Contribution of the ventral hippocampus to impulsive behaviour in the rat

Stephen Dougherty
Department of Psychology
McGill University, Montreal, Canada

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ABSTRACT

In addition to its role in spatial memory, in rats, recent studies have implicated the hippocampus in impulse control. There is evidence to suggest that such differences in the function of the rodent hippocampus can be attributed to its dorsal and ventral subdivisions. To further explore the contribution of the hippocampus to the control of impulsive behaviour, the present study examined the behavioural effect of dorsal and ventral hippocampal lesions in the 5-choice reaction time task (5-CRTT), a test of visuospatial attention and inhibitory control. Following initial behavioural testing of lesioned animals, we administered a systemic dopamine reuptake inhibitory (GBR 12909) and a selective serotonin reuptake inhibitor (escitalopram), in an attempt to ameliorate any deficit that may arise. Our results showed that bilateral lesions of the ventral hippocampus produced an increase in impulsive responses in the 5-CRTT. By contrast, rats with lesions to the dorsal hippocampus were unimpaired in any aspect of task performance. Escitalopram and GBR 12909 produced distinct effects in animals with HC lesions. Systemic treatment with escitalopram reduced the number of premature responses in all groups; by comparison GBR 12909 increased premature responses in animals with hippocampal lesions. These data indicate a selective role of the ventral hippocampus in impulsive behaviour in the rat. Furthermore, the results suggest the possibility that SSRI’s might normalize inappropriate impulsive responding.
RÉSUMÉ

En plus de son rôle dans la mémoire spatiale, chez les rats, les études récentes ont impliqué le hippocampe dans la commande d'impulsion. Nous pouvons démontrer que des différences dans la fonction de l'hippocampe des rongeurs pourraient être attribuées à ses subdivisions dorsales et ventrales. Pour explorer en profondeur la contribution de l'hippocampe à la commande du comportement impulsif, la présente étude a examiné l'effet comportemental des lésions hippocampales dorsales et ventrales dans la tâche de temps de réaction des 5 choix (5-CRTT), qui est un test de l'attention visuospatiale et la commande inhibitrice. Après le test comportemental des animaux avec des lésions, nous avons essayé d'améliorer n'importe quel déficit qui peut survenir par l'administration d'un inhibiteur systémique de reuptake de dopamine (GBR 12909) et d'un inhibiteur sélectif de reuptake de sérotonine (escitalopram). Nos résultats démontrent que les lésions bilatérales de l'hippocampe ventral ont produit une augmentation sélective des réponses impulsives dans le 5-CRTT. En revanche, les rats avec des lésions à l'hippocampe dorsal étaient intacts dans tous les aspects d'exécution de tâche. Escitalopram et GBR 12909 ont produit des effets distincts chez les animaux avec des lésions de HC. Le traitement systémique avec l'escitalopram a réduit le nombre de réponses prématurées dans tous les groupes ; par comparison GBR 12909 a augmenté des réponses prématurées chez les animaux avec les lésions hippocampal. Ces données indiquent un rôle sélectif de l'hippocampe ventral dans le comportement impulsif dans le rat. En outre, les résultats suggèrent la possibilité que la force de SSRI normalisent la réponse impulsive inadéquate.
INTRODUCTION

The case of patient H.M. triggered a surge in interest in the hippocampus (HC) as a brain structure essential to learning and memory. In 1953, patient H.M. underwent medial temporal lobe resection to control severe epileptic seizures. The surgery had the intended effect of curing the seizures, however, it left the patient with severe and selective amnesia. H.M’s case led to extensive research in humans and animals aimed at explaining the relationship between the brain and the organization of memory. One of the most significant findings revealed that damage to the HC was necessary to cause a memory impairment in other patients who underwent medial temporal lobe resection for the treatment of seizures (Penfield & Milner, 1958). While these investigations in humans provided a foundation for the role of the HC in learning and memory, the non-specificity of the lesion warranted further investigations to elucidate the specific role of the HC relative to adjacent cortical structures, as well as the nature of the memory deficit.

Role of the Hippocampus in Spatial Memory

A large body of evidence has accumulated in animals indicating a critical role for the HC in spatial memory. One influential theory suggests that the HC is involved in constructing “cognitive maps” or spatial representations of experienced environments (Olton & Samuelson, 1976; O’Keefe & Nadel, 1978). The success of this theory derives from its ability to explain a substantial portion of the deficits that arise from HC damage in animals. A broad range of studies have shown that animals with HC lesions are impaired in tasks that require the animal to utilize spatial cues from the environment to navigate for reward or to avoid aversive stimuli. The Morris water maze is used to assess an animal’s ability to locate a hidden platform in a circular pool by using spatial cues in the surrounding test environment (Morris, 1984). In this task, rats with HC lesions are impaired in their ability to locate the hidden platform as a consequence of their inability to navigate their
environment using distal spatial cues. This conclusion is supported by testing on a cued version of task in which the platform is signaled by a local visual cue. Under these conditions, performance is unaffected by HC damage (Jarrard, 1983, Morris et al., 1990). Other studies utilizing maze tasks have reinforced the view that the HC plays a central role in spatial memory. In the radial arm maze for example, rats learn to navigate eight arms of a circular maze by entering each arm only once. Similar to the Morris water maze, accurate performance on the radial arm maze can only be accomplished by relying on spatial cues in the test environment. Rats with HC lesions are significantly impaired in this task because they enter previously visited arms leading to many errors (Olton & Samuelson, 1976).

The discovery of place cells in the rodent HC strengthened the view that the HC is central to spatial memory. Place cells are neurons that fire when an animal is in a particular place in its environment (O’Keefe & Dostrovsky, 1971). This was an important breakthrough because it provided a mechanism at the cellular level for hippocampal encoding of the spatial environment. Together, these studies offer convincing evidence for a critical role of the HC in the spatial domain.

Role of the Hippocampus in Non-Spatial Tasks

Dusek and Eichenbaum (1998) developed an important paradigm for evaluating the HC in the non-spatial domain, called transverse patterning. The transverse patterning paradigm provided a means to evaluate non-spatial associative learning using olfactory, rather than visual stimuli. The basic structure of the task takes the form of three separate discrimination problems: A > B, B > C and C > A where each letter stands for an olfactory stimulus and the symbol ‘>’ signifies, “is selected over”. Animals first learn to solve simple discriminations, for example A versus B, B versus C, two problems that can consistently be solved by employing a linear strategy. That is, “select A, and avoid C, whenever one is present”. Subsequently, the task demands increase and the animal must then discriminate between A versus B, B versus C and C versus A. The addition of the C
versus A problem requires the formation of a more complex configural association (Dusek & Eichenbaum, 1998). Eichenbaum and colleagues have examined the effect of fornix transection on performance in this task. The fornix transection serves to functionally disconnect the HC from the entorhinal and perirhinal cortices, while ensuring the cortical regions adjacent to the HC remain intact. In this study, the fornix lesioned group displayed significant impairments in learning this form of complex association, while leaving simple discrimination learning intact (Dusek & Eichenbaum, 1998). Thus, the transverse patterning data demonstrates a role for the HC in complex associative learning not limited to the spatial domain.

Early studies investigating hippocampal function provided evidence that the HC was important for performance in non-spatial tasks. For example, Kimble and Pribram (1963) trained monkeys on a discrimination task in which they made responses to two visual stimuli (touch sensitive response panel) in a specific sequence, when the stimuli were presented simultaneously. Initially, all monkeys tended to respond to the visual stimulus that immediately preceded the reward from the previous trial instead of pressing the panels in the correct sequence, however, control animals quickly learned to press the response panels in sequence. By contrast, the HC-lesioned monkeys could not learn to make the responses in sequence. Although the hippocampal deficit was interpreted as a failure in learning sequences, the deficit could also be interpreted as a failure to inhibit responding to a prepotent stimulus. Similarly, early studies in rodents revealed a pattern of deficits following hippocampal lesions that could not be attributed to specific spatial memory impairments. Several researchers observed that HC-lesioned animals made repeated entries into previously reinforced arms thereby elevating error scores (Kimble, 1963; Leaton, 1965; Niki, 1966). In addition, HC-lesioned rats made repeated errors in the T-Maze spontaneous alternation task in which rats must alternate between left and right responses on each trial (Douglas & Isaacson, 1964). Some researchers have explained this inflexibility of behaviour as the inability to cease responding to prepotent environmental stimuli (Kimble,
1968). Although these early ideas have not received much attention, they are relevant to a comprehensive theory, which views the HC as the central component of a system of interconnected structures that form the behavioural inhibition system (BIS).

The Hippocampus and Behavioural Inhibition

According to the theory proposed by Gray (1982), a key feature of the BIS is based on the observation that anxiolytic drugs, such as benzodiazepines produce behavioural effects that are strikingly similar to HC lesions. The anxiolytic effects of HC lesions have been observed in a range of experiments. In rats, lesions to the HC cause a reduction in freezing response to a shock probe (Blanchard et al., 1970) and footshock (Blanchard et al., 1977). In addition, rats with HC lesions show reduced freezing in the presence of a predator (Kim et al., 1971). Another central feature of this theoretical framework suggests that in tasks that require an animal to learn that a particular behaviour will lead to negative outcomes (i.e. nonreward and punishment), signals from the HC cause a decrease in approach when this behaviour is no longer reinforced. Accordingly, the HC produces inhibition of prepotent behaviour by modifying the salience of negative information (McNaughton, 1997). Deficits in behavioural inhibition following HC lesions have been well documented. For example, Davidson and Jarrard (2004) initially trained rats to discriminate between two auditory cues, one associated with a sucrose pellet and the other with no reward. Although rats with HC lesions did not differ from controls in their ability to discriminate the cues, they did show an increased rate of responding to the original cue associated with reward when the reward contingencies were reversed. Thus, these findings suggest that the anxiolytic effects of HC lesions are related to impairments in behavioural inhibition. It would be logical to posit then, that classical anxiolytic drugs such as diazepam and chlordiazepoxide exert a pharmacological effect on the HC. Indeed, one of the critical sites of action for anxiolytic drugs is the HC where they act on gamma-aminobutyric acid (GABA) receptors (McNaughton & Gray, 2000). Interestingly, novel anxiolytics such as
buspirone produce similar behavioural effects yet the drugs target the serotonergic system rather than GABA. The emergence of selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression and anxiety disorders, have provided considerable evidence that serotonin is a critical regulator of emotion (Merens et al., 2007; Cools et al., 2008). It is now becoming increasingly apparent that SSRIs exert an important pharmacological effect on the HC. For example, Malberg & Duman (2003) showed that exposing rats to inescapable shock causes both the display of learned helplessness behaviours (i.e. failure to attempt escape from shock) and a reduction in cell proliferation in the HC. Interestingly, administration of the SSRI fluoxetine hydrochloride (i.e., Prozac) reverses both learned helplessness and the reduction in cell proliferation (Malberg & Duman, 2003). Evidence from this study highlights the central role that serotonin plays in emotional processing while also suggesting that the HC is a critical site of action where fluoxetine and other SSRIs may exert their therapeutic effects.

To summarize, the behavioural inhibition model (Gray & McNaughton, 2000) views the HC as a critical regulator of emotion and behavioural inhibition. Furthermore, the model interprets the poor performance of HC-lesioned animals in tasks that rely heavily on spatial memory as not necessarily attributable to spatial memory impairments, per se, but rather a loss of behavioural inhibition. Recent studies have been undertaken in an attempt to provide a novel theoretical framework of HC function, which incorporates the view that the HC is important for behavioural inhibition and emotional processing, while maintaining its central role in spatial memory.

The Dorsal and Ventral Subdivisions of the Hippocampus

In light of the mounting evidence that the function of the HC is not restricted to spatial processing, and evidence supporting its role in behavioural inhibition and emotional processing, it follows that the function of the HC may not be unitary in nature. Indeed, several groups of researchers have argued that the function of the HC can be dissociated
along its dorso-ventral axis (Moser & Moser, 1998; Bannerman et al., 1999; McHugh et al., 2008; for reviews, see Bannerman et al., 2004; Bast, 2009). This dissociation is supported by anatomical studies that have traced the connections of the HC. Evidence from anterograde tracing experiments have shown that the ventromedial prefrontal (vmPFC) region in rats (which includes the prelimbic, infralimbic and orbital frontal cortex) receive direct hippocampal projections from the ventral half of the HC (Jay & Witter, 1991). Retrograde tracers injected into the vmPFC show labeling in the ventral HC (HCv) (Ishikawa & Nakamura, 2006) and electrophysiological stimulation of the vmPFC cause activation in individual HCv neurons (Ishikawa & Nakamura, 2006). Thus, the HCv and the PFC are highly interconnected structures. Furthermore, the HCv has direct connections with the nucleus accumbens (Kelley & Domesick, 1982), the hypothalamic-pituitary-adrenal axis (Jacobsen & Sapolsky, 1991) and the amygdala (Krettek & Price, 1977). In contrast, the dorsal HC (HCd) receives its main sources of input from the association cortex and entorhinal and perirhinal areas (Dolorfo & Amaral, 1998; Burwell & Amaral, 1998), structures with a well-established role in the processing of visuospatial information (Room & Groenewegen, 1986). Furthermore, the dorsal region of the HC does not have any direct reciprocal connections with the amygdala but only connects indirectly via the HCv (Pitkanen et al., 2000).

According to this anatomical organization, the HCd seems to be well situated to support spatial memory rather than emotional processing. In fact, deficits in the Morris water maze occur after excitotoxic lesions of the HCd but not the HCv (Bannerman et al., 1999; Moser & Moser, 1998). Although studies have shown that place cells exist in the HCv, the place cells found in the HCd have more well-defined place fields and occur in higher proportions (Poucet et al., 1994). By contrast, HCv lesions have been associated with behavioural abnormalities of fear and/or anxiety. For example, rats with selective neurotoxic lesions of the HCv, but not the HCd, show similarities to the anxiety reducing effects of benzodiazepines: HCv lesions reduce unconditioned defensive behaviours during exposure
to cat-odor as well as during conditioned defensive behaviours following administration of footshock (Pentkowski et al., 2006). In addition, rats with HCv lesions show reduced fear in the open field and the elevated plus-maze (Kjelstrup et al., 2002). The elevated plus-maze is used to assess innate fear by measuring the number of entries into the exposed arms of the maze. Rats with HCv lesions make more entries into open, exposed arms, indicating a higher expression of fearless behaviour (Kjelstrup et al., 2002). Nevertheless, it is also possible to interpret the deficit on the elevated plus-maze as related to a loss of inhibitory function. That is, the propensity of a rat to enter an exposed arm may be related to a failure to inhibit a behavioural response, rather than a failure to show the appropriate anxious response.

Recent studies have explored the role of the HC in tasks specifically targeting inhibitory control processes. For example, in the delay-discounting task rats learn to associate one lever with an immediate small reward and another lever with a large reward after a delay. Animals with HC lesions can learn to discriminate between the levers based on reward magnitude, but when the delay is introduced prior to receiving the large reward, rats with HC lesions display a stronger preference for the lever associated with the small immediate reward, relative to controls (Cheung & Cardinal, 2005). This behavioural evidence is corroborated by early studies using a differential reinforcement of low rate (DRL) schedule. In this task, rats are trained to respond by pressing a lever after a certain time interval has elapsed, in order to obtain a food reward. Responses made before the minimum period has elapsed are recorded as premature responses and cause the interval to reset (Clark, 1965). As in the delay-discounting task, animals with HC lesions are unable to inhibit their impulsive urge to respond as evidenced by an elevated level of responding during the interval (Clark & Isaacson, 1965; Jarrard, 1965; Sinden et al., 1986). One recent study utilized the T-maze apparatus to conduct a temporal discounting task. Instead of using levers as in the operant version of the task, animals learned to discriminate between two arms of a T-maze based on size of reward. The results from the study indicate that lesions
to both the HCd and the HCv lead to impulsive choice behaviour, i.e. both lesion groups preferred to enter the arm that resulted in delivery of the small immediate reward rather than enter the arm that led to the delivery of a large reward after a delay (McHugh et al., 2008).

In consideration of these studies, which implicate the HC in inhibitory control function, further investigation is necessary in order to specify the precise contributions of the HCd and HCv. Since many of the studies that provide the foundation for this view have utilized maze tasks and two lever operant tasks, they lack the ability to assess other functions that may contribute to inhibitory control deficits. For example, inhibitory control deficits might be due to impairments in visual attention or working memory, or increases in general activity. In fact, it is widely held that complex decision-making behaviours are supported by several cognitive functions, which require the animal to plan and organize behaviour to achieve optimal performance. The 5-Choice Reaction Time Task (5-CRTT) is a rodent test of visuospatial attention and inhibitory control. In this automated, operant task, rats are required to monitor five spatial locations and detect a brief (0.5 sec duration) stimulus light. In addition to accurate stimulus detection, impulsive premature and compulsive perseverative responses serve as measures of inhibitory control. Indices of response time and reward collection serve as important measures in evaluating motor behaviour, motivation and decisional processes. The 5-CRTT has been used extensively to assess the effects of lesions and pharmacological manipulations on cognitive function (for review see Robbins, 2002).

The present study set out to further examine the role of the HCv and HCd in inhibitory control and visuospatial attention by making excitotoxic lesions of each structure and evaluating the effects on performance in the 5-CRTT. First, rats with selective HCd or HCv lesions were tested on the standard schedule of the 5-CRTT to test their ability to detect the visual target that occurred randomly in one of five spatial locations. Second, several manipulations to the baseline schedule were instituted to evaluate rats’ performance under cognitively challenging conditions. Third, the rats were treated with systemic doses of
a dopamine reuptake inhibitor (GBR 12909) and a selective serotonin reuptake inhibitor (escitalopram), and tested on the baseline schedule of the task. Considerable evidence has accumulated which suggests that serotonin modulates behavioural inhibition, especially impulsive responses (Puumula & Sirviö, 1998; Evenden, 1999). In light of evidence that SSRIs may exert their effects, in part, by targeting different regions of the HC, we were interested in evaluating the modulatory effect of systemic escitalopram on inhibitory control in animals with selective HC lesions. Furthermore, dopamine serves a critical role in modulating inhibitory control processes in the 5-CRTT (Robbins, 2002), yet it is unknown whether the dopaminergic pathways in selective regions of the HC are involved in regulating these processes. Finally, because there is some indication that HC lesions can cause hyperactivity (Bast & Feldon, 2003), which could potentially influence accurate assessment of premature or perseverative responses, rats were examined on a test of spontaneous locomotor activity.

In light of the evidence implicating a role for the HC in behavioural inhibition, we hypothesized that lesions of the HCv, but not the HCd, would lead to increased premature responses in the 5-CRTT. Furthermore, since impulsive behaviour may interfere with attentional processing, we also predicted that animals with HCv lesions would be impaired in their ability to accurately detect the visual target. Finally, if a deficit arises from lesion of the HCv, it is anticipated that increasing monoamine transmission will have the effect of normalizing levels of premature responding.

**MATERIALS AND METHODS**

**Animals**

This study used male Long-Evans rats (Charles River, Wilmington MA, USA) that were 2 months old and weighed between 250-275 grams at the start of testing. Rats
were housed in pairs on a 12 h light/dark cycle with testing during the light phase. During the experiment rats were maintained on a restricted diet at 85% of their free-feeding weight but had access to water *ad libitum* in their home cages. All experimental procedures were approved by the McGill University Animal Use Committee.

**Apparatus**

The test apparatus consisted of four individually housed aluminum chambers (Lafayette Instruments, Lafayette IN, USA) located inside a cabinet, which was sound attenuating and ventilated by low-level noise fans. Each operant chamber measured 25cm x 25cm x 2.5cm (width x length x height). The rear wall of each chamber was concavely curved and contained nine apertures (2.5cm x 2.5cm). Holes 1,3,5,7,9 were exposed for the rat to nose poke. Holes 2, 4, 6, 8 were blocked with a metal cover (see Figure 1). Responses in the apertures were detected by photocells located at the entrance of each aperture. Illumination of the aperture was achieved through a standard 3-W bulb located at the rear of the hole. The food magazine was located on the opposite wall and was covered by a hinged door with a white light emitting diode. The chamber was illuminated by a house light located on the ceiling of the apparatus. The apparatus and on-line data collection was controlled by Whisker© Control software (Lafayette Instruments, Lafayette IN, USA).

**Behavioural Procedure**

*Habituation*

Rats were exposed to the test chamber for 30 min. The house light remained on for the entire session and food magazine contained 10-15 food pellets. Sticky tape was
used to hold open the panel door for the rats to get easy access to the pellets. In addition, 1-2 pellets were placed in each aperture to ensure the rats were able to make nose poke responses. If any pellets were left uneaten after the session, rats were given an additional day to habituate to the test chamber. The following day animals were habituated to the sound of the pellet dispenser. This was achieved by dispensing 15 pellets every 15-20 seconds. Rats were allowed a maximum of 20 min to consume the pellets. In addition, 1-2 pellets were placed in the apertures to continue shaping this behaviour.

**Behavioural Training**

Rats were trained to discriminate a light stimulus in one of the five apertures. A nose poke in the food magazine initiated the first trial, which consisted of a pre-stimulus interval (5 s) followed by the random illumination of one of the five apertures. Rats were given 5 s to make a response after the stimulus turned off (limited hold). If the rat made a nose poke response in the illuminated aperture either during stimulus presentation or within the limited hold period, a pellet was dispensed and a correct trial was recorded. A nose poke into an aperture that was not illuminated was recorded as an incorrect response. Failure to respond within the limited hold period was recorded as an omission. Incorrect and omitted responses were followed by a time-out period in darkness for 5 s. Each training session ran for either 100 trials or 30 minutes, whichever came first. For the first session of training, the stimulus duration and limited hold periods were both set at 60 s, and the pre-stimulus interval and time-out periods set at 5 s. The duration of the light stimulus and the limited hold were gradually reduced over sessions according to the animals performance until the rat was able to detect a stimulus duration of 0.5 s. Rats were required to satisfy a baseline criteria of 80% target accuracy with less than 20%
omissions within the 30 min time limit. Once rats attained this criterion, they were given ten preoperative baseline sessions. It took approximately eight weeks for the animals to attain criterion. Animals were then ready for surgery.

After surgery animals were given seven days to recover. Animals were then tested on 10 consecutive sessions to establish a post-operative baseline. After completion of baseline sessions the rats were given a series of task manipulations to challenge attentional and inhibitory requirements. First, to examine the effect of increasing the attentional demands of the task, animals were exposed to one session of reduced stimulus duration (0.25s). Following this, rats were evaluated on one session with variable short pre-stimulus intervals (0.5, 1.5, 3.0, 4.5s) and one session of variable long pre-stimulus intervals (4.5, 6.0, 7.5, 9.0s) presented randomly an equal number of times over the session. For the long pre-stimulus interval session the time limit was increased to 45 minutes. Each of these manipulation sessions was preceded by at least one session in which the standard parameters were restored to ensure the animals returned to baseline performance.

**Drug Administration and Procedure**

Following manipulation of the stimulus parameters, baseline performance was re-established. The effects of GBR 12909 (Sigma-Aldrich, Canada), a dopamine reuptake inhibitor and escitalopram oxalate (NIMH Chemical Synthesis and Drug Supply Program), a selective serotonin reuptake inhibitor, were evaluated using standard baseline parameters. GBR 12909 was dissolved in sterile water and escitalopram was dissolved in 0.9% saline and administered 20 min prior to the start of the test session. First animals
received GBR 12909 (0, 2.5, 5, 10 mg/kg, i.p.) and then escitalopram (0, 2.5, 5, 10 mg/kg, i.p.) according to a Latin Square design. Rats were given a week off and then put back on baseline conditions between the GBR 12909 and the escitalopram drug conditions. Doses of GBR 12909 and escitalopram were selected from previous studies in the literature (van Gaalen et al., 2006; Mansari et al., 2005).

Performance Measures

The following measures were recorded and calculated:

• Accuracy: proportion of correct responses to total correct + incorrect responses, expressed as a percentage.
• Omissions: proportion of omission trials to total trials (correct + incorrect + omission trials), expressed as a percentage.
• Premature responses: number of responses made in the apertures during the pre-stimulus interval.
• Perseverative responses: number of responses in the apertures following a correct response.
• Correct Latency: time between stimulus onset and a nose poke in the correct hole.
• Magazine Latency: time between a nose poke in the correct hole and opening of the magazine panel to collect the food pellet.

Locomotor Activity

Animals were assessed in locomotor activity cages (Dimensions L x W x H: 43 x 23 x 28cm). Infrared photocell beams detected and recorded the rats’ activity in the cage
using specialized MotorMonitor software (Lafayette Instruments, Lafayette IN, USA).
Counts were recorded over a 2 h period, and were expressed as the total number of beams
crossed by the animal. The total number of beam breaks was recorded in 15 min time
intervals.

Data Analysis

Data from all measures obtained were analyzed using the SPSS statistical
package, version 16.0 (SPSS Inc., Chicago IL, USA). Data were explored using tests of
homogeneity of variance to identify data that did not satisfy the distribution requirement
of the ANOVA and were transformed appropriately (arcsine, square root, or logarithmic).
Homogeneity of variance across groups was assessed by Mauchly’s Test of Sphericity. If
significance was obtained using Mauchly’s Test, indicating a violation of the sphericity
required by the two-way repeated measures ANOVA, the Huynh-Feldt epsilon was used
to calculate the more conservative $P$-value for each $F$-ratio. The between subject factor in
the repeated measures ANOVA was group (3 levels: HCv, HCd or sham). The within-
subject factor was session (10 days), variable pre-stimulus interval (4 intervals), dose (4
doses) and locomotor activity time interval (15 min, 8 intervals). The criterion for
significance was set at the $p < 0.05$ level. Post-hoc tests (Tukey’s test) were performed to
evaluate significant within- and between-variable differences.

Surgical Procedures

Forty-three rats were used in this study. Five rats did not reach performance criteria
and were removed. The remaining 38 rats were assigned to one of three groups. Sixteen
animals received bilateral lesions of the HCd, 14 animals received bilateral lesions of the HCv, and 8 rats received the same surgical procedure but were infused with saline at the same coordinates (counterbalanced for lesion type) and therefore served as sham controls. Nine animals died during surgery either due to severe seizures following surgery or due to complications with anesthesia, 4 animals from the HCv group and 5 animals from the HCd group. All rats were anaesthetized with isoflurane gas with oxygen as the carrier gas and placed in a head holder fitted with atraumatic ear bars in a stereotaxic frame (David Kopf Instruments, Tujanga, CA, USA). The incisor bar was set at -3.0mm. An incision was made along the midline to expose the skull. A drill was used to remove the portion of bone covering the injection sites. All dorso-ventral coordinates were taken from dura. Excitotoxic lesions were made by injection of 0.09M N-methyl-D-aspartic acid (NMDA; Sigma-Aldrich, Canada) dissolved in 0.9% saline, at the coordinates specified in Table 1. Injections of NMDA (0.4-0.5uL) were made over 2 min using an SGE microsyringe mounted on a stereotaxic frame (Canadian Life Science, Peterborough ON, Canada). The syringe was left in place for 1 min after each injection to allow diffusion of the excitotoxin. Rats receiving sham surgery were injected with saline at the same coordinates. Following surgery, animals were administered Rimadyl (analgesic) at a dose of 5mg/kg and Tribrissen (antibiotic) at a dose of 0.125ml/kg. Transient seizures were observed following surgery. To reduce seizure activity rats were treated with midazolam at a dose of 0.5mg/kg.

Histology

At the completion of behavioural assessment, rats were injected with a terminal
dose of sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% formal saline. Brains were removed and fixed in formal saline solution for at least 24 h. Brains were then dehydrated in a solution of 20% formal sucrose 48 h prior to freezing and 40-µm sections were sliced with a freezing cryostat. Every other section was mounted on glass slides and stained with thionin.

**RESULTS**

**Histological Analysis**

The cytoarchitecture was taken from the rat brain atlas by Paxinos and Watson (2005). In the HCd group, four animals were removed from analysis due to extensive damage to the cortical areas and/or incomplete damage to the HCd region. The remaining seven animals in the HCd group showed extensive cell loss in the dorsal portion of the HC, which included the dentate gyrus and the CA1-CA3 subfields. The lesion started at AP -1.8mm and extended posterior to AP -4.92mm from bregma. There was minimal damage to the fornix. A few animals showed cell damage that encroached on cortical regions including the visual and motor areas, however accuracy and latency scores were reviewed to ensure there was no resulting deficit. HCd lesion reconstructions are represented in Figure 2.

In the HCV group, one animal had extensive damage, which extended into the subthalamic nucleus region. This animal was removed from analysis. Another animal from the HCV group was removed due to an incomplete HCV lesion. The remaining eight animals in the HCV group showed extensive neuronal loss in the HCV region. The lesion
started at AP -4.20mm and extended posterior to approximately AP -6.00mm. The lesions included the CA1-CA3 regions of the HC, the dentate gyrus and the ventral subiculum with minor encroachment on the most lateral portion of the dorsal sector and minimal, if any, damage to extrahippocampal structures. H Cv lesion reconstructions are represented in Figure 3. Thus, the total numbers comprising each group were HCd n=7, H Cv n=8, Sham n=8.

Preoperative Performance

Prior to surgery, the groups were matched on all behavioural measures for the last ten sessions. No significant differences were observed for target accuracy \([F(2,20) = 1.6, p > .05]\) or premature responses \([F(2,20) = 1.2, p > .05]\). There were no differences in the number of omissions, perseverative responding, speed of responding or latency to collect reward \([all \ p > .05]\).

Postoperative Performance

Overall, there were no significant group differences in terms of target accuracy \([F(2,20) = 1.564, p > .05]\). However the H Cv group performed worse relative to the sham and the HCd group \([Sham: 66.6\%±1.4; HCd: 71.5\%±1.1; H Cv: 60.8\%±1.67]\). All groups improved in target accuracy across baseline sessions \([F(9,180) = 11.23, p < .001]\). The number of omissions did not differ between groups \([F(2,20) = .70, p > .05]\). The number of omissions declined over baseline sessions \([F(9,180) = 5.72, p < .001]\). The H Cv lesioned animals exhibited high levels of premature responses over the ten post-operative sessions relative to sham controls and the HCd group. This impairment was revealed by a
group by session interaction \( [F(18,180) = 2.96, p < .01] \). This effect can be attributed to the number of premature responses made by the HCv group relative to the sham group for session three \( [F(2,20) = 4.047, p < .05; \text{Tukey’s } p < .05] \), and session four \( [F(2,20) = 4.979, p < .05; \text{Tukey’s } p < .05] \) as shown in Figure 4C. The number of perseverative responses did not differ between groups across the baseline sessions \( [F(2,20) = .453, p > .05] \). There were no differences on measures of speed of responding \( [F(2,20) = 1.43 p > .05] \), or latency to collect reward \( [F(2,20) = 1.65, p > .05] \).

**Variable Long Pre-Stimulus Intervals**

Increasing the pre-stimulus interval caused a reduction in accuracy for both lesion groups, but an improvement in target accuracy for the sham group. There was a significant group by pre-stimulus interval interaction \( [F(3,60) = 3.8, p < .01] \). This can be attributed to the reduced accuracy of the HCv group relative to the sham group at the longest pre-stimulus interval \( [F(2,20) = 4.075 p = .033; \text{Tukey’s } p < .05] \). Specifically, the sham group improved at the longest pre-stimulus interval while the HCv group was impaired (see Figure 5A). The number of omissions decreased with duration of pre-stimulus interval for all groups \( [F(3,60) = 48.33, p < .001] \). The number of premature responses increased as the duration of the pre-stimulus interval increased \( [F(3,60) = 302.16, p < .001] \). In addition, there was a trend toward a difference in the number of premature responses of the HCv group relative to the sham group \( [F(2,20) = 2.69, p = .09] \) (see Figure 5B). Likewise, the number of perseverative responses increased as duration of pre-stimulus interval increased \( [F(3,60) = 19.930, p < .001] \). There was also a significant group effect of perseverative responses \( [F(2,20) = 3.561, p < .05] \); further
analysis revealed that this was due to higher levels of perseverative responses of the H Cv group relative to the HCd group at the first three pre-stimulus intervals [Tukey’s $p = .038$] (see Figure 5C). There were no significant differences in speed of responding or latency to collect reward.

**Variable Short Pre-Stimulus Intervals**

Reducing the pre-stimulus interval resulted in an overall decline in accuracy across groups [$F(3,60) = 34.861, p < .001$]. There was a trend towards a group effect [$F=3.185, p = .063$], which was due to lower accuracy scores of the H Cv group relative to the HCd group [Tukey’s $p = .05$] (see Figure 6A). There was an overall effect of pre-stimulus interval on omissions [$F(3,60) = 141.85, p < .001$]; the number of omissions increased as the pre-stimulus interval decreased. The number of premature responses declined as the pre-stimulus interval increased [$F(3,60) = 20.33, p < .001$]. There was a significant group difference in the number of perseverative responses that was independent of pre-stimulus interval [$F(2,20) = 3.54, p < .05$]. This was due to higher levels of perseverative responses of the H Cv group relative to the HCd group for the first three pre-stimulus intervals [Tukey’s $p = .038$] (see Figure 6B). There was an overall decrease in latency to respond as pre-stimulus interval increased [$F(3,60) = 50.52, p < .001$], but there was no change in latency to collect reward.

**Reduced Stimulus Duration**

Reducing the stimulus duration to 0.25 sec resulted in an overall reduction in target accuracy ($F(3,60) = 26.880, p < .001$) but no group differences were observed.
There was no change in the number of omissions overall nor were there any group
differences in number of omissions. The HCv group continued to display high levels of
premature responding during the reduced stimulus duration condition as shown in Figure
7. There was a trend toward a group difference between the HCv group and the HCd
group \( F(2,20) = 2.73, p = .089, \) Tukey’s \( p = .09 \). There were no changes in perseverative
responding, speed of responding or latency to collect reward.

**Effects of Systemic Escitalopram**

Escitalopram did not produce any effects on target accuracy, however, it
significantly increased the number of omissions for all groups \( F(3,60) = 2.84, p < .05 \).
This effect occurred at the high 10mg/kg dose relative to vehicle \( F(2,20) = 5, p < .05 \).
Escitalopram significantly reduced the overall number of premature responses for all
animals \( F(3,60) = 5.42, p = .012 \). Further analysis revealed that there were also
significant effects that were group dependent. That is, the number of premature responses
was reduced for the HCd group at the 10mg/kg dose compared to vehicle \( F(1,7) = 7, p <
.05 \) and for the HCv group at the 5mg/kg dose relative to vehicle \( F(1,7) = 7.126, p <
.05 \) (see Figure 8). Escitalopram did not significantly affect levels of perseverative
responding. Latency to collect reward increased with drug for all groups \( F(3,60) = 9.87,
p < .001 \). Further analysis revealed that this effect occurred at all doses relative to
vehicle \( all \ p < .05 \). There were no significant changes in speed of responding.

**Effects of Systemic GBR 12909**
GBR 12909 did not affect target accuracy or the number of omissions, however, it significantly increased the overall number of premature responses for all groups \([F(3,60) = 3.58, p < .05]\). There was also a significant dose by group interaction \([F(6,60) = 2.67, p < .05]\). Post-hoc analysis revealed that the HCv group made significantly more premature responses at the 10mg/kg dose relative to the HCd group \([F(2,20) = 5.32, p < .05;\) Tukey’s \(p = .01\)] (see Figure 9A). There were no significant dose effects on perseverative responses. There was a significant group effect across doses \([F(2,20) = 6, p = .009]\); the HCv group had significantly higher levels of perseverative responding relative to both shams and the HCd group \([Tukey’s \(p = .028\) and \(p = .014\)], respectively (see Figure 9B). There were no changes in speed of responding or latency to collect reward.

**Locomotor Activity**

Animals with lesions to the ventral and dorsal hippocampus were not significantly different in terms of spontaneous locomotor activity compared to sham controls \([F(2,20) = 2.33; p > .05]\).

**DISCUSSION**

**Effects of HCv lesions on the 5-CSRTT**

Lesion to the dorsal region of the HC did not produce a significant increase in impulsive behaviour on the 5-CSRTT. By contrast, animals in the HCv lesion group displayed an elevated level of premature responses under baseline conditions. In terms of accuracy effects, the HCv group displayed lower accuracy scores relative to sham controls and the HCd group; this effect was moderate and did not reach statistical significance under
baseline conditions. This is in opposition to the predicted outcome that HCv lesions would produce an accuracy impairment under standard task parameters. Nevertheless, the HCv group displayed reduced target accuracy at the longest interval of the variable long pre-stimulus interval session, whereas the sham group improved in accuracy at this same interval. Furthermore, there were differences in levels of perseverative behaviour between the HCd and HCv group and a trend toward significant differences between the HCv and sham groups. Thus, animals with HCv lesions are particularly vulnerable to circumstances in which the stimulus becomes long and unpredictable. Under these conditions, this deficit may arise either from an inability to schedule behaviour under varying time constraints, or because the interfering effect of premature responses on target accuracy becomes more pronounced. It is unlikely that the HCv plays a substantial role in attentional processes, although it is possible that the high levels of premature and perseverative responses may interfere with optimal task performance. Moreover, the high levels of perseverative responses may indicate a generalized disinhibitory effect of the HCv lesion, yet further investigation is required in order to support this assertion.

The evidence obtained from this study, that the HC is involved in impulsive behavior, is consistent with previous studies. Specifically, animals with HC lesions are more likely to choose a small immediate reward over a larger reward following a delay period in an operant temporal discounting task (Cheung & Cardinal, 2002) and in a T-maze version of the temporal discounting task (McHugh et al., 2008). Furthermore, in a DRL task, animals with HC lesions exhibit an increased number of “premature” responses before the completion of the specified time interval leading to lower levels of reinforcement (Sinden et al., 1986).

There are a number of important points to consider in the interpretation of these results. First, since HC lesions have been associated with hyperactivity under certain conditions (Richmond et al., 1999), it is important to rule out increased motor activity as a possible cause for the observed increase in premature responding. In our study, no differences in motor behaviour were observed as assessed by speed of responding and
latency to collect reward. Furthermore, spontaneous locomotor activity was assessed and there were no significant differences in levels of activity. Another possibility is that changes in motivation might cause alterations in the pattern of response behaviour. Nevertheless, this is an improbable explanation for elevated premature responses given that there were no observed changes to indices of motivation, i.e. latency to collect reward or number of omissions. Therefore, it is unlikely that any changes in motor behaviour or motivation were responsible for the deficits observed.

Although it is possible that the effect of the H Cv lesion on its own is enough to cause impulsive deficits, a possible explanation can be derived from the anatomical organization of the H Cv. The H Cv is well connected with areas that have been associated with the regulation of impulsive behaviour such as the nucleus accumbens core (Brog et al., 1993). In fact, lesions of the nucleus accumbens core cause both impulsive choice for small immediate rewards (Cardinal et al., 2005), and an increase in premature responses in the 5-CRTT when the pre-stimulus interval is made unpredictable (Christakou et al., 2004). Importantly, the H Cv projects to the core region of the nucleus accumbens (Brog et al., 1993), albeit less densely than its projections to the shell, which is not implicated in impulsive choice behaviour (Cardinal et al., 2001). Another possibility may be that output from H Cv to the vmPFC serves as an important circuit for the control of impulsive behaviour. Indeed, a dense direct projection from the H Cv to the vmPFC has been well characterized (Jay & Witter, 1991; Hoover & Vertes, 2008). In addition, there is evidence that the vmPFC contributes to the regulation of impulsive behaviour. Specifically, in the 5-CRTT, rats with lesions targeting the infralimbic region of the vmPFC, also display elevated levels of premature responding in a similar manner to that of H Cv lesions (Chudasama et al., 2003). In addition, rats with prelimbic lesions of the vmPFC lesions exhibit executive dysfunction, that is they display deficits in attentional performance and they are disinhibited (Pasetti et al., 2002).
**HCd lesions do not disrupt performance in the 5-CRTT**

In our study, we have shown that there are no significant changes in task performance following HCd lesion. Thus, our findings are in line with the present hypothesis that the HCd does not lead to changes in impulsive behaviour or attentional processing in the 5-CRTT. Previous investigation of the involvement of the HCd in impulsive behaviour has been limited. Nevertheless, there have been indications that the HCd is involved in regulating impulsive behaviour. In one previous study conducted in a T-maze, lesions to the HCd have been shown to result in impulsive choice or an increased preference for an immediate small reward over a large reward after a delay (McHugh et al., 2008). The HCd plays a critical role in spatial memory (Moser & Moser, 1998; Bannerman et al., 1999) and although McHugh and colleagues (2008) provided support for the role of the HCd in impulsive choice, it is also necessary to consider that this task was performed in a maze, as such, the spatial demands of the maze environment may recruit the dorsal regions of the HC.

Another possibility is that the HCd may be critical for some forms of impulsivity while not contributing to others. In support of this, previous authors have argued that impulsivity is not a unitary construct (Evenden, 1999; Winstanley et al., 2006). For example, impulsive behaviour in the 5-CRTT has been labeled an impulsive action (an inability to withhold from making a motoric response) (Aron et al., 2003), whereas in temporal discounting tasks, preference for an immediate small reward over a larger delayed reward is labeled impulsive choice (Cardinal et al., 2001). Further study is necessary to delineate the precise neural correlates of these forms of impulsivity. To this end, it would be interesting to determine whether the HCd is involved in impulsive choice in an environment with limited spatial demands.

**Escitalopram Reduces Premature Responding**

Administration of the SSRI escitalopram had the effect of reducing the number of premature responses in all groups. The HCv and HCd showed a larger reduction in
premature responses relative to the sham group. This drug effect was independent of changes to accurate stimulus detection. In addition to a reduction in premature responses, escitalopram increased the overall number of omissions as well as the latency to collect reward. Based on evidence supporting the relationship between serotonin and appetite, it is likely that the appetite suppressant effects of SSRIs can account for these motivational changes (for review see Curzon, 1990). In terms of premature responding, previous studies have established that global decreases in serotonergic transmission increase impulsive action in rodents (Harrison et al., 1997). In addition, there is evidence that indicates that escitalopram can improve cognitive functioning in patients with major depression (Wroolie et al., 2006). The mechanism by which escitalopram may modulate cognitive function is unknown but could be related to its effect on the PFC. In fact, escitalopram has been shown to facilitate NMDA-induced currents in pyramidal cells of the medial PFC, as well as glutamate-driven burst firing in mesolimbocortical dopamine neurons (Jardemark et al., 2003). Here, we have shown that escitalopram can normalize the decline in cognitive function at a locus outside of the HC system. Nevertheless, more investigation is required to elucidate the mechanisms for this effect.

**GBR 12909 Increases Premature Responding**

Systemic GBR 12909 increased premature responses for all rats. There is some support for the observation that increased dopamine transmission increases impulsive behaviour in the 5-CRTT. For example, it has been shown that increased dopamine transmission by systemic amphetamine administration robustly increases premature responding (Pattij et al., 2007). Although the pharmacological mechanisms by which these drugs exert their effects are not identical, both drugs interact with the dopamine transporter. Further investigation is necessary to pinpoint the specific effect of GBR 12909 on inhibitory control. Interestingly, both GBR 12909 and amphetamine decrease impulsive decision-making in a delayed reward task (van Gaalen et al., 2006), offering further support
for distinct forms of impulsivity.

Limitations

It should be noted that while our results provide evidence that the H Cv is involved in the control of impulsive behaviour in the rat, it is difficult to determine the extent of its role, given that the group effect was observed on only two sessions during baseline conditions. In addition, manipulation sessions showed trends toward a group effect but there was considerable variance in the data, which is, in part, attributable to the small group sizes in this study. Thus, further exploration of the relationship between the H Cv and impulse control is warranted.

Future Directions and Clinical Implications

Future research should aim to explore the interaction of the H Cv with the vmPFC. A disconnection study would provide a means by which the circuit between the two structures could be destroyed while leaving intact the unilateral structures on opposite hemispheres. A similar behavioural deficit following H Cv-vmPFC disconnection would provide additional evidence for the involvement of these hippocampal-prefrontal circuits in impulse control. Furthermore, it would also be useful to test the effect of damage to the H Cv and the H Cd on a temporal discounting paradigm in a test environment with a minimal spatial bias. This would allow further examination of these structures in other aspects of impulsivity, which may elucidate the neural mechanisms by which the H Cv contributes to inhibitory control processes.

At the clinical level, this study has particular relevance to research into emerging views of the brain pathology responsible for the symptoms associated with schizophrenia. It has long been known that hippocampal and prefrontal pathology are central to a range of neuropsychiatric disorders. In recent years, HC-PFC circuits have been implicated in schizophrenia. Weinberger and colleagues (1992) have shown that monozygotic twins
discordant for schizophrenia have reduced brain area in the anterior hippocampal formation; they show that the more an affected twin differed from the unaffected twin in left hippocampal volume, the more they differ in prefrontal physiological activation during the Wisconsin Card Sort Task. The authors conclude that their findings are consistent with the view that schizophrenic pathology involves a broad neocortical-limbic neural network that is involved in working memory and other key cognitive tasks (Weinberger et al., 1992).

Furthermore, schizophrenia has been related to dysfunction of dopamine transmission. It is now believed that dysfunction results from abnormalities in the dopamine system at the level of the HC, PFC and other relevant brain areas (Goldman-Rakic, 1994). A more complete understanding of these neural circuits and their relationship with dopamine neurons will create opportunities for the development of novel therapeutic interventions targeting these specific regions.

**Summary and Conclusions**

In the present study, rats with bilateral excitotoxic lesions of either the HCd or HCv were compared with rats that received sham surgery on their performance in the 5-CRTT. The results indicate that HCv lesions produce premature ‘impulsive’ responses under baseline conditions. By comparison, rats with HCd lesions did not show behavioural deficits in any aspect of performance under standard task parameters or in the manipulation sessions relative to sham controls or the HCv group. Collectively, these results support the dissociation of function along the dorsoventral axis while also indicating a role of the HCv in impulsive behaviour.
REFERENCES


TABLE AND FIGURE CAPTIONS

Table 1. Stereotaxic coordinates and injection volumes for HCd and H Cv lesions. AP, anterior-posterior; ML, medial-lateral; DV, dorsoventral.

Figure 1. Schematic illustration of the experimental chamber of the 5-CRTT in cross section (top) (adapted from original illustration by John Romford, Department of Zoology, Cambridge University, UK). The standard procedure during one trial in the 5-CRTT (bottom).

Figure 2. Diagrammatic reconstructions of coronal sections of the H Cd lesion adapted from Paxinos and Watson (2005). The grey and black shaded regions represent the largest and smallest extent of the lesion, respectively.

Figure 3. Diagrammatic reconstructions of coronal sections of the H Cv lesion adapted from Paxinos and Watson (2005). The grey and black shaded regions represent the largest and smallest extent of the lesion, respectively.

Figure 4. Mean (±S.E.M.) performance of Sham controls (diamond), H Cd group (square) and H Cv group (triangle) on 10 days of post-operative baseline on the 5-CRTT: (A) percent accuracy, (B) percent omissions, (C) premature responses, (D) perseverative responses. (*) Significantly different from sham controls.

Figure 5. Effects of variable long pre-stimulus interval on 5-CRTT performance of Sham, H Cd and H Cv group. Each bar represents mean ± SEM: (A) percent accuracy, (B) premature responses, (C) perseverative responses. (*) Significantly different from sham controls. (†) Significantly different from H Cd group.

Figure 6. Effects of variable short pre-stimulus interval on task performance of Sham, H Cd and H Cv group. Each bar represents mean ± SEM: (A) percent accuracy, (B) perseverative responses. (†) Significantly different from H Cd group.

Figure 7. Effects of reduced stimulus duration on premature responses of Sham, H Cd and H Cv group. Each bar represents mean ± SEM.

Figure 8. Effects of systemic administration of escitalopram on premature responses of Sham, H Cd and H Cv group. Each bar represents mean ± SEM. (‡) Significantly different from vehicle.

Figure 9. Effects of systemic administration of GBR 12909 on premature responses and perseverative responses of Sham, H Cd and H Cv group. Each bar represents mean ± SEM: (A) premature responses, (B) perseverative responses. (†) Significantly different from H Cd group.
TABLES AND FIGURES

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