est très sensible à la récompense.

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Table of Contents

General Introduction

1.1 Overview

Scoville and Milner's clinical work on "Patient H.M." has been the driving force of memory research for the last 60 years. They reported that bilateral damage to the hippocampus leads to an amnesia of events and facts but spares both intelligence and attention (Scoville & Milner, 1957; Penfield & Milner, 1958). However, a recent histological analysis of patient H.M.'s brain reveals that the hippocampal damage was not as extensive as originally thought and included the anterior portion of the hippocampus for the most part (see figures 2-3, page 4-5 from Anesse et al., 2014) with encroachment into the entorhinal cortex, amygdala and other adjacent areas (Anesse et al., 2014). What then is the contribution of the hippocampus to memory, and importantly, what other functions does the hippocampus subserve? Recent work in our lab has focused on this latter question by investigating hippocampal executive functions by virtue of its connections to the prefrontal cortex. This thesis uses a rodent model to examine the contribution of the hippocampus to two executive domains, those of behavioural control and decision-making.

While the mnemonic role of the hippocampus has dominated the literature in neuropsychology, animal studies have provided direct evidence that the hippocampus supports a diversity of functions. A wide selection of rat lesion studies points to hippocampal contributions in areas such as decision-making (Abela & Chudasama, 2013; Mariano et al., 2009); Pavlovian conditioning (Berger & Orr, 1983; Solomon, 1977); and associative learning (Jagielo et al., 1990) in particular discrimination learning (Becker & Olton, 1980; Murray & Ridley, 1999; Sakimoto et al., 2015). Furthermore, there is significant evidence that the hippocampus can be functionally differentiated along its dorso-ventral or longitudinal axis, which encourages a reassessment of the traditional

view of the hippocampus as a unitary structure (Fanselow & Dong, 2009). Recent work has shown that the dorsal (dHC) and ventral (vHC) poles of the hippocampus are differentially connected to the ventral prefrontal cortex (Jay & Witter, 1991), nucleus accumbens (Kelley & Domesick, 1982) and thalamus (Ishikawa & Nakamura, 2006; Prasad & Chudasama, 2013). The interconnections between the ventral hippocampus and ventral prefrontal cortex (vPFC) may explain the similarity in perseverative behaviour that is found after cytotoxic lesions to these areas (Chudasama & Robbins, 2003; Chudasama et al., 2012; Abela et al., 2013). Similarly, both vHC and nucleus accumbens lesions affect optimal decision-making in tasks that require waiting for a better outcome, making these animals impulsive in their choices (Rawlins et al., 1985; Abela, Duan & Chudasama, 2015; McHugh et al., 2008).

There is a rich historical literature on the effects of hippocampal damage on behavioral control in rats (e.g., Kimble and Kimble 1965, Jarrard et al., 1964). These studies showed that hippocampal ablation caused animals to be hyperactive and encouraged a tendency to repeat responses as if they were perseverating. Most of this work focused on the dorsal hippocampus which is much easier to access when conducting brain manipulations. As such, there was an absence of research on the effects of vHC lesions. In addition, the lesions were conducted using an aspirative or radiofrequency approach which inevitably caused large indiscriminate lesions and damaged fibres of passage. Particularly surprising is the recent finding that the dHC, known to be a spatial memory structure (Moser & Moser, 1998), can also impair performance in delay discounting decision-making tasks similar to the effects of vHC lesions (McHugh et al., 2008). In this case, however, the researchers used a T-maze to conduct the study and therefore the animals' behavior might have been influenced by the spatial components of the task. This is potential confound as the dHC is heavily involved in spatial memory (O'Keefe & Nadel, 1978).

The experiments presented in this thesis address the confounds and major gaps present in the literature. Rats were tested on a non-spatial version of a visual discrimination and delay discounting task using an operant touchscreen platform. Importantly, unlike previous studies in our lab (e.g., Abela et al., 2013; Abela & Chudasama, 2013) which examined the vHC alone, here rats with dorsal or ventral hippocampal lesions were directly compared on the same task. I report several findings including an impairment in decision-making and inhibition regardless of the location of the hippocampal lesion. These findings indicate that hippocampal lesion deficits extend beyond the spatial domain, and that both poles of the hippocampus may contribute to executive action. I start with an anatomical overview of the rodent hippocampus with emphasis on the longitudinal axis, which will give a framework for understanding the diversity of hippocampal contributions to behaviour.

1.2 General anatomy of the rodent hippocampus and its long-axis

The hippocampus is a large medial temporal lobe structure conserved throughout the mammalian line. The hippocampus proper comprises the Cornu Ammonis subfields (CA1 – CA4), but the entire hippocampal formation includes the dentate gyrus and subiculum as well (Amaral & Lavenex, 2006; Strange et al, 2014). The continuation of CA3 that inserts into the dentate gyrus does not share the pyramidal cytoarchitecture of the other subfields, and is sometimes referred to as CA4, or the hilus when considered part of the dentate gyrus (Amaral, 1978). Adjacent to the hippocampus is the entorhinal cortex (see Figure 1), which provides the main inputs of the structure. Along with the fornix the entorhinal cortex is also the target of most hippocampal efferents (Amaral, 1978). In this way the entorhinal cortex is an intermediary or node between the hippocampus and other cortical areas.

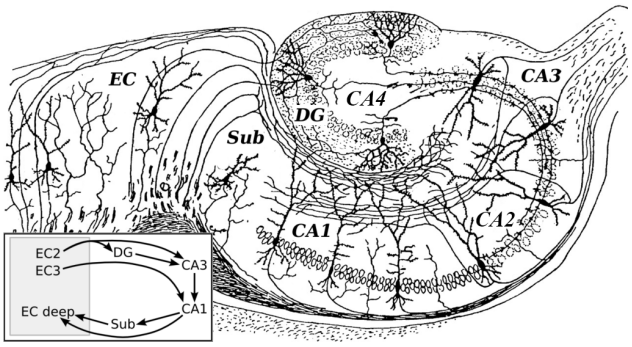

Figure 1. Diagram of the hippocampus showing anatomical subdivisions.

Coronal section of the hippocampal region showing the hippocampus proper as well as the entorhinal cortex and subiculum. Insert shows connections and direction of information flow. CA1-4; *Cornu Ammonis* subfields, DG; dentate gyrus, Sub; subiculum,

The overall structure of the hippocampus is a 3-dimensional system with information flow between the longitudinal and transverse axes (Amaral & Lavenex, 2006). The hippocampus is composed of lamellar sections, perpendicular to the longitudinal axis that work somewhat independently (Amaral & Lavenex, 2006). Its organisation is referred to as the 'trisynaptic circuit'; a synaptic pathway conserved across the entire longitudinal axis, and likely performs similar computations regardless of the function of the particular hippocampal region (Fanselow & Dong, 2009).

In terms of the longitudinal axis, the hippocampus is classically divided into two sections: the dorsal and the ventral hippocampal poles (see Figure 2). These divisions are commonly spoken of as being analogous to the posterior and anterior hippocampus in humans, respectively (Fanselow & Dong, 2009). However, while the human and rodent hippocampus has a similar longitudinal structure during the first 14 weeks of gestation, thereafter the human dorsal hippocampus shrinks to a thin band above the corpus callosum (Kier et al., 1995). The remaining ventral portion develops into the entire human hippocampus. As such, developmental aspects of the hippocampus ought to be considered when making transspecies comparisons (Strange et al., 2014).

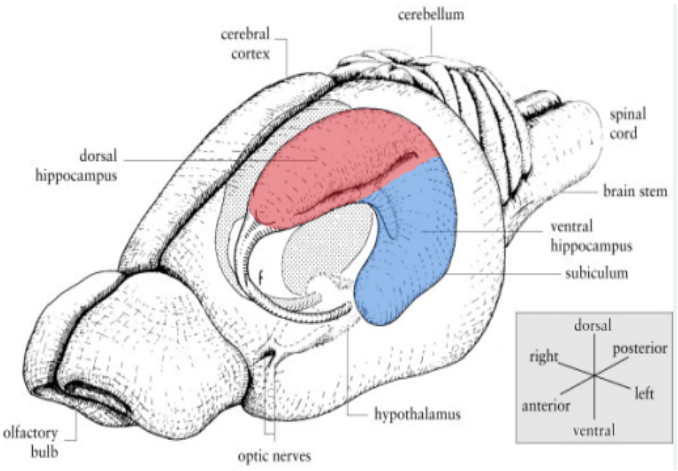

Figure 2. Schematic illustration of the rodent hippocampus

Figure shows entire hippocampus embedded within cortex The longitudinal axis of the hippocampus comprises a dorsal (red) and ventral (blue) division. The dorsal segment is also known as the septal hippocampus while the ventral segment is often known as the temporal hippocampus. Figure adapted from (Amaral & Witter, 1995).

One major distinction between the dorsal and ventral hippocampal poles in rodents is the gradient of projections to other brain areas. For example, as one moves along the hippocampus from dorsal to ventral, projections to the nucleus accumbens (NAc) shift from lateral to medial (Brog et al., 1993). Moreover, while the ventral 2/3s of the hippocampus projects to the amygdala, there are no projections from the dorsal hippocampus whatsoever (Kishi et al., 2006). Again, an embryological perspective is useful here; although neurogenesis occurs at the same time along the dorsoventral axis of the hippocampus, there is a pattern linking the location of efferents to their targets in other brain structures (Bayer & Altman, 1987). Dorsal hippocampal neurons project to those brain areas that develop earlier, and ventral hippocampal neurons to those that develop later in embryogenesis (Bayer & Altman, 1987). This topographic organization is carried forward to second-order synapses as well so that, for example, the vHC projects to the ventral lateral septum which in turn projects to ventral areas of the hypothalamus (Risold & Swanson, 1996). In this way each hippocampal pole carves out unique circuitry within the brain.

Although afferents to the hippocampus also show topographical gradients along the dorsoventral axis, two of these connectivity patterns have been used to functionally differentiate

the hippocampus poles in the past. In general, the dorsal half of the hippocampus receives the majority of sensory (especially visuospatial) information and the ventral half receives the majority of somatic and affective information (Amaral & Witter, 1995; Gray & McNaughton, 2000). This is thought to explain why dorsal damage leads to impairments of spatial memory (Moser et al., 1995, and ventral damage to impairments of anxiety and emotional behaviour (Henke, 1990). The combination of anatomical and functional evidence has led to the dominant perception of a strict spatial/emotional segregation of the two hippocampal poles.

1.3 Hippocampal-prefrontal connections

The connections of the hippocampus to the prefrontal cortex are of particular interest to executive control functions. Anatomical studies using conventional (lectin) tracers have shown that the ventral CA1 region of the hippocampus sends direct projections to the ventral prefrontal cortex (vPFC) (Jay & Witter 1991; Hoover & Vertes, 2007). We now know that although ventral hippocampal projections to the vPFC are unidirectional, there are no direct return projections from the vPFC back to the vHC. Instead, this communication is indirect, mediated through the thalamus or entorhinal cortex (Jones & Witter, 2007; Hoover & Vertes et al, 2007; Prasad & Chudasama 2013). Modern approaches using transynaptic viral tracers have shown that different prefrontal areas target different regions of the hippocampus (see Figure 3). Specifically, the ventral prelimbic and infralimbic regions project to the vHC via the midline thalamus or ventromedial entorhinal cortex, while dorsal anterior cingulate cortex and retrosplenial cortex target the dorsal hippocampus (dHC) via the anterior thalamus or dorsolateral entorhinal cortex (Prasad and Chudasama, 2013). This anatomical organisation reveals parallel disynaptic pathways to the dorsal and ventral regions of the hippocampus providing an important framework for understanding the

hippocampal contributions to behaviour (see Figure 3). The finding that ventral hippocampal-prefrontal circuitry mediates response control in the rat (Chudasama et al., 2012) suggests that inhibitory control deficits following hippocampal lesions cannot be easily explained by prevailing theories of its role in spatial mapping.

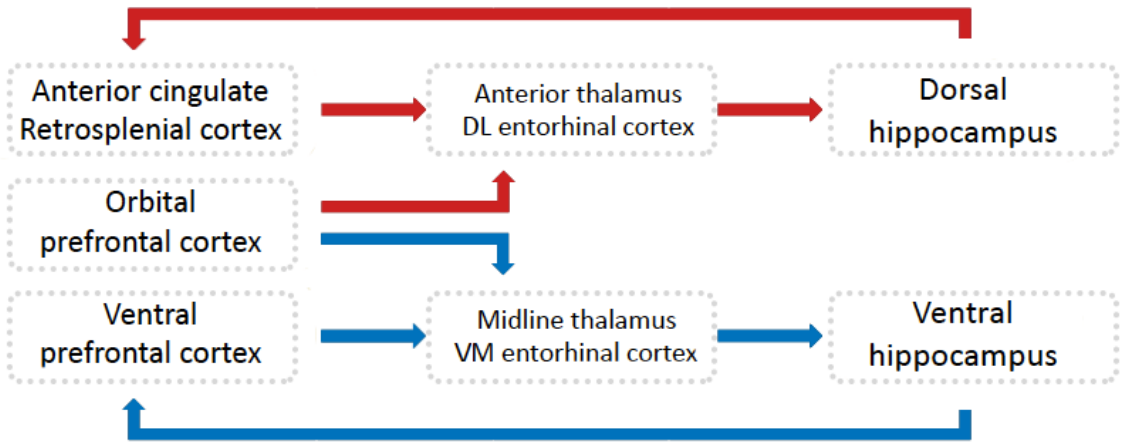

Figure 3. Diagram shows disynaptic input from prefrontal and limbic areas to different regions of the hippocampus

Red arrows show pathways from neocortical areas to and from the dorsal hippocampus, while blue arrows show pathways to and from the ventral hippocampus. While hippocampal inputs from frontal areas as well as the retrosplenial cortex are mediated by the thalamus or entorhinal cortex, return projections are direct (figure adapted from Prasad and Chudasama, 2013).

1.4 Traditional views of hippocampal function

A series of single-unit recording experiments in rats (O`Keefe and Dostrovsky, 1971; O`Keefe, 1976) showed that a certain class of hippocampal cells fired based on the animal's location in space. This was systematized into the cognitive map theory of hippocampal function (O'Keefe & Nadel, 1978). The concept of 'place cells' was developed, which had receptive fields that mapped onto physical regions of the animal's environment. The large effect sizes reported from hippocampal lesion studies of spatial learning instantly drew a large amount of interest in the discovery (Bannerman et al., 2004).

One commonly used test of spatial memory is the 'Morris water-maze'. In this task, rats are required to spatially navigate a large circular arena of water in which they must locate a hidden platform. Rats with hippocampal lesions are severely impaired in their navigation skills when dissociated from any motor, motivational, or reinforcement confounds (Morris et al., 1982). Moser et al. (1993) used this task to compare navigational performance in rats with aspirative dorsal and ventral hippocampal lesions. While they found that the entire ventral hippocampus could be removed without deficit in the water maze, the dHC is highly sensitive to spatial information such that a small amount of damage to the dHC produced a major spatial memory impairment. Importantly, Moser et al. (1995) repeated their findings in rats with excitotoxic hippocampal lesions, confirming that aspiration lesions destroying fibres of passage were not the cause of the reported hippocampal deficits in spatial learning.

As consistent as these data are, it must be remembered that place cells in the hippocampus form a gradient with increasing receptive field (i.e. coarseness) from the dorsal pole to the ventral pole (Kjelstrup et al, 2008). This may explain why the vHC is sufficient for spatial navigation in a water maze task if given additional training days (Hoz et al., 2003). In fact, as long as dHC-

lesioned rats are trained in the task before surgery, the rats will not be impaired (Hoz & Martin, 2014). Although the dHC may be sufficient for spatial memory, it is not necessary.

The spatial attributes of vHC function are a recent addition to the literature. Until recently, the ventral pole of the hippocampus was associated with anxiety and affect. Early reports show an important role for the hippocampus in emotion from case studies (Klüver & Bucy, 1937; Papez, 1937). The vHC, likely through its regulatory control of the hypothalamic-pituitary-adrenal axis and connections to the amygdala, has been shown to be involved in stress and defensive behaviours (Henke, 1990; Gray & McNaughton, 2000). Primate studies show direct inhibition of fear responses in lesioned animals, such as when a monkey is exposed to a potential predator like a fake snake (Chudasama, Wright & Murray, 2008). In rats, ventral hippocampal lesions reduce conditioned freezing responses (Richmond et al., 1999). They also show lower defecation rates and lower corticosterone levels compared with either controls, or rats with dHC lesions (Bannerman, 2004). The effect can be readily seen when vHC lesioned rats are placed in partially open-topped mazes. Rodents are averse to exposed locations, and they tend to hide from sight. However, rats with lesions are more willing to explore open arms of mazes and have lower hormonal stress than control rats (Kjelstrup et al., 2002). Again, while some evidence points to a role for the dHC in anxiety control, as seen in some conditioned freezing drug-studies (Carvalho et al., 2008), but this just underscores the complexity and flexibility of hippocampal function.

1.5 Hippocampus and behavioural inhibition

In natural settings, whether it be foraging behaviour, motor planning, or social interaction, animals must be flexible in their behaviours to adapt to changing circumstances. This includes the ability to inhibit behaviour that is no longer fruitful. However, in pathological states, this

mechanism may fail, leading to disinhibition. This may lead to perseverative behaviour; some form of inappropriate repetition of a response, behaviour or rule. The control of this type of behavioural flexibility is an executive process largely associated with the orbital prefrontal cortex (OFC). For example, old world and new world monkeys with OFC lesions can readily acquire simple stimulus-reward associations but make many errors when the stimulus-reward contingencies are reversed, (Jones and Miskin 1972; Dias et al., 1996; Izqueirdo and Murray, 2004). The same is true for mice (Graybeal et al., 2011) and rats (Chudasama et al., 2003) with OFC lesions. An increase in the number of errors might also be due to a failure to encode and update the value of reward (Rudebeck & Murray, 2014). This argument implies that animals with OFC lesions are not disinhibited, *per se*, but unable to learn from their mistakes and use negative feedback to guide behaviour.

While the OFC has dominated research on the concept of perseveration and inhibitory control, the hippocampus has long been implicated in behaviours that require control of actions (Altman et al., 1973; Douglas, 1967; Kimble, 1968). Consistent with this notion, data from our own lab (Abela et al., 2013), and the labs of others (e.g., Bannerman, 2004) have shown that rats with hippocampal lesions exhibit a difficulty in suppressing responses in a variety of contexts, and do so in a manner that resembles the effects of OFC lesions. This is typically assessed in so-called discrimination and reversal learning tasks. Typically, the animal is given two stimuli of which only one is associated with reward. The apparatus can be the in the form of a spatial T-maze or Y-maze, which allows a binary choice at the intersection point, or operant chambers containing levers or touchscreens which allow animals to make a specific action (e.g., depress a lever, nosepoke a visual stimulus on a touchscreen). Once the animal has acquired the stimulus reward association, the animal is subjected to a reversal whereby the stimulus-reward contingency is reversed. In other words, the rewarded stimulus is no longer rewarded, and the previously unrewarded stimulus

becomes rewarded. The animal must now inhibit responding to the previously rewarded stimulus and reverse its response.

A prevalence of disinhibition characterized by perseverative errors to the previously rewarded stimulus was reported in the early hippocampal literature (e.g., Kimble, 1963; Douglas & Isaacson, 1964). These results led to several early behavioural inhibition theories of hippocampal function across species noting the similarities between frontal and hippocampal lesions (Altman et al., 1973; Douglas 1967; Kimble, 1968). However, given the prevailing view of the hippocampus being the locus of the spatial map, several objections to a purely inhibitory theory of hippocampal function were raised (Nadel et al., 1975) with the argument that many of the deficits in reversal learning could be easily explained as a mix of spatial habits and crude lesion methods (radiofrequency, ablation, aspiration) that damaged fibres of passage and cerebrovasculature (Munoz & Grossman, 1981; Nadel et al., 1975). Nevertheless, even when spatial cues are removed, hippocampal-lesioned rats continued to make perseverative errors (Murray & Ridley, 1999; see also Becker and Olton, 1980). Moreover, work in our lab has shown that only rats with ventral hippocampal lesions are impulsive and compulsive in a visuospatial test of attention and inhibitory control (Abela et al., 2013). These data prompted us to re-evaluate the role of the dorsal and ventral hippocampus in tests of inhibition and control (Experiment 1).

1.6 Hippocampus and decision-making

Animals often use environmental cues to guide behaviour (e.g. chemotaxis) when multiple alternatives are available. However, decision-making is a distinct cognitive function that weighs potential choices with regard to their costs and benefits (Mischel, 1966; Mazur, 1989, 1997; Sozou, 1998). The principle brain areas studied in decision-making research are the orbital prefrontal

cortex, also known to play an important role in inhibitory control (Mar et al., 2011; Rudebeck et al., 2006) and the nucleus accumbens (Cardinal et al., 2001; Cardinal & Howes, 2005). That these two structures are anatomically interconnected with the hippocampus raises the possibility that they may subserve similar functions (Brog et al, 1993; Jay & Witter, 1991; Verwer et al., 1997). In fact, recent studies in humans have shown that the hippocampus may play a role in decision-making through prospective planning. Patients with hippocampal pathology fail to make strategic choices involving a trade-off between short-term and long-term gains in neuropsychological tests of 'gambling' (Gutbrod et al., 2006; Sinz et al., 2008; Gupta et al., 2010; Labudda et al., 2009). In other words, humans with hippocampal damage only take into account the most recent reward or punishment to make their decision (Gupta et al., 2009). This impairment may have to do with the hippocampus' role in updating values of decks across trials, as more information is discovered with each ensuing choice (Guitart-Masip et al., 2013). In general, patients with bilateral hippocampal damage have trouble with learning from feedback and in making decisions with incomplete information (Delazer et al., 2010).

Similar studies have been conducted in rodents. The most commonly used test of decision-making is the delay or temporal discounting task. Typically, the animal is presented with a choice between a stimulus that delivers a small immediate reward, or one that delivers a large reward but after a delay (for review, see Floresco et al., 2008). The term "discounting" is used because normal subjects have a tendency to undervalue the delayed rewards and tend to prefer the small reward option as the delay increases. Steeper "discounting curves" with increasing delay indicate some level of impulsive choice, in other words, that the animal is delay intolerant and switches to the immediate reward option. Previously, we have shown that rats with vHC lesions were delay intolerant resembling the effects of both OFC and nucleus accumbens lesions (Abela and

Chudasama, 2013; Mobini 2002; Rudebeck et al., 2006; Mar et al. 2011). To date, the effects of dHC lesions on decision-making tasks have not been systematically explored. In the past, lesion size was confounded by the aspiration methods, or the test was influenced by spatial cues, short delays (e.g., 10 sec vs 60 sec) or large reward magnitude (e.g., 4 pellets vs 10 pellets) (Rawlins, 1985; Rudebeck et al., 2006; Mariano et al., 2009). Consequently, we were interested in comparing the effects of selective dHC and vHC lesions in a delay discounting task using operant touchscreen platform (experiment 2). The visual stimuli allowed us to institute a variety of manipulations including controlling for space (i.e, left or right) as well as time, as each stimulus could represent a different delay.

1.7 Specific aims and objectives

In the next two experiments, I present evidence for hippocampal involvement in non-traditional areas such as behavioural control and decision-making. Previous work in our lab showed that the vHC is important in at least some tasks that require inhibitory control (Abela et al., 2013). In a decision-making task, we showed that animals with vHC lesions were delay intolerant, choosing options that deliver small immediate rewards rather than waiting for large delayed ones (Abela & Chudasama, 2013). We did not test the effects of dHC lesions at the time but there is evidence in the literature in rats and monkeys that hippocampal lesions cause perseveration in spatial reversal tasks (Kimble & Kimble, 1965; Silveira & Kimble, 1968; Jones and Mishkin, 1970), as well as in decision-making tasks (McHugh et al., 2008). My experiments directly compare the ventral and dorsal hippocampus in a discrimination learning and delay discounting task, thereby expanding our knowledge of the role of this important medial temporal lobe structure.

2.0 Experiment 1: Hippocampal lesions and behavioural control

In this experiment, we used an operant touchscreen platform to conduct a discrimination and reversal task to assess adaptation to changes in reward contingency. In brief, rats with dorsal and ventral hippocampal lesions were required to discriminate two visual stimuli by forming stimulus-reward associations, and then adjust their response when the stimulus-reward associations were reversed. Perseverating responses to the previously rewarded stimuli indicates a disinhibition of the erroneous response and failure to adapt to changes in the environment.

2.1 Methods and materials

Subjects

A total of 48 male Long-Evans rats, acquired from Charles River Laboratories (Kingston, New York, USA) were used for this study. The animals were 250-270g at the start of testing. They were housed in pairs in cages in a climate controlled room with a 12-hour day/night cycle. The animals were kept at 85% of their free-feeding weight and received water *ad libitum*. All experimental protocols were approved by the McGill University Animal Care Committee, in accordance with the guidelines of the Canadian Council on Animal Care.

Surgery

Cytotoxic lesions were performed using standard stereotaxic procedures. Rats were anaesthetized with isoflurane gas and secured in a stereotaxic frame with atraumatic ear bars. The animals were randomly assigned to receive either excitotoxic N-methyl-D-aspartic acid (NMDA)

lesions to the ventral hippocampus, dorsal hippocampus, or sham control surgery. Table 1 details the coordinates used in all surgeries. Approximately 0.5 μl of 0.09 M NMDA (Sigma-Aldrich, Canada) in 0.9% saline per injection was administered bilaterally in the lesion group. The volume of injected toxic was determined by results from previous pilot surgeries. Sham controls received the same surgical procedure but were injected with 0.9% saline only. Bregma was used as the origin for all anterior-posterior and medial-lateral readings, and dorsal-ventral readings were made with respect to the dural surface (Paxinos & Watson, 2005).

Table 1: Stereotaxic coordinates for dorsal and ventral hippocampal regions

Region	Stereotaxic coordinates	Volume of neurotoxin (µl)
vHC	AP -4.6; ML ±5.0; DV -6.7	0.5
	AP -4.7; ML ±4.4; DV -6.7	0.5
	AP -4.8; ML ±4.6; DV -7.5	0.4
dHC	AP -2.4; ML ±1.4; DV -3.3	0.5
	AP -2.7; ML ±1.6; DV -3.0	0.5
	AP -3.2; ML ±2.6; DV -2.8	0.5
	AP -3.6; ML ±3.0; DV -2.8	0.5
	AP -4.0; ML ±1.4; DV -3.4	0.5
	AP -4.4; ML ±4.4; DV -3.0	0.5

AP, anterior-posterior; dHC, dorsal hippocampus; DV, dorsal-ventral; ML, medial-lateral; vHC, ventral hippocampus

Apparatus

Four identical automated operant chambers (see Figure 4) were used (Lafayette Instruments, USA). The front walls were fitted with a touch-sensitive monitor protected by a black plastic shield. Two windows in the shield allowed the rat to make nose-poke responses to the stimuli. The rear aluminum walls were fitted with a food magazine attached to a pellet dispenser. LEDs illuminating the food magazine indicated that a trial could be initiated. Magazine entries while the LEDs were on triggered stimuli to appear on the screen. The touchscreen stimuli

consisted of white geometric symbols on black backgrounds. According to the task, certain nose-poke responses led to sucrose pellet(s) (45mg each) being dispensed into the food magazine. The testing was automated using the Monkey CANTAB software running on the Whisker control system (Cardinal and Aitken, 2010).

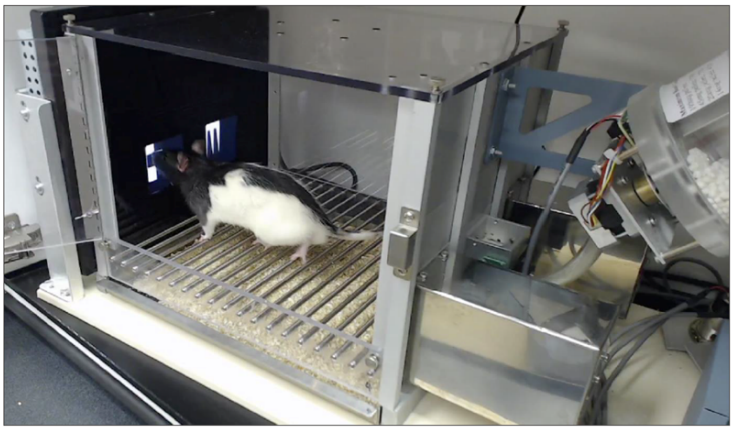

Figure 4. Automated touchscreen testing operant chamber

Photo shows operant chamber with two computer graphic stimuli displayed on the touchscreen, one of which the rat is responding to. Each chamber was housed a cabinet, ventilated by low-level fans to mask background noise. A house-light was provided by a 3W bulb located on the ceiling of the cabinet.

Habituation and Pre-training

Rats were habituated to the testing apparatus and then trained in the operation of the touchscreen. First they were given one 30 min session to habituate to the testing chamber and consume pellets freely available in the food magazine. Rats were then shaped to collect pellets that were delivered every 15s upon illumination of the magazine. When rats consumed approximately 80 pellets during a 20 min session, they were trained to respond to the stimuli presented on the touchscreen. In this procedure, a white square was randomly presented on the left or right of the screen. The stimulus remained on the screen until the rat responded to it by making a nose-poke response which dispensed a one pellet reward in the food magazine. After a 5s inter-trial interval (ITI), the rat was allowed to initiate the next trial by making a head entry into the lit food magazine. When the rat was reliably collecting 50 pellets within a 20 min session, the rat underwent surgery

to receive a dorsal or ventral hippocampal lesion, or sham control surgery. On average each subject took one week to complete all the pre-training steps. Since the same rats were used in both tasks, pre-training was conducted only once.

Acquisition

Approximately one week after surgery, rats were trained to discriminate two visual stimuli on the touchscreen (see Figure 5A). A session started with the illumination of the house light and the food magazine light. After a 5s ITI, the rat initiated a trial by making a food magazine entry. This resulted in two white shapes being presented on the screen simultaneously (see Figure 5B). One stimulus was positively associated with a pellet reward (the A^+ stimulus). The other stimulus was never associated with a reward (the B^- stimulus). The rewarded stimulus was counterbalanced across subjects. The same pair of stimuli was presented on every trial, but the left/right positions of the stimuli were determined pseudorandomly with no configuration occurring more than twice in a row.

A correct response to A^+ was followed by the disappearance of the stimulus and the delivery of a pellet concomitant with the illumination of the food magazine. After a 5s ITI the rat was allowed to initiate the next trial, up to a maximum of 100 trials in a session. However, an incorrect response to B^- resulted in a 10s timeout with all lights extinguished and stimuli removed, followed by the initiation of a correction trial. In correction trials, the same left/right stimulus configuration was presented successively until the rat responded correctly. There was no limit to the amount of correction trials that could be triggered, and they were not included in the criterion measure (see performance measures). A successful correction trial was followed by a trial with the opposite

stimulus configuration. Rats were advanced to the next stage only if they achieved a criterion of 85% accuracy in 100 non-correction trials on two consecutive days.

Reversals

Once rats had successfully acquired the stimulus-reward association (i.e., discrimination), the stimulus- reward associations were reversed so that the previously non-rewarded stimulus (B^-) became the rewarded stimulus (B^+) and vice versa ($A^+ \rightarrow A^-$). Other than the reversed contingencies, no other changes were made from the initial acquisition phase. Rats were again required to attain an average criterion of 85% accuracy in 100 trials over 2 consecutive days. If the rat reached criterion it was moved on to the final stage, and the reward contingencies were reversed again (i.e., Reversal 2). Note that reward contingencies for Reversal 2 were the same as the initial acquisition (see Figure 5B).

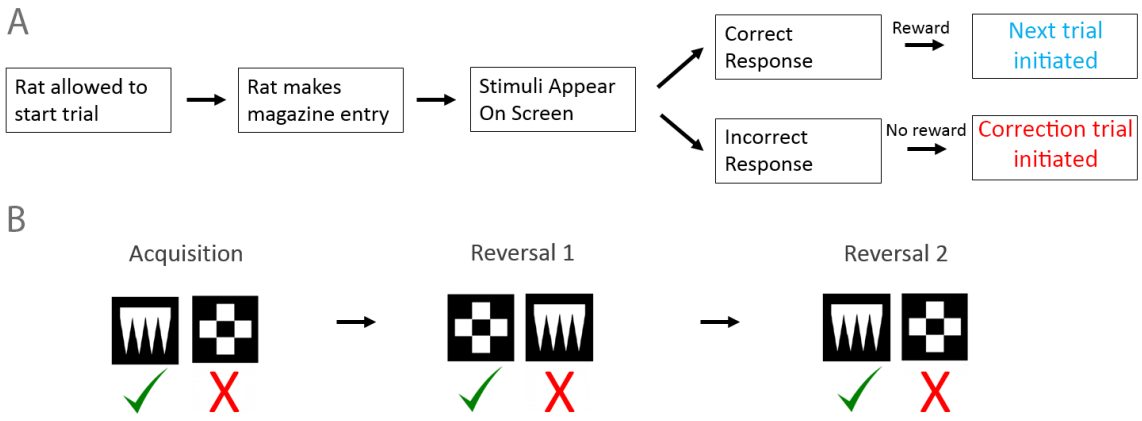

Figure 5. Diagram of the discrimination and reversal task structure

A) The sequence of events that occur on every trial. The rat has a choice between two stimuli once they appear on screen. If the rat makes an incorrect response the trial repeats with stimuli in the same left/right position; the correction trial. (B) The stimuli used in the experiment. Green "tick" represents the correct rewarded stimulus during acquisition, and each reversal. Which particular stimulus is correct was counterbalanced between subjects.

Performance measures

The number of sessions to attain criterion performance was used as a measure of general learning ability. Subjects that did not achieve criterion within 30 sessions were excluded from the remainder of the experiment. Non-correction trials were additionally analyzed with the following measures: (1) the number of errors made during a session; (2) response latency, which was the time between stimulus presentation and a nose-poke response; and, (3) magazine latency, which was the time from making a correct nose-poke response to the food magazine entry to collect food.

Along with these non-correction trial data, the number of errors during a session were also analyzed during correction trials. Errors made during a correction trial provided a general measure of perseveration, since the stimulus configurations remained in the same spatial positions over consecutive trials. That is, these errors indicated responses to the previously rewarded stimulus (A^-) or to a specific side (left or right). However, errors made during non-correction trials provided a measure of *stimulus* perseveration as these errors were directed towards the stimulus, irrespective of side.

Histology

At the conclusion of the experiments all rats were intracardially perfused with a solution of 0.9% saline, followed by 4% paraformaldehyde (Sigma-Aldrich, Canada). The brains were extracted, and dehydrated by immersion in a 10% sucrose solution, followed by a 30% sucrose solution. The brains were then sectioned on a cryostat (40 μm thickness) and every second section mounted on glass slides and stained with cresyl violet.

Data Analysis

Data for all performance measures were analyzed using one-way ANOVAs. The Homogeneity of variance assumption was tested using Levene's test, and data failing this test were log transformed. Significant results were followed by Fisher's Least Significant Difference (LSD) post hoc tests. The between-subject factor was group at three levels (Sham, vHC and dHC lesion). All data were analyzed in R 2.3.2 (R Core Team, 2013).

2.2 Results

Histological analysis

Figure 6 shows the histological analysis of the dorsal (red) and ventral (blue) hippocampal lesion. The lesion of each animal was reconstructed and superimposed on coronal rat sections. The lesions of rats in both groups overlapped considerably, and included the CA fields 1-4 as well as the dentate gyrus, and subiculum.

In the dorsal hippocampal group (Figure 6A), the lesion started at approximately -2.28 mm caudal to bregma, and continued until -5.28 mm. In one rat, the lesion unilaterally encroached into the ventral tip of the hippocampus, but was not significant enough for exclusion. In four rats, the most caudal extent of the dHC showed sparing of the CA2 and CA3 subfields. There was no damage to areas beyond the intended target, such as the amygdala or fornix, in these rats.

In animals with vHC lesions (Figure 6B), the lesion started at approximately -4.44 mm caudal from bregma and continued until -5.60 mm. Some rats showed sparing of intermediate areas of the hippocampus when moving caudally. One rat showed unilateral encroachment into the tip of the dHC in the rostral portion of the lesion, this rat was not excluded. However, two rats showed incomplete unilateral lesions to the vHC and encroachment into the amygdala and were excluded

from analysis. Again, there was no damage to parahippocampal structures such as the entorhinal or perirhinal cortex. An additional two rats (one vHC, one sham) died of unknown causes during night hours so their brains could not be retained for analysis. No sham control rats showed any neuronal damage to the hippocampus or other areas. Therefore, the total number of subjects that were retained for statistical analysis were as follows: sham controls, n = 17; dHC lesion, n = 9; vHC lesion, n = 7.

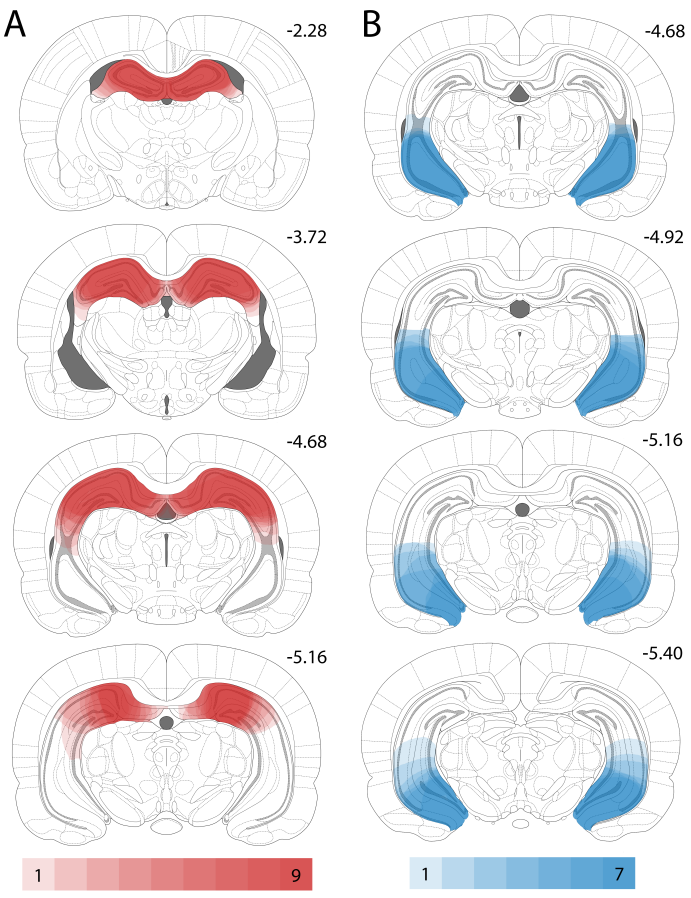

Figure 6. Histological analysis of dHC and vHC lesions

Digital reconstructions of coronal brain sections showing the extent of lesions to the dorsal (A, red) and ventral (B, blue) hippocampus. The lesion of each animal, depicted in translucent colours, were superimposed to reveal a heat map of the lesion extent. Darker colors indicate a greater overlap of lesions. Colour scale bars indicate the number of rats. Negative numbers indicate the location of the section relative to bregma according to Paxinos & Watson (2005).

Behavioural Analysis

In the discrimination and reversal learning task, rats were required to learn a stimulus-reward association (acquisition) and then to change their response with the reward contingencies were reversed (reversal). Rats were given a total of two reversals.

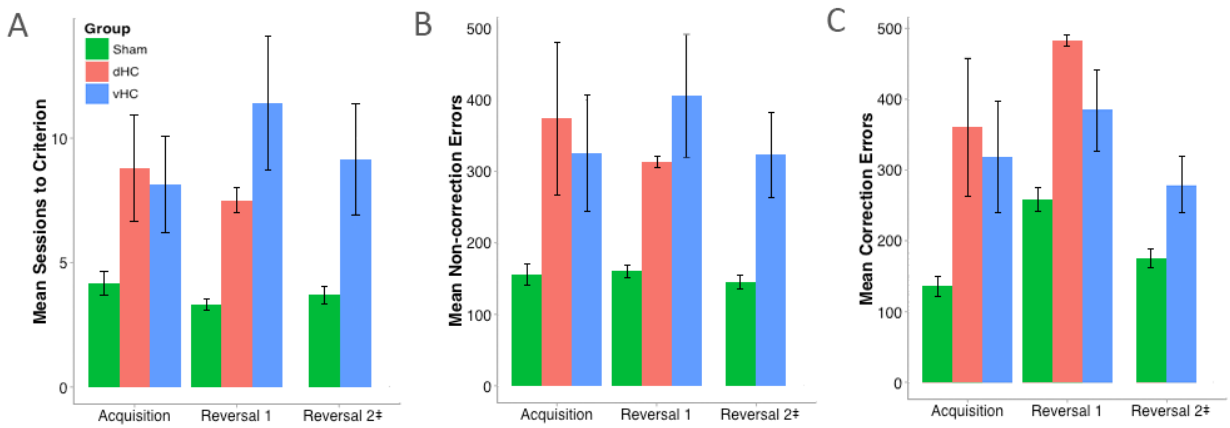

Figure 7. Mean performance (±SEM) of lesion and sham groups on the visual discrimination and reversal learning task

A) Mean number of sessions required to attain 85% criterion, **B)** Mean number of errors during non-correction trials only, a measure of stimulus perseveration, C**)** Mean number of errors committed during the correction trials, a measure of generalized perseveration. ‡ Note that only two rats reached criterion in reversal 2 constituting an insufficient number of rats to plot mean, standard errors or conduct analyses.

The dorsal and ventral hippocampal-lesioned rats took many sessions to reach criterion level of performance relative to sham controls (see Figure 7A) for the initial acquisition ($F_{2,26} = 5.32$, $P < 0.05$) and each reversal (reversal 1: $F_{2,22} = 11.3$, $P < 0.01$; reversal 2: $F_{2,21} = 33.6$, $P < 0.001$). Subsequent post hoc analysis confirmed that while the lesion groups did not differ from each other (LSD, all $P > 0.05$), they were significantly different from sham controls (LSD, all $P < 0.05$).

A similar pattern was observed in terms of errors committed (Figures 7B and 7C). Specifically, non-correction trial errors were substantially increased in the dHC- and vHC-lesioned groups during acquisition ($F_{2,26} = 5.91$, $P < 0.01$), reversal 1 ($F_{2,22} = 10.02$, $P < 0.01$), and reversal

2 ($F_{2, 21}$ = 31.7, P < 0.001). Post hoc analysis confirmed that each lesioned group made errors substantially greater than controls (LSD, all P > 0.05) indicating the dorsal and ventral hippocampal lesion made rats highly perseverative.

Correction trial errors provided a generalized measure of stimulus or side perseveration. Again, during acquisition, both lesioned groups did worse than controls ($F_{2,26}$ = 7.30, P < 0.01; LSD, P < 0.01), but did not differ from each other (LSD, P > 0.05). When the reward contingencies were reversed, the number of correction errors increased for all animals ($F_{2,22}$ = 7.28, P < 0.01). However, figure 7C shows that those animals with a dHC lesion made significantly more errors than sham controls (LSD, P < 0.01) and substantial, but not significantly, greater numbers of errors than vHC lesioned rats (LSD, P > 0.05). In reversal 2, shams committed fewer errors than the vHC-lesioned group ($F_{2,21}$ = 11.4, P < 0.01) suggesting that these animals maintained their perseverative tendency despite having learnt the same stimulus-reward contingency during acquisition. Intriguingly, only two of the dHC animals reached criterion performance on reversal 2. The substantially reduced sample size did not permit viable statistical analyses. In terms of raw numbers however, these two animals made an average of 692 non-correction errors and 480 correction errors over 30 sessions, substantially higher than the vHC rats or shams.

Latency to respond and collect food rewards are summarised in the Table 2. A main effect of lesion was obtained for response latency during acquisition only; dHC-lesioned rats were faster to make a response than the other groups (Sham 3592.0 ± 247.89 ms; dHC 2270.51 ± 277.52 ms; vHC 3284.73 ± 348.76 ms; LSD, all P < 0.05). All other latency measures during acquisition and reversal were in the normal range.

Table 2: One-way ANOVA results for latency measures in experiment 1

Measure	Acquisition	Reversal 1	Reversal 2
Response Latency	($F_{2,26} = 3.75$, $P < 0.05$)	($F_{2,22} = 3.23$, $P > 0.05$)	($F_{2,21} = 2.12$, $P > 0.05$)
Magazine Latency	($F_{2,26} = 3.02$, $P > 0.05$)	($F_{2,22} = 0.04$, $P > 0.05$)	($F_{2,21} = 0.33$, $P > 0.05$)

2.4 Summary of findings

The aim of this experiment was to examine the contribution of the dorsal and ventral hippocampus to associative learning and response control by use of a visual discrimination and reversal learning task. Sham control rats showed normal learning of this task but both lesioned groups showed substantial deficits in learning the association, and in adjusting their response during reversal, indicating a high degree of perseveration and disinhibition. Of particular significance however was the finding that the dHC lesion caused a substantial increase in correction trial errors indicating that despite needing many repeat trials to correct their errors, they were committed repeatedly to the same space. In other words, rats with the dHC lesions were sensitive to the left/right locations of the stimuli and this was sufficient to cause major deficits in this group.

Although the findings of this experiment diverge from a previous experiment in which vHC lesions failed to cause perseverative impairments (Abela et al., 2013), the current data are consistent with a multitude of historical studies that reported perseverative tendencies following hippocampal lesions in both primates and rodents during discrimination and reversal learning (Kimble and Kimble 1965; Jones and Mishkin 1972; Murray & Ridley, 1999). An extended discussion of these data and their potential implications both theoretically and clinical is provided in the General Discussion (Section 4).

3.0 Experiment 2: Hippocampal lesions and decision-making

In this experiment I investigated another aspect of executive function, that of decision-making. Using the same operant touchscreen system as in experiment 1, we tested rats on a novel temporal discounting task. To summarize, lesioned rats and sham controls were required to make a choice between a small immediate reward and a large delayed reward. Rats were advanced through four successive stages in this task: 1) At first delays were set to zero, in order for the rats to learn to discriminate the reward sizes; 2) delays were introduced and progressively increased in each block (0s, 8s, 16s, 32s) to observe discounting behaviour; 3) delay order was randomized (e.g. 16s, 8s, 0s, 32s) to test if rats learned the association between stimuli and delay; and 4) the small reward was removed as rats were required to choose between 2 large delayed rewards, in order to test the rats ability to judge differences in time.

3.1 Methods and Materials

The animals from experiment 1 were used for this study. At this point, the rats were approximately 320 g in weight and 9 months in age. All aspects of housing and feeding schedule were identical to Experiment 1. In addition, the apparatus was the same so rats were not subjected to habituation or pre-training as was required before the first experiment. Below I describe the novel delay discounting task that was designed and implemented specifically for this project.

Temporal discounting task

At the conclusion of Experiment 1, the rats were placed on free-feeding diet before being again reduced to 85% of their free-feeding weight and trained on the temporal discounting task. The experimental set-up was identical to the first experiment but each stimulus was associated

with a different delay. All stimuli were different from those used in experiment 1. The diagram in Figure 8 provides the series of events for each trial.

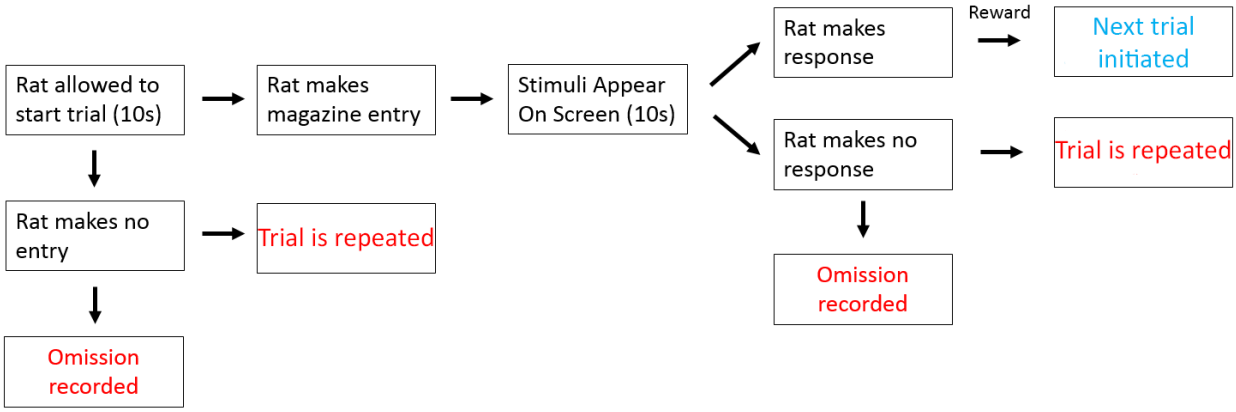

Figure 8. Structure of a trial in the delay discounting experiment

As in the discrimination task, the rat chooses when to begin a trial by making an entry to the food magazine. However, in this experiment the rat has a limited time to do so. If the rat does not start a trial, or does not make a choice on the touchscreen after a trial begins, the program begins a correction trial procedure as in experiment 1.

An illuminated food magazine signalled to the rat that he could initiate the trial. A nose-poke entry into the lit food magazine within 10 seconds presented the stimuli on the screen. Rats were required to make a choice response within 10 seconds which subsequently delivered a small 1 pellet reward immediately or a large 4 pellet reward after a delay. Failure to initiate a trial, or make a response within a 10 s response time, terminated the trial and was recorded as an omission. Trials began every 70 seconds regardless of the choices the rat made. Figure 9 illustrates the different configurations of stimuli and delays presented in each stage.

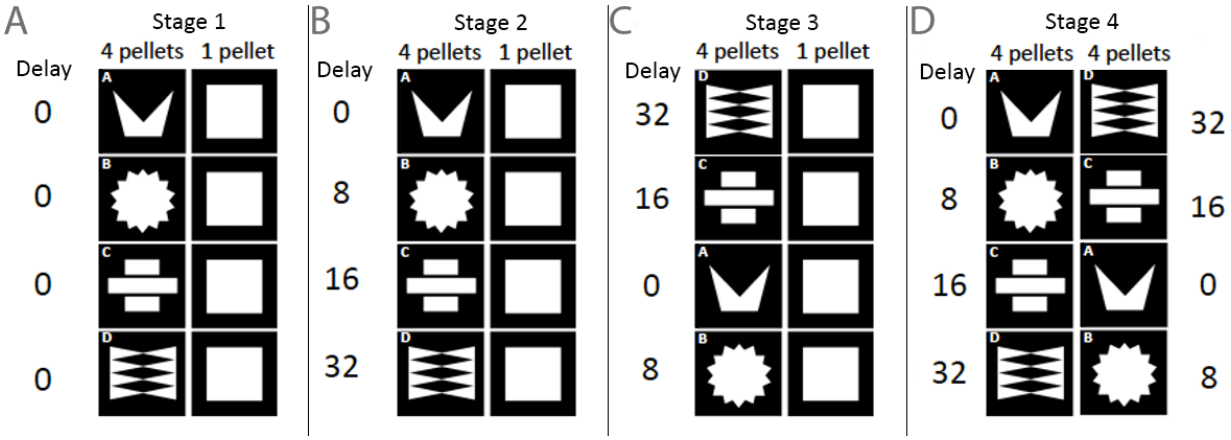

Figure 9. Illustration of the stimulus pairs in each of the four stages

A) No delays were present. The rat had a choice between a large 4 pellet reward and a small 1 pellet reward. **B)** Each stimulus was associated with a delay to reward delivery (0s, 8s, 16s, 32s). Each stimulus pair was presented in blocks of 10, in a linear fashion (see methods). **C)** The order of stimuli presentations and their associated delays was randomized. **D)** The small pellet reward associated with the white square was removed so that reward was constant. The stimuli were paired in different combinations so that the animal had to discriminate the stimuli by delay to reward.

Stage 1: No delays

In the first stage rats had a choice between a small reward (1 pellet) and a large reward (4 pellets). There were no delays associated with reward delivery. This stage was implemented to ensure that the rat understood the task structure and, importantly, discriminate the two reward magnitude or values. The small reward was always represented by a white square, and the alternative large reward was represented by different shape that varied (see Figure 9). During each session of testing, the rat was presented with 4 blocks of 12 trials each. The first two trials in each block were "forced-choice" trials. Here only one stimulus was presented and the rat was forced to respond. The forced choice trial procedure informed the rats what the reward and stimulus contingencies were for that block. For the remaining 10 trials the rat had a "free choice" as to which stimulus to choose, by making a head-on nose-poke response to their chosen stimulus. The large reward stimulus changed in each successive block in the order presented in Figure 9. Each subject was tested until it made 8 or more large reward choices in each block.

Stage 2: Increasing Delays

We now increased the delay to reward delivery for the large reward stimulus. The large reward of four pellets was associated with four different stimuli. In each block, the delays increased in sequence: 0s, 8s, 16s, and 32s. The same stimuli were associated with the same delays for all rats. The purpose of this stage was to determine if subjects discounted the value of future rewards as a function of their delay. The criterion for this stage was determined by analyzing the last 5 sessions for stability. If a repeated measures ANOVA showed a main effect of delay (indicating discounting) and no main effect of session (indicating stability) the rat was advanced to the next stage.

Stage 3: Randomized blocks

The third stage randomized the order that the delays were presented, and did not include forced-choice trials. In this way, rats had to understood the relationship between the stimulus and the delay. Subjects could rely neither on the order that delays occurred in, nor the contingencies displayed by forced-choice trials. Again a stability criterion was used to determine if rats completed the task.

Stage 4: Paired delays

Finally, in the fourth stage the small reward option was removed and the rats were presented 6 successive blocks of paired stimulus each associated with a different reward delay: 0s/8s, 0s/16s, 0s/32s, 8s/16s, 8s/32s, and 16/32s. As all delayed stimuli were associated with large reward, the ideal action would be to always choose the lower delay. This would indicate that the rat can conceptualize the magnitude of an 8, 16, 24, or 32 second difference. This was an exploratory

stage aimed at developing an indicator of hippocampal involvement in the perception of time. As such, rats were only tested for 10 days to gauge behaviour.

Performance Measures

For the 10 free-choice trials in stages 1-3, the following measures were collected: (1) the total number of large reward choices per block (out of 10); (2) the number of omissions per session (out of 40); (3) the response latency, or the time between stimuli appearing on screen and a nose-poke response; and (4) the magazine latency, or the time between reward delivery and pellet collection. For stage 4, since both options gave the large reward, the number of choices for the longer delay (out of 10) were recorded.

Complications in behavioural testing

As stated above a stability criterion was used during the second and third stages of the delay discounting experiment. The inherent difficulty of this novel task led to a large number of both sham controls and lesion rats failing to meet criterion even after 30 days. As such the number of animals available for analysis in certain groups is very low. If this experiment was replicated it would be preferable to test the rats for a fixed period (perhaps 25 days) on every session instead of using a criterion. This would have provided us with sufficient data for appropriate statistical analyses.

Data Analysis

Data for all performance measures were analyzed using either one-way or repeated-measures ANOVAs. The Homogeneity of variance assumption was tested using Levene's test, and data

failing this test were log-transformed. Likewise, repeated measures data were tested with Mauchly's sphericity test; the Huyn-Feldt epsilon correction was used for data that did not meet this assumption. Significant results were followed by Fisher's Least Significant Difference (LSD) post hoc tests. The between-subject factor was group, and the within-subject subject factor was delay. All data were analyzed in R 2.3.2 (R Core Team, 2013).

3.2 Results

First, rats were given a choice between a large 4 pellet reward and a small 1 pellet reward across 4 blocks of 10 free choice trials each. With no delays present, both sham and lesion groups were capable of discriminating reward size and tended to choose the high reward option ($F_{3,84}$ = 0.432, P > 0.05). Nonetheless, as Figure 10A shows, the lesioned animals chose the high reward less often than controls ($F_{2,28}$ = 8.86, P < 0.01). Nonetheless, both lesioned groups chose the large reward option well above chance performance (dHC = 75%; vHC = 80%). Post hoc comparisons confirmed that sham rats were more consistent in choosing the high reward option (LSD, all P < 0.05).

When the delays were introduced in stage 2, all rats irrespective of lesion, were less willing to choose the large reward option as the delay increased from 0 to 32 seconds ($F_{3,75}$ = 20.5, P < 0.01; Figure 10B). However, there was no main effect of group ($F_{2,25}$ = 2.359, P > 0.05), or group x delay interaction ($F_{6,75}$ = 1.55, P > 0.05). We note however, that rats with dHC lesions failed to choose the large reward stimulus option above chance levels in the first block of trials when the delay was zero, as in stage 1 suggesting a change in strategy which was detrimental to their overall decision making. Rats did not differ on any other measure in this stage; response latency ($F_{2,25}$ =

0.29, P > 0.05), magazine latency ($F_{2,25}$ = 1.18, P > 0.05), or number of omissions ($F_{2,25}$ = 0.748, P > 0.05).

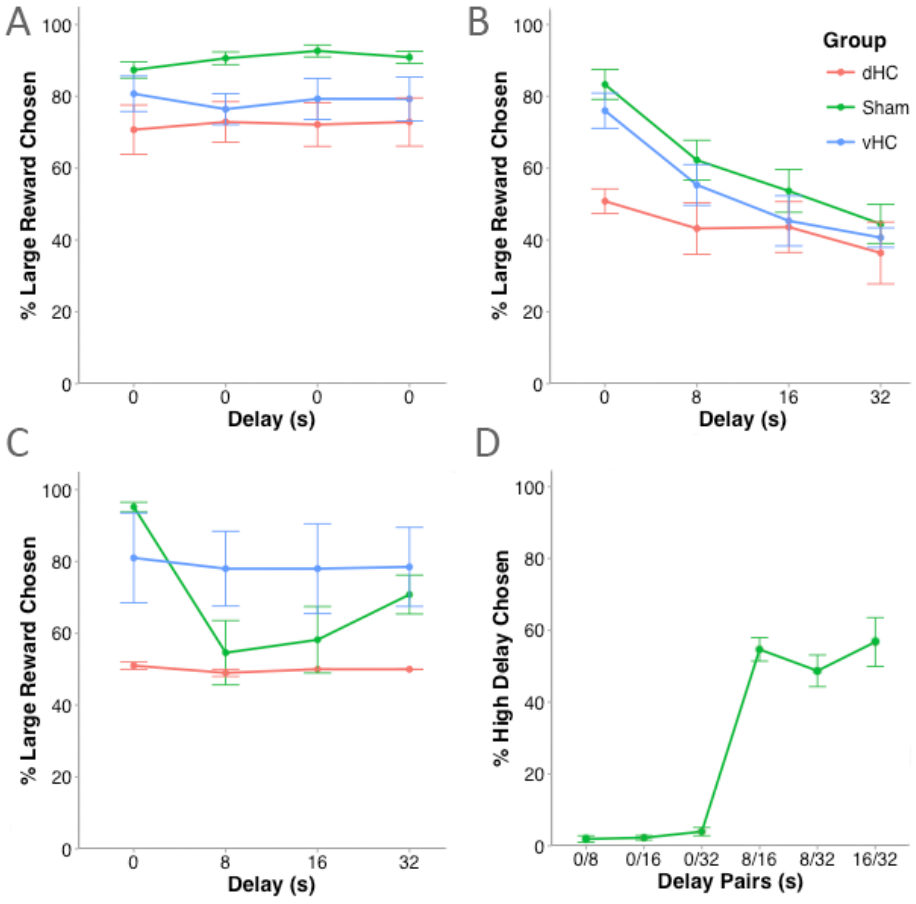

Figure 9. Mean high reward choice in the delay discounting task

Mean choice of the large 4 pellet reward (±SEM) in, **A)** a condition with no delays, **B)** increasing delays, **C)** randomized trials. **D)** In stage 4, delays were paired and only the large reward was available. Only sham rats were tested.

In the third stage of testing, the stimuli-delay pairs remained the same, but the block structure was randomized. Therefore, the rat could encounter any level of delay in any particular trial. In this stage the choice strategy of the rats took on very different characteristics as shown in Figure 10C. Just like their performance in stage 2, rats in the dHC group chose randomly, maintaining 50% choice across every block. In contrast, the vHC group chose the high reward consistently,

irrespective of delay suggesting that these animals successfully learnt the stimulus-delay associations. A main interaction between group and delay was obtained ($F_{6,39} = 3.017$, $P < 0.05$) which was due to the vHC lesion causing a major bias towards the large reward option compared with the dorsal group (LSD, all delays $P < 0.05$). The sham controls did not differ from the dHC-lesioned rats at intermediate delays (LSD, both $P > 0.05$), but they chose the large rewards most of the time at the extremes of 0s and 32s delay (LSD, both $P < 0.05$). However, at the extreme delays, shams were indistinguishable from vHC-lesioned rats (LSD, both $P > 0.05$). No other main effects were observed: response latency ($F_{2,13} = 0.49$, $P > 0.05$), magazine latency ($F_{2,13} = 1.04$, $P > 0.05$), or omissions ($F_{2,13} = 0.673$, $P > 0.05$).

Finally, a cohort of sham rats was tested on stage 4 where the small reward option was removed and rats had to choose between stimuli that always offered the large delayed reward (see Figure 10D). Every possible delay pairing was presented for 6 blocks of 10 trials each. There were no forced choice trials. An interesting pattern of data emerged in this stage. When the choice was between a stimulus that presented a large reward at 0 seconds, compared with a stimulus that presented a large reward after a delay (i.e., 0 vs 8s, 0 vs 16s and 0 vs 32s), the rats chose the low (i.e., 0 sec) delay option ($F_{5,25} = 75.15$, $P < 0.01$). However, when the choices were between stimuli associated with different delays (i.e., 8 vs 16, 8, vs 32, and 16 vs 32), they switched to a random choice strategy suggesting that control rats have a difficulty in making optimal choices when the times is not separated or distinct enough.

3.2 Summary of findings

We tested rats on four successive stages in a delay discounting task with increasing difficulty. The objective was to discover how the dorsal pole of the hippocampus contributes to decision-

making in a non-spatial task, given that previous work has shown dHC lesions cause impulsive-type choices in maze tasks (McHugh et al., 2008). Due the difficulty of reaching criterion at each stage, there was a high attrition rate. However, choice curves in stage 1 with no delays, confirmed that all rats could distinguish between low and high reward options. The relatively low rate of large reward choices following dHC lesions might be related to the poor learning rate, as observed in experiment 1. In other words, there appears to be a deficit in stimulus-reward association in the dHC group. Deficits in remembering stimulus-reward relationships have been found previously in rats with conventional hippocampal lesions (Rawlins et al., 1991). Alternatively, dHC spatial sensitivity may be so great that the left/right positioning of the stimuli, in a small operant chamber, may impair learning in the subject. It should be noted however, that while we expect sham rats to choose the large reward option at all times, especially after many days of testing, this is not always the case presumably due to the motivational factors (see also Figure 3C, on page 2228, in Abela, Duan & Chudasama, 2015).

When the delays were introduced all animals showed delay-dependant choice behaviour as evidenced by the presence of downward discounting curves. However, the data did not show the effect of impulsive choice as reported in previous experiments following hippocampal lesions (McHugh et al., 2008; Abela & Chudasama, 2013). Moreover, when the order of the delays was randomized, the groups showed unique effects. Dorsal hippocampal rats appeared entirely unable or unwilling to choose based on reward size or delay. Likewise, the vHC rats adopted a strategy that was irrespective of time, choosing the high reward around 80% of the time. It is clear that these rats understand the stimulus associations but the randomization apparently inhibited their ability to react to different delays. As for the shams, their behaviour may well be related to changes in motivational state, that is, rats may have been hungrier earlier on the session before consuming

many reward pellets. Since the delays were randomised, a 32s delay may have been appetizing if this trial was presented near the start of the session.

In the fourth stage, sham rats showed rational choosing when the immediate large reward delivery stimulus was present but began choosing randomly when two delayed reward stimuli were paired. The data indicate the use of different strategies to optimize reward although rats find it difficult to distinguish two similar durations.

4. General Discussion

The role of the hippocampus in memory has been the chief focus in the clinical literature since the days of Scoville, Milner and Penfield. Although an amazing diversity of function for the hippocampus has been shown in animal studies, we are still unclear about the extent of its contributions to behaviour. This is especially the case when it is treated not as a unitary structure but one with distinct functional zones along its longitudinal axis (Fanselow & Dong, 2009).

Problems with behavioural control, a common symptom of several neuropathologies, is currently an important field of study as there are very few treatments and interventions available (Hamilton & Brigman, 2015). Work in our lab in particular has shown that the dorsal and ventral hippocampus can be distinguished in terms of their contribution to inhibitory control. For example, when tested on the 5-choice reaction time task, a test of visuospatial attention and inhibition, rats with vHC but not dHC lesions showed high rates of impulsive and compulsive responses indicating a failure to control actions and inhibit inappropriate responses (Abela et al., 2013). Additionally, the same vHC-lesioned rats did not show deficits in a standard discrimination and reversal task, as used in the experiment 1, suggesting that the vHC is not sensitive to all types of inhibition (Abela et al., 2013). Moreover, the animals used by Abela et al (2013) were extensively trained having

made up a thousand errors when just acquiring the task, suggesting that the cohort of animals used in the Abela et al (2013) cannot be matched directly with the animals in the current study.

On the other hand, inhibition control problems following hippocampal lesion have been reported extensively in past experiments, all of which show the majority of damage localised to the dorsal hippocampus (Kimble & Kimble, 1965; Silveira & Kimble, 1968; Murray & Ridley, 1999). In this study, I directly compared the two hippocampal poles and found that these areas produced very similar deficits. Both operated groups not only found it hard to acquire the stimulus-reward association, they showed evidence of perseveration when the visual contingencies of the task were reversed indicating repeated responses to the previously rewarded stimulus. The reason for the divergence between this experiment and the related study by Abela et al. (2013) may be due to the specific stimuli, or slight variations in damage to the hippocampus. Some have suggested that the intermediate zone of the hippocampus may be the cause of some confusion in the literature. Researchers identified several papers that purported similar functions between hippocampal poles but were confusing dHC and vHC contributions with that of the intermediate zone (Fanselow & Dong, 2009). The specific cause of the diverging results merits further investigation.

We additionally investigated decision-making behaviour mediated by the hippocampus. This experiment presented an entirely novel delay discounting task for rodents. The primary results are hard to interpret due to the inherent difficulty of the task. For example, in stage 1, both lesion groups chose the high reward less often than shams. There simply may not be enough subjects, to differntiate the performance level of the lesion groups in this stage. Nonetheless, this experiment paves the way for implementing similar styles of behavioral testing which provide a great degree of control and avenues of analysis. I feel confident that with some more piloting and exploration of the training parameters, these animals would learn the task successfully.

Unlike previous tasks (Cheung & Cardinal, 2005; Abela and Chudasama, 2013), in which the small and large reward were spatially positioned to the left or right side of the chamber, we introduced different delays, each associated with a unique stimulus. Thus rats were required to make several different time-stimulus associations. This allowed us to subsequently perform tests, in stage 4, to establish representations of time. Although results for stage 4 were inconclusive, it's possible that the time difference between the two delayed options (e.g., 16-32 s) were not distinguishable enough for the rats. An experiment specifically manipulating time or length of delay might reveal a rodent sensitivity to the absolute value of time. An interest in the role of the hippocampus in time is a growing subject of research; it has been suggested that the hippocampus comprises "time-cells," which is just as much a unique component of the hippocampus as "place cells" (Eichenbaum, 2012). Perhaps this novel task can be used in the future to explore this new avenue.

However, as was stated in the methods the criteria and training regimen for this experiment was adequate to show strong effects between the groups. The difficulty of the task as well as the small sample size hampered the investigation of the most interesting questions (such as stage 4). While evident that both the dHC and vHC play a role in decisions with respect to time, more work will have to be done, taking into account the lessons learned from this experiment. In potential replications, or similar experiments in the future, it would be very useful to perform a full battery of tests on the rats prior to training. For example, we already know of tasks that are differentially impacted by dorsal or ventral lesions. The Morris Water Maze described above separates out dorsal lesion rats as only they should show spatial memory impairments (Morris et al., 1982). Likewise, ventral but not dorsal, lesioned rats show anxiolytic effects in several tests of fear and anxiety

(McHugh et al., 2004). By testing all rats on these tasks prior to training, one could ensure the prototypicality of the lesions and be better able to explain variations in the data.

Apart from these alternations to the existing experimental design, there are several possible directions in which to continue this research. First, there have been several neurotransmitter systems potentially involved in hippocampal discrimination tasks. Both glutamatergic (Murray & Ridley, 1999) and GABAergic (Sakimoto, et al. 2014) mechanisms have been suggested. Although the effects of serotonergic and dopaminergic drugs have been tested with temporal discounting tasks (Abela and Chudasama, 2014), their effects on discrimination-reversal tasks have not been established. Determining which drugs aid or impair hippocampal function in executive tasks will facilitate our understanding the neurochemical mechanisms underlying this behaviour, and provide a basis for therapeutic strategies for disorders of inhibition.

Second, probability discounting has been investigated in hippocampal-lesioned animals but dorsal and ventral lesions have not been compared in a touchscreen task (Abela and Chudasama, 2013; McHugh et al., 2008). In probability discounting, subjects must choose between a small certain reward and a large uncertain one (Rachlin et al., 1986). The tendency to take risks with high payoffs provides a measure of impulsive and risky behaviour. Previous work has shown that rats with hippocampal lesions prefer certain rewards to uncertain ones, at least in situations where time is not a factor (Rawlins et al., 1985). However, reward probability was not an experimental variable in this experiment partly because we have developed a new task that required validation. We know that vHC lesions do not affect probability discounting behavior in an experiment similar to that reported in this study (Abela et al., 2013; Abela, Duan and Chudasama, 2015), but to date, the effect of dHC lesions on reward uncertainty has not been systematically explored. Furthermore,

by using distinct shapes as stimuli it would be possible to compare different probabilities directly as in stage 4 of the delay discounting experiment.

Third, several recent papers have suggested new genetic subdivisions of the hippocampus across the longitudinal and transverse axes (Fanselow & Dong, 2009; Strange et al., 2014). Ion channel biophysics appear to change in a gradient down the longitudinal axis. This indicates that not only do dorsal and ventral cells receive distinct inputs, but they may process information in computationally distinct ways (Hönigsperger et al., 2015). Investigating complexity at this level may be past the limit of cytotoxic lesion approaches. Just as early aspiration lesions were not suitable for investigating the dorsoventral axis, the potentially functionally distinct genetic domains will require cutting-edge methods such as optogenetics and DREADDs to investigate. Both of these methods induce the expression of cell-specific membrane proteins, which when followed by light pulses or drugs can activate the neurons (Zhao et al., 2011; Roth, 2016). Perhaps with the ability to have tight spatial and temporal control of neuronal activation, the question of hippocampal function diversity may finally be resolved.

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