Abstract

The neonatal ventral hippocampal lesion (NVHL) is the most well-characterized neurodevelopmental animal model of schizophrenia. NVHL animals are known to display marked deficits in cognitive flexibility and working memory (WM), which are largely reminiscent of cognitive deficits seen in human patients. Though WM deficits are a well-characterized feature of the NVHL model, our study was the first to use magnetic resonance imaging (MRI), on live rats, to determine the relationship between lesion extent and the working memory deficit in a variable delayed non-match to sample (vDNMS) task. Similar to the existing literature, NVHL animals showed a significant deficit in performance when compared to sham operated animals. Interestingly, however, the magnitude of deficit in WM performance in NVHL animals was stable, regardless of delay length. We suggest that this delay-independent WM deficit reflects inefficiency during the encoding stage of WM processes in NVHL animals. Additionally, we found no evidence of a relationship between ventral hippocampal (VH) lesion volume and the magnitude of WM deficit, suggesting that there may be a threshold level of VH damage, beyond which no further WM impairment is produced.
Preface

The experiments presented in this dissertation were designed together by my supervisor, Dr. Jeremy Seamans, my colleague, Dr. James Hyman, and myself. I conducted all aspects of the experiment, such as animal surgeries and behavioural testing, and performed all data analyses, under the guidance of Drs. Seamans and Hyman.

All experiments described here were conducted in accordance with the Canadian Council of Animal Care, and with the approval of the Animal Care Committee at the University of British Columbia. The animal protocol numbers, under which all experiments were conducted, was A10-0055 and A14-0084.
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<tr>
<td>AH</td>
<td>Anterior hippocampus</td>
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<tr>
<td>aCSF</td>
<td>Artificial cerebrospinal fluid</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
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<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
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<td>DMPFC</td>
<td>Dorsomedial prefrontal cortex</td>
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<tr>
<td>GAD 67</td>
<td>Glutamic acid decarboxylase 67</td>
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<tr>
<td>IBO</td>
<td>Ibotenic acid</td>
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<tr>
<td>LFC</td>
<td>Lateral frontal cortex</td>
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<tr>
<td>mPFC</td>
<td>Medial prefrontal cortex</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NAA</td>
<td>N-Acetylaspartate</td>
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<td>NVHL</td>
<td>Neonatal ventral hippocampal lesion</td>
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<td>PD</td>
<td>Postnatal day</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<tr>
<td>RARE</td>
<td>Rapid acquisition with rapid enhancement</td>
</tr>
<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
</tr>
<tr>
<td>vDB</td>
<td>Vertical limb of the diagonal band</td>
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<tr>
<td>vDNMS</td>
<td>Variable delayed non-match to sample</td>
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<tr>
<td>VH</td>
<td>Ventral hippocampus</td>
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<tr>
<td>WCST</td>
<td>Wisconsin card-sorting task</td>
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<td>WM</td>
<td>Working memory</td>
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Dedication

For my mother and father. May you have the strength to stay awake as you read this dissertation in its entirety.
Chapter 1: General introduction

Characterized by significant disturbances of mental functions and subtle brain abnormalities, schizophrenia is a complex psychiatric disorder that affects roughly 1% of the global population (Tseng, Chambers, and Lipska, 2009). Despite relatively low incidence rates of schizophrenia (median value 15.2 per 100,000 persons per year; McGrath et al., 2004), the disorder is a major contributor to the global burden of disease (Murray and Lopez, 1996). Psychosis is typically the most salient clinical feature of schizophrenia, however, deficits in such cognitive processes as working memory, sustained attention, and cognitive flexibility are thought to be the core features of schizophrenia (Elvevag & Goldberg, 2000). These cognitive deficits are known to manifest years before the onset of psychosis, to persist throughout the course of the illness, and are considered to be the best predictors of long-term functional outcome in patients (Gold, 2004). Yet, despite the clinical significance of cognitive symptoms in schizophrenia, less research has been conducted on this cluster of symptoms when compared to the negative and positive symptoms. However, the prefrontal cortex (PFC) and hippocampus are two brain regions that have been consistently implicated in contributing to symptom severity in schizophrenia patients.

Evidence of compromised PFC function in schizophrenia, includes findings from studies of cognitive and neuropsychological function (Barch and Carter, 1998; Gold et al., 1997; Goldberg et al., 1987, 1988; Goldberg and Weinberger, 1988; Keefe et al., 1995; Mahurin et al., 1998; Park and Holzman 1992; Stone et al., 1998; Weickert et al., 2000; Wexler et al., 1998), neuroimaging (Andreasen et al., 1996, 1997; Berman et al., 1992; Callicott et al., 1998, 2000; Carter et al., 1998; Catafu et al., 1994; Curtis et al., 1998; Ingvar and Franzen, 1974; Kawasaki et al., 1993; Manoach et al., 1999, 2000; Stevens et al., 1998;
Volz et al., 1997; Weinberger et al., 1986, 1988, 1992), eye movements (Cegalis and Sweeney 1979; Holzman et al., 1974; Jacobsen et al., 1996; Lieberman et al., 1992; Litman et al., 1997; Shagass et al., 1974), and electrophysiology (Abrams and Taylor, 1979; Guenther et al., 1988; Hoffmann et al., 1996; Karson et al., 1987; Tauscher et al., 1998). Indeed, it is widely held that compromised PFC neural systems may provide some explanation of the salient prefrontal-dependent cognitive symptoms, such as working memory (WM) deficits, displayed by those with schizophrenia.

As with the PFC, there is a growing body of literature implicating the hippocampus in the pathophysiology of schizophrenia. The most significant evidence comes from MRI studies, which show a significant reduction in hippocampal volume of schizophrenia patients when compared to healthy controls (Lawrie and Abukmeil, 1998; Wright et al., 2000; Davatzikos et al., 2005; Narr et al., 2004; Shenton et al., 2001). Specifically, the anterior hippocampus of schizophrenia patients has shown pronounced differences in volume and shape, with such differences seen less frequently in the posterior hippocampus (Csernansky et al., 2002; Narr et al., 2002; Ho and Magnotta, 2010). Interestingly, reduced hippocampal volumes have also been observed in prodromal and first episode patients (Bogerts et al., 1990; Lawrie et al., 1999; Joyal et al., 2002; Pantelis et al., 2003), which suggests that hippocampal involvement in schizophrenia is not secondary to the illness or its treatment, but may be developmentally linked to schizophrenia pathogenesis.

Despite such a well-characterized knowledge of the neuroanatomical and physiological traits associated with schizophrenia, little is known about the mechanisms underlying the pathophysiology of the disorder. Therefore, animal models of disease are critically important for investigating the mechanisms underlying human diseases and for the
design of new therapies. Until the early 1990’s, animal models of schizophrenia were primarily pharmacological constructs and typically involved direct manipulation of glutamate or dopamine neurotransmitter systems (Tseng, Chambers, and Lipska, 2009). Unfortunately, these early models overlooked key features of schizophrenia, such as the developmental pathogenesis, the cognitive symptoms, and potential anatomical features—a major heuristic limitation (Tseng, Chambers, and Lipska, 2009). However, a relatively recent model known as the neonatal ventral hippocampal lesion (NVHL) model of schizophrenia has proven capable of capturing many important aspects of the disorder that may not be adequately addressed by pharmacological and even genetic models.

Below I will provide an overview of the literature concerning the cognitive symptoms of schizophrenia. Next, both the PFC and hippocampus will be discussed in terms of their involvement in schizophrenia, particularly the role that each region is thought to play in contributing to the severity of cognitive symptoms of the disorder. Finally, the NVHL model will be discussed in detail. This chapter will conclude with an outline of the overall objectives of this thesis.

1.1 Cognitive deficits in schizophrenia

1.1.1 General overview of cognitive deficits

Schizophrenia is a complex brain disorder characterized by clinical heterogeneity of symptom presentation including deficits in cognitive abilities, such as WM, sustained attention, and other executive functions (Lee and Park, 2005). Although cognitive dysfunction in schizophrenia may sometimes be overlooked in light of the more salient positive and negative symptoms, cognitive dysfunction is now being seen as a core feature of
the disease and major predictor of functional outcome (Green et al., 2000). Indeed, there exists a large volume of empirical evidence in support of the notion of cognitive dysfunction as a core feature of schizophrenia. For example, several studies have shown that patients will present symptoms indicative of cognitive deficits, such as problems with working memory, sustained attention and declining IQ, well-before the clinical onset of the illness (e.g., Aylward, Walker, and Bettes, 1984; Kremen et al., 1998). Similarly, impaired cognitive functioning is also evident in the non-affected biological relatives of patients, although to a slightly milder degree (Cannon et al., 1994; Goldberg et al., 1993). Finally, despite changes in the magnitude of cognitive dysfunction with changes in clinical state, cognitive dysfunction is still known to be largely persistent even during times of positive and negative symptom remission (Gold and Harvey, 1993). Thus deficits in cognitive performance represent a stable, central core feature of schizophrenia.

1.1.2 Developmental course of cognitive deficits in patients with schizophrenia

Cognitive impairments are commonly reported in first-episode patients with schizophrenia (Bilder et al., 1992; Hoff et al., 1992; Censits et al., 1997) and these impairments persist into the chronic stages of the disease (Goldberg and Seidman, 1991; Heaton et al., 1994). In fact, cognitive impairment often significantly predates the onset of clinical diagnosis. For example, children who later went on to develop schizophrenia were found to have on average lower scores on scholastic tests than comparison children at ages 8, 11, and 15 years old (Jones et al., 1994). Further, the cognitive impairment noted in these children appeared to worsen with time, as the most robust findings were seen when children were measured at 15 years of age (Jones et al., 1994). Similarly, Ambelas (1992) discovered
that children who later developed schizophrenia had lower IQ scores and impaired speech, language, and reading performance, when compared to similarly aged children who did not develop schizophrenia. Interestingly, in a large scale study of healthy male adolescents, a linear relationship was found between cognitive functioning and later risk of developing schizophrenia (David et al., 1997; Davidson et al., 1999). Thus, premorbid cognitive impairment is a well-documented feature of schizophrenia (Fuller et al., 2002).

The early emergence of cognitive impairment and its robust relationship to the risk of later developing schizophrenia implicates the involvement of aberrant neurodevelopmental processes, in what is known as the neurodevelopmental theory of schizophrenia. This theory suggests that both genetic and environmental factors confer vulnerability to developing schizophrenia, and occur well before the clinical manifestation of schizophrenia. These factors may alter the normal course of neural development and produce changes in specific types of neurons and circuits (Murray and Lewis, 1987, Weinberger, 1987). According to this theory, abnormal genetic and/or epigenetic factors work synergistically to disrupt processes of early brain development, such as cellular proliferation, differentiation, migration, and synaptogenesis (Murray and Lewis, 1987; Weinberger, 1987; Ho, Andreasen, Nopoulos, et al., 2003). Processes occurring later in brain development, such as synaptic pruning and apoptosis, as well as other neuropathological events, such as stress, are then thought to interact with the abnormally developed brain to produce the characteristic attributes of schizophrenia in late adolescence to early adulthood (Ho, Andreasen, Nopoulos, et al., 2003).

Imaging studies have implicated developmental abnormalities in regions essential to cognitive functioning, such as the hippocampus (Narr et al., 2002; Narr et al., 2004) and PFC (Catafau et al., 1994; Weinberger and Lipska, 1995). Abnormalities in these regions have
been shown to manifest in a variety of different ways, such as through altered cortical thickness (Kuperberg et al., 2003), hippocampal volume and shape (Johnson et al., 2013), and cerebral asymmetry (Crow et al., 1989). Volumetric differences have been noted in both, drug-naïve and first-episode patients (Gur et al., 1999; Szeszko et al., 2003), with some patients even showing these deficits before the onset of psychosis, in the prodromal stage of the illness (Pantelis et al., 2003). Additionally, there is substantial evidence (e.g., Bogarts et al., 1985; Benes, Sorensen, and Bird, 1991; Arnold et al., 1995) implicating the involvement of microscopic, histological changes in the brains of schizophrenia patients that further implicate aberrant neurodevelopmental processes as a major contributor to the onset of cognitive symptoms.

One of the most notable histological findings in schizophrenia is that of aberrant neuronal clustering or migration in the lateral temporal lobe and parahippocampal white matter (Akbarian et al., 1993), entorhinal cortex (Arnold et al., 1991; Harrison and Weinberger, 2005), and prefrontal white matter (Akbarian et al., 1996). Such aberrations implicate an early neurodevelopmental event affecting the proper migration, connectivity, and ultimate survival of developing neurons (Roberts, 1990; Harrison, 1997). These developmental neuropathological changes may be responsible for the cognitive symptoms of schizophrenia.

1.1.3 Presentation of a working memory impairment in schizophrenia patients

Cognitive symptoms, notably WM deficits, are thought to be core feature of schizophrenia (Goldman-Rakic, 1996). Broadly defined, WM is a capacity-limited system, which allows an individual to hold task-relevant information while he/she works towards
solving a problem (Baddeley, 1992). Following the cessation of sensory input, information is transiently represented in WM and used to plan or decide on a course of action towards completing a goal (Goldman-Rakic, 1994; Silver et al., 2003). Baddeley (1986) initially suggested that WM consists of a central executive and two slave systems: a visuospatial scratch pad and a phonological loop capable of retaining modality-specific information over short periods of time.

WM is fundamental to numerous cognitive and executive processes and dysfunctional WM in schizophrenia may underlie the cognitive organization deficits, impaired goal-oriented behavior and failure of self-monitoring (Silver et al., 2003). If WM deficits are a salient and central feature of schizophrenia, then identifying the factors that impair WM function becomes a critical step in uncovering the underlying disease process. However, gaining a clear and decisive understanding of WM deficits in schizophrenia patients is hindered by the lack of homogeneity in patient samples and working memory testing procedures (Forbes et al., 2009). However, despite such heterogeneity, meta-analyses by Forbes et al. (2009) and Lee and Park (2005) found consistent deficits in schizophrenia patients across phonological, central executive, and visuospatial domains of WM across diverse methodologies and approaches. In addition, the study of Lee and Park (2005) also noted that the WM deficit in patients was typically delay-independent. Specifically, it was found that increasing the duration of the delay period in a WM task beyond one second did not lead to any more robust WM deficits in schizophrenia patients compared to controls (Lee and Park, 2005). Such reliable delay-independent performance deficits seem to argue against the idea that WM representations are inherently unstable or prone to decay in patients. Rather, this pattern of performance deficit may implicate a more basic problem in the encoding stage of the WM task, such as difficulties
attending to task-relevant information (Adler et al., 1998)—an idea that will be returned to in Chapter 4 of the current dissertation.

1.2 Evidence of prefrontal cortex dysfunction in schizophrenia

PFC dysfunction remains a core component of current theories of the pathophysiology of schizophrenia (Lewis, Hashimoto, and Volk, 2005; Harrison and Weinberger, 2005). The PFC plays an essential role in flexible decision making (Ragozinno et al., 1999), conflict resolution, specific memory processes, and executive functions—capacities that are compromised in schizophrenia (Rushworth and Behrens, 2008). Indeed, certain cognitive symptoms of schizophrenia, such as working memory deficits, reflect alterations in executive control processes that rely on a properly functioning dorsolateral prefrontal cortex (DLPFC; Miller and Cohen, 2001). The most significant evidence that individuals with schizophrenia have abnormal DLPFC activity comes from experimental paradigms that incorporate neuroimaging techniques with neuropsychological testing. For example, healthy control subjects performing the Wisconsin Card Sorting Task (WCST)—a task requiring effective problem solving skills, abstract reasoning, and working memory (Weinberger and Lipska, 1995)—show an increase in prefrontal activation as task difficulty increases (Weinberger, Berman, and Zec, 1986). Conversely, schizophrenia patients performing the same task show an inverse relationship between task demands and activation of the frontal cortex (i.e., hypofrontality; Weiberger, Berman, and Zec, 1986). In fact, the severity of the deficit in DLPFC activation, but not activation of any other cortical region(s), predicts the severity of cognitive disorganization symptoms in schizophrenia patients (Silver et al., 2003).
Studies of regional cerebral blood flow (rCBF) in monozygotic twins discordant for schizophrenia show an attenuated prefrontal blood flow index for schizophrenic twins compared to non-schizophrenic twins (Berman, Torrey, and Weinberger, 1992). Specifically the ratio of prefrontal rCBF to non-frontal rCBF was relatively more reduced in the schizophrenic versus non-schizophrenic twin. This approach has been applied to broader cohorts of patients and reduced DLPFC rCBF has been observed in acute, chronic, medicated (Weinberger and Lipska, 1995), and neuroleptic-naïve patients (Catafau et al., 1994). These findings suggest that a sizeable group of schizophrenia patients display reduced frontal lobe activation across a variety of cognitive tasks and this phenomenon has become known as hypofrontality.

However, hypofrontality is not always observed in patients and several functional imaging studies have found the opposite or hyperfrontal cortical activation in response to task demands (Manoach et al., 1999; Callicott et al., 2000; Callicott et al., 2003). For example, Manoach et al. (1999) found that relative to control subjects, schizophrenia patients displayed hyperfrontal left DLPFC activation in response to low WM demands, but hypofrontal left DLPFC activation at high WM demands. Thus patients may be able to compensate by increasing DLPFC activation up to a critical point, and beyond that, their WM capacity is tapped (Manoach et al., 1999). This is consistent with the idea that prefrontal pathology in patients confers an abnormal or ‘inefficient’ neural strategy for dealing with WM information (Callicott et al., 2003).
1.2.1 Neuroanatomical characteristics and post-mortem findings in prefrontal areas of schizophrenia patients

Anatomical and epidemiological studies in schizophrenia led to the hypothesis that disruption of normal, orderly brain development may be a major contributor to the etiology of the disorder (Bunney and Bunney, 2000). In large part, this hypothesis was motivated by findings that, in schizophrenia, abnormal patterns of neuronal migration may affect the development of the cerebral cortex, thus providing a mechanism by which cortical connectivity and associative functions, like cognitive processes dependent on the communication between the PFC and hippocampus, could be disturbed (Jones, 1997; Bunney and Bunney, 2000). That is, cognitive disturbances in schizophrenia are thought to be, in part, a result of a developmental disconnection of fronto-temporal cortices (Weinberger and Lipska, 2005). For example, in the cingulate cortex of schizophrenia patients a laminar shift in neuronal density (Benes et al., 1991) appears to implicate improper or failed migration of developing neurons into their appropriate cortical target sites (Weinberger and Lipska, 1995). Indeed, this impaired ability of prefrontal neurons to reach their appropriate target sites likely also means that these neurons are unable to make their appropriate, designated connections with other neurons (Rakic, 1988). This would affect both afferent and efferent projections of the PFC to key regions associated with higher order cognitive processes (Schubert, Martens, and Kolk, 2014). As a result, the ability of functionally and anatomically distinct systems in the brain to communicate with one another, coordinate activity, and process information is disrupted, likely manifesting in inefficient or noisy processing abilities and impaired cognitive performance (Weinberger and Lipska, 1995).
Studies of post-mortem tissue in schizophrenia show that patients’ brains differ from those of control subjects with regards to neuronal density, patterns of neuronal cell settling, and in gene expression of neurotransmitter-related molecules especially in the PFC (Lewis, Hashimoto, and Volk, 2005). In particular, mRNA for glutamic acid decarboxylase 67 (GAD67), a GABA-synthesizing enzyme, are significantly reduced in the PFC of schizophrenic patients (Akbarian et al., 1995; Volk et al., 2000; Guidotti et al., 2000). These differences may ultimately contribute to the cognitive deficits seen in the disorder. For example, GABAergic DLPFC neurons may be necessary for normal WM performance by either tuning or synchronizing WM related activity (Lewis, Hashimoto, and Volk, 2005; Constantinidis, Williams, and Goldman-Rakic 2002).

1.3 Evidence of a dysfunctional hippocampus in schizophrenia

1.3.1 Contributions of aberrant hippocampal activity to cognitive dysfunction in schizophrenia

The hippocampal formation has also been implicated in the pathophysiology of schizophrenia. Indeed, many of the memory impairments noted in patients with schizophrenia are thought to be a direct consequence of dysfunction in hippocampal circuits (Boyer et al., 2007). Consistent with this view, the memory deficits in patients are often in the realm of contextual or spatial information (see: Cohen and Servan-Schreiber, 1992; Rizzo et al., 1996; Bazin et al., 2000). Moreover, the context memory hypothesis of schizophrenia proposed by Rizzo et al., (1996), suggests that patients struggle in encoding or binding together contextual information to form an intact memory representation. For example, patients with schizophrenia show poor performance on tasks requiring them to remember both a word
previously presented on a screen as well as its spatial location (Rizzo et al., 1996). Similarly, Burglen et al., (2004) tested contextual binding in schizophrenia patients and found them particularly deficit in the combination condition, relative to the recognition of either the target or its location alone.

The hippocampus may also contribute to WM retrieval and Heckers et al. (1998) proposed that hippocampal dysfunction could contribute to errors during the retrieval stage of a WM task in patients with schizophrenia. Heckers et al. (1998) found hippocampal recruitment in patients was significantly reduced during a task that required high-level semantic encoding followed by later conscious recollection. Conversely, at baseline and low task demands, patients displayed hippocampal hyperactivity compared to control subjects. Additionally, when task demand was high, patients showed increased regional cerebral blood flow (rCBF) to several prefrontal regions, which was thought to reflect an increase in cognitive effort during the task. This increased frontal activity was interpreted as an attempt to compensate for failing to efficiently recruit the hippocampus. These findings are in line with the studies cited above (e.g., Manoach et al., 1999; Callicott et al., 2000; Callicott et al., 2003) in which patients displayed hyperfrontal activation in response to WM task demands.

1.3.2 Neuroanatomical characteristics and post-mortem findings in hippocampi of schizophrenia patients

Evidence from histopathological studies indicates anatomical abnormalities in the hippocampi of schizophrenia patients, such as a reduction in hippocampal area or volume (see: Bogarts, Meertz, and Schonfeldt-Bausch, 1985; Jeste and Lohr, 1989; Suddath et al., 1990; Weiss et al., 2005). Further, evidence of generalized hippocampal atrophy, decreased neuronal density (Falkai and Bogerts, 1986) and size (Arnold et al., 1995), and pyramidal cell
disorganization (Kovelman and Scheibel, 1984; Conrad et al., 1991) have also been shown to be present in the brains of schizophrenia patients. However, volume reductions and shape deformations in the hippocampal formation are one of the most frequently stated findings in post-mortem (Bogerts et al., 1990), anatomical imaging (Narr et al., 2004; Davatzikos et al., 2005), and twin (Goldberg et al., 1994) studies of schizophrenia patients. A meta-analysis by Adriano, Caltagirone, and Spalletta (2012) of 44 structural MRI studies showed significantly reduced bilateral hippocampal volume in schizophrenia patients. Interestingly, these hippocampal volume abnormalities often precede the clinical onset of schizophrenia (Lawrie et al., 1999). Studies of hippocampal shape analysis have shown that deformations are most significant in the anterior hippocampus of patients with schizophrenia (Csernansky et al., 2002; Narr et al., 2004; Ho and Magnotta, 2010). This region projects to the PFC and is thought to be involved in associative memory (Strange et al., 1999; Bannerman et al., 2004). Interestingly, such anatomical changes have also been shown to correlate with specific cognitive deficits and illness severity (Boos et al., 2007; Hasan et al., 2011; Adriano, Caltagirone, and Spalletta, 2012). For example, Hasan et al. (2011) described a significant correlation between left hippocampal volume and verbal memory performance in first-episode schizophrenia patients, but failed to show any correlation between hippocampal volume and performance on tests of cognitive flexibility or working memory.

Contributing to the reduction of hippocampal volume are findings of ventricular enlargement in patients with schizophrenia. Johnstone et al. (1976) showed institutionalized schizophrenia patients have significantly enlarged ventricular size when compared to healthy controls. This study also noted a significant relationship between ventricular size and impairment on cognitive testing. Similarly, Bilder et al. (1995) found an inverse relationship
between anterior hippocampal volume and performance on tasks of executive functions in patients with first-episode schizophrenia. However, it should be noted that the relationship between hippocampal volume and cognitive impairment is not entirely clear, as there is also evidence of a lack of a relationship between hippocampal abnormalities and symptom severity or illness duration. For example, Csernansky et al. (2002) found that, despite the presence of abnormalities in areas of the hippocampus that project to the PFC, the magnitude of these abnormalities in hippocampal shape or asymmetry in schizophrenia patients was unrelated to either symptom severity or illness duration.

There is also a body of evidence suggesting that abnormalities in the cytoarchitecture of hippocampal cells exists in patients with schizophrenia (Arnold et al., 1997). Altered cytoarchitecture of hippocampal cells may adversely affect the ability of neurons to entrain task-related field potential oscillations—potentially influencing the overall functionality of the hippocampus, including its ability to efficiently communicate with other regions (Boyer et al., 2007). A study by Benes et al. (1991) showed schizophrenia patients have significantly smaller pyramidal neurons in CA1-CA4. Similarly, Zaidel et al. (1997) reported reduced neuronal size in the left CA1, left CA2, and right CA3 subfields of patients with schizophrenia. Further evidence of altered hippocampal cytoarchitecture comes from Nesvaderani, Matsumoto, and Sivagnanasunduram (2009), who found evidence of aberrant protein expression profiles in the anterior hippocampus of patients. This evidence of altered cytoarchitecture in the anterior hippocampus is consistent with the hypothesis that aberrant wiring within the anterior hippocampus and its efferent projections, particularly to the PFC, may underlie the cognitive deficits in schizophrenia (Goldman and Mitchell, 2004; Harrison, 2004).
Given the importance of potentially aberrant wiring within the human anterior hippocampus to its efferent projection areas like the PFC in schizophrenia, it is necessary to gain a better understanding of the impact of these wiring changes on cognitive abilities in schizophrenia patients. To do so, one must turn to animal models, which capture the anatomical and functional connectivity between the anterior hippocampus and the PFC in humans. Indeed, such a pathway, referred to as the H-PFC pathway, exists in the rat (Godsil et al., 2013). The H-PFC pathway is composed of neurons projecting from the subiculum and CA1 of the VH directly to the PFC (Jay, Glowinsky, and Thierry, 1989; Godsil et al., 2013) and thus, effectively captures the connectivity seen between the human anterior hippocampus—the structural analog of the rat VH (Bannerman et al., 2004)—and the PFC. Given the direct connectivity between the hippocampus and PFC through the H-PFC pathway, it is unsurprising to learn that this network supports both up and down regulation of synaptic connectivity and is also recruited during WM performance (Jay et al., 2004). Thus, the study of this pathway may help advance the current understanding of WM deficits in psychiatric disorder in schizophrenia. Indeed, such a model exists.

1.4 NVHL model

Until roughly 20 years ago, animal models of schizophrenia were largely pharmacologically based and focused on direct manipulation of dopamine and glutamate neurotransmitter systems (Tseng, Chambers, and Lipska, 2009). These approaches were consistent with a large body of evidence implicating dysfunction of the dopaminergic system with schizophrenia (Chambers et al., 1996; Lipska and Weinberger, 2000; Chambers, Krystal and Self, 2001; Harrison and Weinberger, 2005; Tseng, Chambers, and Lipska, 2009). Even
though dopamine-based models have great predictive validity given the efficacy of dopamine antagonists as anti-psychotics (McKinney and Moran, 1981; Costall and Naylor, 1995; Cools et al., 1990), these drugs tend to be ineffective in attenuating cognitive symptoms of schizophrenia (Tseng, Chambers, and Lipska, 2009). Lesion models of schizophrenia, however, are often better at encompassing aspects of the neurobiological characteristics of the disease as well as the cognitive symptoms. Specifically, the NVHL model, created by Lipska and Weinberger (1993), involves a developmentally-timed excitotoxic lesion to the VH, which results in the emergence of several behavioural, neuro-anatomical and –chemical alterations in early adulthood. As such, the NVHL model is the most capable heuristic model of the cognitive deficits in schizophrenia. With more than 100 empirical studies using the NVHL model to-date, it is the also the most thoroughly characterized neurodevelopmental model of the disorder (Tseng, Chambers, & Lipska, 2009). In fact, its ability to capture the developmentally delayed onset of symptoms in a manner similar to the onset of symptoms in human schizophrenia patients is one of its notable advantages (Marcotte, Pearson, and Srivastava, 2001).

1.4.1 Producing the NVHL model of schizophrenia

Ventral hippocampal lesions are made in postnatal day 7 (PD7) rat pups using the NMDA receptor agonist, ibotenic acid (α-amino-3-hydroxy-5-isoxazoleacetic acid; IBO). PD7 was chosen as a developmental time point as it is analogous to the second trimester of human fetal brain development—a sensitive period of hippocampal network formation (Chambers and Lipska, 2011). IBO, when delivered in concentrated doses to specific brain areas, induces excitotoxic cell death. However, one advantage of IBO is that it spares axons of passage (Chambers and Lipska, 2011). Thus, IBO infusion to the VH of PD7 rat pups kills
only projection neurons and hence the efferent projections of the VH to other cortical regions, most notably, the PFC.

VH lesions in PD7 rat pups compromise the architectural integrity of the mPFC (Carr and Sesack, 1996), which is thought to play a role in mediating the post-pubertal emergence of the behavioural symptoms in NVHL animals (Lipska, Al-Amin, & Weinberger, 1998). This is congruent with the hypothesis that neurodevelopmental abnormalities that disrupt the normal development of the human anterior hippocampus—which is analogous to the VH in rats—may underlie the cognitive deficits that rely on fronto-temporal communication (Bilder et al., 1995).

1.4.2 Behavioural features of the NVHL model of schizophrenia

As previously mentioned, the rat VH corresponds to the human anterior hippocampus, which has consistently been associated with cognitive symptoms in schizophrenia patients (Csernansky et al., 2002; Narr et al., 2002; Tseng, Chambers, & Lipska, 2009; Ho and Magnotta, 2010). Unlike other animal models of schizophrenia, the developmentally timed VH lesion produces analogous cognitive symptoms to those seen in human patients with schizophrenia. The relevance of the model to positive and negative symptoms of schizophrenia is a matter of debate but will be discussed briefly below.

Dopamine (DA) dysfunction is thought to underlie at least some of the positive symptoms of schizophrenia largely by virtue of the fact that the DA releaser amphetamine can reproduce psychotic-like symptoms in normal adults and because all effective antipsychotic agents block D2 receptors (Seeman, 1987). NVHL animals may also have altered DA system function given their hypersensitivity to amphetamine or apomorphine challenge in the post-adolescent period (Lipska, Jaskiw, & Weinberger, 1993; Lipska and Weinberger 1993).
NVHL animals also have an enhanced immobility response to neck clamping, when compared to control animals, which is suggested to be a result of aberrant mesolimbic DA transmission (Flores et al., 2005b). Lipska and Weinberger (1993) also found NVHL animals to be less cataleptic in response to haloperidol injection, when compared to sham lesioned animals. Aside from the potential parallels to direct changes in the DA system, NVHL animals also exhibit deficits in the expression of pre-pulse (Becker et al., 1994), and latent (Grecksch et al., 1999) inhibition, which are also observed in schizophrenia patients and thought to reflect enhanced behavioural reactivity and reduced ability to ignore irrelevant stimuli, respectively (Tseng, Chambers, and Lipska, 2009).

Evidence of negative-like symptoms in NVHL animals has also been documented. NVHL animals demonstrate abnormal social behaviours, such as frequent displays of aggression and spend less time in social interactions (Becker et al., 1999; Tseng, Chambers, and Lipska, 2009). Becker and Grecksch (2000) reasoned that such abnormal social behaviour in NVHL animals may be the result of poor social memory. Interestingly, the abnormalities in social behaviour do not emerge in a developmentally-dependent manner, as is the case for both the positive- and cognitive-like symptoms in NVHL animals (Tseng, Chambers, and Lipska, 2009). Rather, in NVHL animals, social behaviour abnormalities are evident both before and after puberty and are largely unresponsive to antipsychotic medications (Sams-Dodd, Lipska, & Weinberger, 1997). Although the source of these social behavioural abnormalities is uncertain, they are not considered to involve anxiety, as there were no differences between NVHL and sham lesioned animals on performance of an elevated plus maze task (Becker et al., 1999).
While there may be parallels to the positive and negative symptoms, the NVHL model is mainly considered to be a model of the cognitive deficits in schizophrenia. As noted above, NVHL animals display cognitive-like symptom traits, such as show poor performance on tasks of spatial learning and working memory. Specifically, lesioned animals show impaired place learning on the Morris water maze task, despite intact sensorimotor functions and motivation to escape (Lipska et al., 2002; Tseng, Chambers, and Lipska, 2009). In a delayed non-match to sample task of working memory, Lipska et al., (2002) highlighted a delay-dependent deterioration of performance in NVHL animals, which closely resembled the performance of animals with adult lesions of the PFC. Surgical ablation of the PFC in adult NVHL rats was found to normalize some of the behavioural effects of the initial NVH lesion, such as enhanced responsiveness to novelty and amphetamine administration (Lipska, Al-Amin, & Weinberger 1998). These findings seem to suggest that a neonatal insult to the ventral hippocampus impairs an animal’s later performance on tasks that are dependent on the integrity of the PFC and communication between the PFC and VH (Lipska et al., 2002). Importantly, these cognitive-like symptoms in NVHL animals only begin to emerge around the time of puberty and are stable throughout the lifetime of the animal, as seen in patients (Lipska and Weinberger, 2002). Further, since the PFC is a relatively late-maturing structure with extensive excitatory connectivity with the VH (Van Eden and Uylings, 1985), the previous findings also suggest that a dysfunctional PFC in the NVHL rat may be a downstream driving force in the expression of the behavioural changes that emerge in puberty and adulthood (Lipska, Al-Amin, & Weinberger 1998).
1.4.3 Neurobiological features of the NVHL syndrome

PFC disturbance in NVHL animals is considered to be a valid model of the cortical deficits seen in human patients with schizophrenia (O’Donnell et al., 2002). As mentioned above, subsequent lesions of the PFC in adult animals that received a neonatal VH lesion have been shown to restore NVHL-induced behavioural abnormalities. This suggests that a critical requirement for the expression of the NVHL syndrome is the aberrant development of the PFC and that this aberrant development is actually worse than lacking a PFC at all (Tseng, Chambers, & Lipska, 2009). The critical alterations in the PFC that cause the cognitive deficits are not entirely clear, but include structural changes such as a reduction in spine density and dendritic length of PFC pyramidal neurons (Flores et al., 2005a; Alquicer et al., 2008). The brains of NVHL animals also have abnormally low cortical expression of glutamate transporter proteins, reduced levels of N-acetylaspartate—considered to be a marker of neuronal integrity (NAA; Lieberman et al., 2001), brain-derived neurotrophic factor (BDNF), and GAD 67; as well as abnormal cortical expression of activity-dependent transcription factors such as, c-fos and Δ-fosB (Lipska et al., 1995; Lee and Kornetsky, 1998; Bertolino et al., 1999; O’Donnell et al., 1999). Importantly, many of these neuroanatomical alterations have also been reported in human schizophrenia patients (Weinberger and Lipska, 1995; Chambers, Krystal, & Self, 2001; Harrison and Weinberger, 2005). In addition there is a dysfunction in the ability of DA via D2 receptors to modulate fast-spiking interneurons in the PFC of NVHL animals (Gruber et al., 2010; O’Donnell, 2012). A reduced capacity of pyramidal neurons to activate inhibitory interneurons and/or an alteration in the ability of DA to modulate interneurons, may result in a disinhibited or hyperactive PFC, a decreased neural
signal to noise in PFC networks and ultimately deficits in cognitive performance (Winteerer & Weinberger 2004).

1.4.4 NVHL as the optimal model of cognitive symptoms in schizophrenia

By highlighting the neurodevelopmental pathway in the schizophrenia disease process, the NVHL model affords investigation into the complex genetic, developmental, neuronal, and environmental-based foundations of schizophrenia. The downstream neurobiological effects on the development of the prefrontal and hippocampal formations show that a NVHL lesion can alter gene expression and neural morphology without directly manipulating the genome. When compared to cross-sectional genetic animal models of schizophrenia, the NVHL model may, therefore, be better equipped to more accurately reproduce the gestalt of schizophrenia disease process (Tseng et al., 2009). No other animal model of schizophrenia is able to parallel the cognitive deficits in schizophrenia as accurately as the NVHL model.

1.5 Aims of the thesis and use of MRI

A major limitation of lesion studies is the inability of the experimenter to effectively control the size of a lesion. As a result, animals are often excluded from studies based on qualitative differences in lesion size, with no credible effort made to quantify lesion volume and compare this volume against task performance. Nowhere is this more true than in the NVHL literature, where animals are routinely excluded from experiments based on a mere qualitative assessment of lesion size, often as being too small. Not only is this an imprecise practice, but it is also unfounded, as there have been no studies linking the extent of hippocampal damage in NVHL animals with their performance on any behavioural tasks. Thus, the specific aim of this thesis is to assess the relationship between NVH lesion volume
and performance on a variable delayed non-match to sample (vDNMS) task of WM. Based on work investigating the effects of hippocampal IBO lesions on adult primates (e.g., Murray and Mishkin, 1998), we hypothesized that VH lesion volume would be positively correlated with WM performance in our animals.

We assessed VH lesion volume using MRI in living animals. MRI is preferable to histological processing, because the former circumvents potential artifacts of traditional histological analyses such as tissue shrinkage or damage during tissue preparation. The use of MRI to calculate lesion volume in-vivo is a well-validated technique, and is often used in place of post-mortem histological analysis of lesion volume for these reasons (Miyabe et al., 1996; Takano et al., 1998; Nemanic et al., 2002; Kazemi et al., 2004; Sandner et al., 2010). Typically the two procedures are in good agreement however and Bertrand et al. (2010) showed that MRI analysis of lesion extent in NVHL animals was robustly correlated with qualitative lesion extent obtained from histological examination. Further, Malkova et al. (2001) showed that T2-weighted MRI hypersignals were highly correlated with histological analysis of lesion extent (r = 0.95, P < 0.001). Thus, the use of MRI to calculate lesion volume rather than histology, is a more convenient and efficient alternative and is well validated in the literature.
Chapter 2: Methods and materials

2.1 Subjects

Pregnant Long-Evans dams were acquired at approximately 14 days of gestation and housed individually in breeding cages. On PD7, female pups were permanently removed, resulting in litters of 2-10 male pups. Animals were housed in a facility with a 12 hr light/dark cycle; all training and surgeries took place during the light cycle. Once they reached the age required for behavioural training (PD50), pups were singly housed and placed on a food-restricted diet. For the duration of training and testing on our behavioural paradigm, all animals were restricted to 90% of their free-feeding weight. Feeding took place in the animals’ home cage following their daily training session. Water was available ad libitum for all animals. All procedures were carried out in accordance with the Canadian Council of Animal Care and the Animal Care Committee at the University of British Columbia.

2.2 Surgeries

Male rat pups were randomly assigned to lesion (n = 16) or sham (n = 7) status. Surgeries were performed on PD7 as described by Lipska et al. (1993). Briefly, rat pups were anesthetized via hypothermia by placement on wet ice for 15-20 min. After reaching a surgical plane of anesthesia, pups were immobilized by use of adhesive tape to secure them onto a styroform platform that was fixed to a stereotaxic Kopf instrument (David Kopf Instruments, Tunjunga, CA, USA). Following immobilization, a single incision was made in the scalp, exposing the underlying skull. IBO (0.3 µl; Sigma Chemical Co.) was dissolved in
artificial cerebrospinal fluid (aCSF; 10 µg/µl), or 0.3 µl of aCSF alone, was injected bilaterally into the ventral hippocampus (AP −3.0 mm, ML ±3.5 mm, VD −5.0 mm, relative to bregma; Chambers and Lipska, 2011) at a rate of 0.133 µl / min. After the injection was finished, the needle was left in place for 3.5 minutes, to prevent backflow of IBO or aCSF into the needle track, before being withdrawn. Next, the incision site was closed with veterinary wound closure glue, which was allowed to fully dry while pups were placed under a heating lamp. Pups were returned to their mothers 30-45 min after recovering from anesthesia, when they were fully mobile and awake. Pups were weaned at PD24 and housed 2-3 per cage, based on lesion status.

2.3 Apparatus

All rats were trained and tested on a T-maze (Fig. 1), consisting of a 48-inch long main stem, with two 22-inch long choice arms that ran perpendicular to the main stem. Each choice arm was connected to the base of the main stem by a left and right return arm, allowing rats to start a new trial by returning to the base of the stem after making a turn down either the left or right choice arms. The maze contained five experimenter-controlled doors: two doors (“return doors”), located at the left and right of the base of the maze’s stem, could be closed to prevent re-entry into the return arms. Two additional doors (“choice doors”), located at the top of the stem could be closed to direct rats into either the left or right choice arm or, alternatively, could both be kept open, allowing rats to freely enter the arm of their choosing. A fifth door (“stem door”) was located on the stem floor and served to isolate rats to the base of the stem for the beginning of a trial and during delay periods on the vDNMS task (described below). All maze floors measured 5.5-inches wide and were constructed of solid pine wood; floors
were enclosed by 6-inch high walls made of flexible corrugated plastic. All doors of the maze were constructed of 0.25-inch thick plexiglass. The entire maze was painted black and was elevated 48-inches off the floor. Fixed spatial cues (black, pink, or green geometrical designs) were located on three of the surrounding walls of the testing room. The position of the experimenter in the room was fixed thus, serving as an additional spatial cue.

2.4 Behavioural tasks

2.4.1 Pre-exposure and pre-training on T-maze

Training on the T-maze began with 3 days of 15 minute pre-exposure sessions. During these sessions, rats were placed in the maze with all doors opened, and chocolate cereal loops on the maze floor. The experimenter was present in the room during pre-exposure sessions in order to allow the animals to habituate to the presence of the experimenter.

Following pre-exposure, animals were trained to make forced-alternations, where they were directed into the left or right choice arm, by means of closing the door to the opposite choice arm, and received a chocolate cereal loop reward at the end of each arm. Animals were given 5 days of forced-alternation training, with each day’s training consisting of 10-12 alternations.

Upon successful acquisition of the forced-alternation task, animals were trained on a 1 second DNMS task. In this DNMS task, sessions began by placing animals at the base of the maze’s stem; all surrounding doors were closed, effectively containing the animal. Next, the experimenter lowered the base door, allowing the animal to travel down the stem. At the opposing end of the stem, the animal was forced to make either a left or right turn after the
experimenter closed either the right or left choice door, respectively (forced run). Forced runs were not reinforced with chocolate cereal loops. Once the animals had completed the forced run, they returned to the base of the stem by means of the return arm where, once again, all doors would be closed and the animal was contained (delay period). After a one-second delay, the base door was lowered and both choice arm doors were opened (choice run). Animals were only rewarded if they entered the arm opposite to that entered in the previous forced run (non-match to sample). A different pattern of randomly-chosen forced runs with an equal number of left and right turns (e.g., L-L-R-R…) was used everyday, with the same pattern being used for all animals on any given day. Animals were tested daily on this 1 sec DMNS training task until they reached criterion performance of at least 80% correct trials over 3 days. Once criterion had been reached animals were trained on a 10 sec DMNS task until they reached criterion performance, which was defined as less than 10% variance in performance over 5 days, regardless of choice accuracy (Lipska et al., 2002).

2.4.2 vDNMS testing

Upon successfully reaching criterion performance on the 10 sec DMNS task, animals were tested on a vDNMS task. This task was similar to the 1 sec and 10 sec DMNS tasks, with the only difference being that animals were contained in the base of the stem for either 10, 20, or 30 seconds during the delay period. Each vDNMS testing session consisted of 20 trials, containing an equal number of left and right turns, and a roughly equal number of each delay length. Again, a different pattern of randomly-chosen forced runs with an equal number of left and right turns, and roughly equal number of each delay length was used everyday, with the same pattern being used for all animals on a given day.
2.5 In-vivo MRI

In vivo MRI was used to enable visualization, and volumetric calculations, of lesions in NVHL animals at roughly six months of age. Both, NVHL and sham animals were imaged. Rats were first anesthetized using isoflurane inhalation (4 % for induction; 1.5% for maintenance). Body temperature was maintained at 37ºC ± 1ºC by means of a circulating water-heating pad. Breathing rate and heart rate were monitored throughout the course of all imaging sessions.

Rats were positioned in a supine position on a head holder, locally designed and constructed with non-magnetic materials. MR Imaging was performed in a 7T Biospec System (Bruker BioSpin MRI GmBH, Ettlingen, Germany) equipped with a 100 mT/m gradient system. A birdcage coil of 72 mm inner diameter (Bruker BioSpin MRI GmBH, Ettlingen, Germany) was used for transmission. An actively decoupled surface coil (24 mm diameter) was positioned on the head of the animal for signal reception, to enhance signal-to-noise ratio. T2-weighted images were acquired using a rapid acquisition with relaxation enhancement (RARE) sequence, with short localizer scans acquired in the sagittal and axial planes (32 sec; resolution = 0.312 mm x 0.312 mm x 1.5 mm). A higher resolution RARE coronal scan of the brain was acquired with the following parameters: echo time = 34.5 ms; repetition time = 4 sec; number of averages = 2; RARE factor = 8. The field of view was 25.6 mm x 25.6 mm on a 256 x 256 matrix, providing an in-plane resolution of 0.1 mm x 0.1 mm. A total of 26 contiguous slices (1 mm thick) were acquired; the scan time was, on average, 4 min 16 sec per animal.
2.6 Lesion verification and quantitative assessment

Our MRI technique provided 26 serial coronal sections, 150 sagittal, and 100 axial sections. MRI analysis focused on various regions of interest, such as CSF-filled tissue loss in ventral (VH) and dorsal hippocampus, and lateral ventricles (Bertrand et al., 2010). Due to the presence of high concentrations of free water, the lateral ventricles and all lesioned tissue appeared as hypersignals in our T2-weighted images.

Assessment of the lesion volume was performed using the MRI image analysis software, 3D Slicer (www.slicer.org). Briefly, 3D Slicer allows the user to outline lesion boundaries in all three planes of MR images, affording precise volumetric calculations of the ROI. Lesion boundaries were defined as regions of hypersignal on T2-weighted images, which indicated areas of tissue loss and ventricle enlargement. Volumetric calculations of lesion size were performed for all NVHL animals. Later, the calculated volume was correlated with vDNMS task performance.

2.7 Post-mortem histology and lesion assessment

Following completion of all behavioural testing, rats were deeply anesthetized using Isoflurane. Rats were then decapitated, at which time their brains were quickly extracted and placed into individual beakers containing a 4% paraformaldehyde-glucose solution, for at least one week. Coronal sections (40 µm) were collected, beginning when the dorsal-rostral blade of the hippocampus came into view (approximately -2.0 mm AP to bregma; Swanson, 2004). Sections were collected for slides approximately every 400 µm thus, every 9th-10th section was kept (Chambers and Lipska, 2011). Sections were stained using a Cresyl Violet stain, and cover slipped.
Lesions were evaluated by means of a light microscope to examine cresyl violet stained brain sections. The coordinates for the NVH lesion target the region of the hippocampus where the CA3 layers of the dorsal and ventral blades convene (Chambers and Lipska, 2011). Typically, lesioned animals are characterized by one or more of the following neuroanatomical qualities: 1) generalized enlargement of the lateral ventricles; 2) tissue atrophy of the VH; 3) distortion of VH cell layers (Chambers and Lipska, 2011). Animals were disqualified from the current study if there were evidence either or any of the following: 1) that the lesion was a complete hippocampectomy or if the lesion involved significant damage to the dorsal blade of the hippocampus 2) evidence of no lesion or unilateral lesion 3) evidence of significant damage to the surrounding areas (e.g., thalamus).

Although the use of MRI to calculate lesion volume has been validated in previous studies, as discussed above, we wanted to verify the correspondence between MRI and histological quantification of lesion volume. To calculate lesion volume from the histological specimens, we captured digital images of each stained brain slice, and imported them into Microsoft® Paint. Lesion areas were then shaded bright yellow, and imported into MATLAB (The Mathworks, Inc) where a custom script was written to identify and count the number of bright yellow pixels in each histological image of three different animals. Pixels from all images were then added together and a volume was calculated from the total number of pixels by adding the measurements across each section and multiplying by section thickness (40 µm).
2.8 Behavioral analysis

vDNMS performance was analyzed by means of ANOVA with testing day, delay length, and lesion status as the independent factors. The ANOVA was followed by a Fisher LSD post-hoc test. The relationship between VH lesion volume and vDNMS performance was evaluated using Pearson’s correlation.
Chapter 3: Results

3.1 Histology and MRI

Lesion verification using MRI indicated ventricular enlargement, and volume reduction of the ventral hippocampus of NVHL animals, consistent with the existing literature (Lipska et al., 1995; Lipska, Aultman, and Verma, 2002). The present study mainly relied on the use of MRI, as opposed to histological processing, to determine lesion volume as in previous studies (Angst et al., 2007; Macedo et al., 2010; Bertrand et al., 2010; Sandner et al., 2010). While there is generally a good correspondence between lesion volume calculated using MRI and post-mortem histology, we found that the VH lesion volume obtained from histological analysis of three animals to range from 67% smaller to 11% larger than the volume calculated from the MRI, which we believe is attributable to the inherent flaws in the use of histology to calculate lesion volume (see Fig 2; an idea that will be discussed in Chapter 4).

MRI image analysis indicated no damage to the dorsal hippocampus; however, in some animals, portions of the subiculum and dentate gyrus did sustain damage in the form of cell loss and cavitation—a finding that is consistent with the existing NVHL literature (Lipska et al., 2002; Angst et al., 2007; Bertrand et al., 2010). Two NVHL animals were excluded from the current dissertation due to misplaced, or excessively large, lesions that extended into the dorsal hippocampus or thalamus. For all other NVHL animals, lesion volumes varied in size from 11.61 mm$^3$ to 63.01 mm$^3$, or 12% to 65% of total hippocampal volume (Fig. 3).

Fig. 4a – 4n shows MRI-generated 3D models of all 14 lesioned animals along with their respective average performance across five days of vDNMS testing, at all delay lengths.
3.2 Working memory performance

All NVHL and sham animals were able to learn the vDNMS task, which suggests the absence of impairment in sensory integration, acquisition, or any other non-nemonic process (Lipska et al., 2002). Over the three days prior to reaching criterion performance on the 1s DNMS training task, ANOVA indicated significantly lower performance in NVHL animals compared to sham animals ($F_{1,19} = 9.73, p < 0.01$; Fig. 5). However, no effect of day ($F_{2,63} = 0.43, p > 0.05$) or interaction between lesion status and day ($F_{2,63} = 0.19, p > 0.05$) was noted in performance of NVHL and sham animals on the 1s DNMS training task. On the 10s DNMS training task, all animals achieved the criterion of < 10% variability in performance, with a negligible difference in the number of training days required to do so. Additionally, we compared the performance on the last day of testing at the 1s delay (Fig 5, rightmost bar) versus the average performance across the 3 delays on the first day of variable delay testing (vDNMS; Fig 6). An ANOVA uncovered a main effect of both lesion group ($F_{1,19} = 16.03, p < 0.001$) and day ($F_{1,19} = 10.56, p < 0.01$); however, no interaction was found ($F_{1,19} = 2.84, p > 0.05$). This may be a result of reduced statistical power due to the low numbers of sham animals relative to NVHLs. In spite of the non-significant interaction effect, it was nevertheless important to determine whether or not the difference in performance on the day prior (Fig 5) could solely account for the deficit observed on the first day of variable delay testing (Fig 6). In other words, did the lesion animals perform poorly at variable delays simply because they had not acquired the task rule as well as the shams? Tukey’s HSD post-hoc test on these data showed that the sham versus lesion groups did not differ in terms of their performance on the last day of testing at the 1s delay ($p = 0.54$; Fig 5, rightmost bars). However the groups did differ significantly in their average performance on the first day of
vDNMS testing ($p = 0.004$, Fig 6). Likewise, in terms of within group comparisons, the performance of the sham animals did not differ on the last day of testing at the 1s delay versus the first day of vDNMS testing ($p = 0.73$), while the performance of the lesioned animals did ($p = 0.0028$). Therefore, the difference in performance between the groups on the first day of vDNMS testing was much larger than the difference in performance between the groups on the day prior. That is even though the lesioned animals were slightly impaired relative to shams prior to imposing variable delays, the imposition of longer delays impaired the performance of NVHL animals to a greater extent than sham animals.

In comparing vDNMS performance of the NVHL and sham animals across all five days of testing, a three-way ANOVA uncovered significant main effects of lesion status ($F_{1,21} = 12.81, p = 0.002$), delay length ($F_{2,63} = 4.22, p = 0.02$), and day of testing ($F_{4,105} = 3.36, p = 0.01$). That is, NVHL animals performed significantly worse than sham animals at all delay lengths and across all days of testing in our vDNMS task (Fig. 6). However, three-way ANOVA failed to uncover statistically significant interaction effects between the previously mentioned variables. Taken together with our finding of impaired performance on the 1s DNMS task, the findings of the vDNMS task are indicative of a delay-independent WM deficit in NVHL animals. Nevertheless, we probed any potential interaction using Fisher’s LSD post-hoc test and found no significant effect between lesion status and delay length (Fisher’s LSD test; $p > 0.05$).

3.3 Relationship between working memory performance and lesion volume

Within the NVHL group, Pearson’s correlation revealed no statistically significant relationship between lesion volume and vDNMS task performance (see: Fig. 7), at either the
10s ($r = -0.20, p > 0.05$), 20s ($r = 0.12, p > 0.05$), or 30s ($r = 0.15, p > 0.05$) delay length. That is, WM deficits observed in NVHL animals were not significantly influenced by lesion volume. The absence of this relationship was evident at all delay lengths examined. Similarly, no statistically significant correlation was observed between lesion volume and days to criterion on the 10s DNMS task ($r = -0.24, p > 0.05$; Fig. 8).
Chapter 4: Discussion

Findings of the current study indicate that a neonatal ventral hippocampal lesion using the excitotoxin, ibotenic acid, impairs working memory performance in a behavioural paradigm that is reliant on the PFC, consistent with previous reports (Sanchez-Santed et al., 1997; Aultman and Moghaddam, 2001; Lipska et al., 2002). The results suggest a generalized reduction in working memory performance in lesioned relative to sham animals. We observed no evidence of a delay-dependent amplification of the WM impairment as there was no statistically significant difference in NVHL animal performance at 1s, 10s, 20s, and 30s delay lengths. The generalized attenuation of vDNMS task performance in our NVHL animals compared to sham animals is most consistent with a deficit during the encoding period of the WM task.

4.1 Discrepant findings in the NVHL literature

Overall, it is difficult to compare the findings of the current study to the existing NVHL WM literature due to the large degree of heterogeneity in the methodology employed by individual studies. For example, some studies do not look at delay-dependent WM (e.g., Wood, Quirion, and Srivastava, 2003) and, others that do, either use extended delay periods in which the animal is not actively maintaining the WM representation (Lipska et al., 2002; Brady, Saul, and Wiest, 2010; Naert et al., 2013) or do not employ the use of variable delay lengths (Brady, Saul, and Wiest, 2010; Heuer and Bachevalier, 2011; Naert et al., 2013). Because of such differences, we are limited in the number of studies that have assessed delay-dependent spatial WM.
In one related study, Brady, Saul, and Wiest (2010) tested WM deficits of NVHL animals in a radial-arm maze spatial delayed win-shift task. In this task, animals were allowed to explore four arms of a radial-arm maze, which were baited with food, during a sample run. Then, after a 5 min or 30 min delay interval, animals were reintroduced to the maze where all eight arms were opened, and only the four previously closed arms were baited. The authors that NVHL performance was significantly reduced, compared to sham animals, at the 30 min delay (Brady, Saul, and Wiest, 2010). Unfortunately, however, data from the 5 min delay training sessions were not reported thus, preventing any conclusions from being made about the delay-dependency of their NVHL animals’ performance. Marquis, Goulet, and Dore (2008) assessed whether the NVHL manipulation was capable of disrupting delayed alternation performance in rats at PD22, the earliest developmental stage of WM ability. Similar to the findings of the current study, Marquis, Goulet, and Dore (2008) found that PD22 NVHL rats displayed delay-independent deficits in WM, on delays ranging from 0 to 30 seconds, in support of our finding of a delay-independent impairment.

Conversely, a study by Lipska et al. (2002) found that NVHL animals displayed delay-dependent WM deficits on a task similar to the one employed in the current dissertation. A plausible explanation for the discrepant findings between the two studies may lie in the variable delay lengths chosen by each group (additional explanations are presented in the following sections). For example, the current dissertation used closely-spaced delay lengths of 10s, 20s, and 30s on testing days; whereas, Lipska et al. (2002) used more salient delay lengths of 1s, 10s, and 40s. We suggest the possibility that the use of an extended 40s delay period by Lipska et al. (2002) may have resulted in a delay-dependent WM deficit because the likelihood of becoming distracted by both internal or external events may have been
augmented by time. Further, such an effect would be exaggerated in NVHL animals, which have been shown to be particularly vulnerable to interference (Tseng et al., 2009). Thus, we argue that use of such extended delay lengths as those employed by Lipska et al. (2002) and Brady, Saul, and Wiest (2010), impose unrealistic and excessive constraints on WM, especially considering that visual WM representations in most human tasks, are largely used for a period no longer than several hundred millisecond to a few seconds (Luck, 2008).

There are a number of factors, other than delay-length that also need to be considered when comparing studies utilizing the NVHL model. For one, severity of lesion-induced impairments in NVHL animals has been attributed to genetic diversity (Lipska and Weinberger, 1995). Specifically, Lipska and Weinberger (1995) found that Sprague-Dawley, Fisher 344, and Lewis rats receiving small or large NVH lesions exhibited differences in the age of onset and severity of positive-like symptoms. Strain differences in cognitive performance are also well documented, with pigmented and albino rat strains showing differences in performance on spatial memory tasks (Hort et al., 2000). Additionally, differences in morphological changes following hippocampal insult have also been observed across different strains, with Long-Evans rats showing more extensive changes to CA1 and CA3 subfields than Wistar rats, in a model of epileptic seizures (Hort et al., 2000). The above findings suggest that genetic differences among varied strains of rat may confer susceptibility to differential expression of the NVHL phenotype and, potentially, to latent traits seen in schizophrenia (Lipska and Weinberger, 1995; Harrison and Weinberger, 2005; Tseng et al., 2009). Indeed, such inter-strain genetic diversity may contribute to the expression of delay-independent WM deficits seen in our NVHL animals. However, this is not to say that the delay-independent WM deficits we found are simply an artifact of the strain of rat we used.
Rather, what we suggest is the possibility that strain differences in hippocampal and prefrontal response to the NVH insult may partially contribute to the delay-independent WM performance seen in our animals.

Yet another potential contributing factor to our animals’ delay-independent WM performance may result from their age at testing and its effect on the cholinergic system. For example, it has been shown that aged, compared to young, Long-Evans rats display marked reductions in acetylcholine binding in the medial septum and vertical limb of the diagonal band (vDB), a region where basal forebrain cholinergic neurons are located (Gill and Gallagher, 1998). These basal forebrain cholinergic neurons project to the hippocampus, cortex, amygdala, and various other forebrain regions (Lewis and Shute, 1967; McKinney, Miller, and Aagaard, 1983; Amal and Kurtz, 1985). Interestingly, in young rats, AMPA-induced lesions of the vDB produce a marked decrease in choline acetyltransferase activity and acetylcholine levels in both the cingulate cortex and the hippocampus (McAlonan et al., 1995). When tested on a spatial DNMS task, vDB lesioned rats displayed a delay-independent performance deficit (McAlonan et al., 1995), much like our NVHL animals. We propose the possibility that an age related decline in vDB acetylcholine activity (Gill and Gallagher, 1998), in combination with the known reduction in acetylcholine receptor binding in NVHL animals (Laplante et al., 2004; Berg et al., 2014), may lead to a functional lesion of the vDB, thus contributing to the delay-independent WM deficits seen in our animals.

Therefore, while our data are roughly consistent with some but not all past NVHL studies, a number of critical factors must be considered before any definitive cross-study conclusions can be drawn.
4.2 Absence of a relationship between lesion volume and performance deficit

To our knowledge, we are the only group to compare lesion volume against WM deficits, using the NVHL model. The existing literature appears to primarily use MR imaging with the NVHL model as a means of selecting animals, based on laterality and qualitative assessment of lesion size (e.g., small, medium, large) (Macedo et al., 2008; Macedo et al., 2010; Sandner et al., 2012). The sparse NVHL literature that does use MRI to calculate lesion volume, often does so to monitor the size of a lesion over time (Bertrand et al., 2010; Sandner et al., 2010). Nonetheless, our exclusive use of MRI instead of histology to calculate lesion volume is supported by the existing literature (presented earlier). The lack of correspondence between lesion size assessed with MRI versus post-mortem histology was at first glance, troubling. The histologically based lesion volume estimates ranged from 67% smaller, to 11% larger, than those obtained from the same animals using MRI and were on average 30.1% smaller. However, there are a number of possible factors that could have lead to over- as well as under-estimation errors in lesion volume from port-mortem histological analyses. First, in live NVHL animals, CSF flowing through the enlarged ventricles will exert outward pressure on the surrounding tissue. In contrast, in the ex-vivo brain prepared for histology, no such outward pressure exists from CSF, and the brain tissue surrounding the enlarged ventricle collapses inward, distorting lesion volume estimates (Pfefferbaum et al., 2004). Second, due to ventricle enlargement and cell loss, the NVHL brain is extremely fragile and prone to shearing or tearing during cryostat slicing, which can lead to distorted lesion sizes. Third, the fixative itself can introduce tissue shrinkage (Wehrl et al., 2014) and neurites often appear as shriveled rather than straight, which collectively will also distort the gray and white matter in histological sections (Schrag et al., 2010). MRI performed in living animals circumvents all
these issues and therefore gives the most accurate estimate of the true lesion volume in our animals. Nonetheless, if one’s intention is simply to detect the presence or absence of a lesion, then histology is an adequate technique. However, MRI is the most well-suited technique if one’s intention is to accurately assess lesion volume, which was an objective of the current dissertation. For these reasons, we calculated lesion volumes from MRI in all of our analyses.

Our finding that lesion volume does not correlate with WM performance seems to suggest that, beyond a certain point, any developmental disturbance of VH activity, or its connectivity to the PFC, is detrimental to spatial WM performance as an adult. As well, the absence of a correlation between VH lesion volume and spatial WM may be interpreted to suggest that a specific region of the VH network, which was damaged by the developmental lesion, may serve as a critical substrate in spatial WM. That is, there may exist a critical region or threshold volume of VH lesion necessary for the developmentally-delayed expression of the NVHL cognitive phenotype beyond which, there is no additional behavioural deficit as assessed by our vDNMS WM task.

The idea that there exists a volume threshold for effective lesions is also reflected by the work of Moser et al. (1993) who found a threshold value of 20% dorsal hippocampal damage was necessary to produce deficits in spatial learning in Long-Evans rats. Zola-Morgan et al. (1992) also reported that no less than 24% CA1 field damage was required to produce performance impairments in a DNMS recognition memory task in monkeys. Interestingly, Moser et al. (1993) reported a correlation between dorsal hippocampal lesion volume and spatial learning; however, no such correlation was observed with ventral hippocampal lesions. Our smallest lesion encompassed 12% of the total VH volume, which
was able to create a WM deficit similar to an animal whose lesion covered 65% of the total VH volume. The differences between the findings of the current thesis and that of both Moser et al. (1993) and Zola-Morgan et al. (1992) may likely be explained either by the difference in age at which animals were lesioned, the lesion target, how the lesion was achieved, or a combination of all three factors. For example, both Moser et al (1993) and Zola-Morgan et al. (1992) inflicted aspiration and ischemic lesions, respectively, to adult animals thus, overlooking the impact that a neonatal excitotoxic lesion would have on prefrontal development. As a result, the NVHL model may serve as a more sensitive tool to study WM deficits in schizophrenia, due to the seemingly lower threshold volume necessary to achieve an effective lesion.

In line with the idea of a threshold volume or critical region of VH lesion, the absence of a correlation between lesion volume and WM performance may result from how the functional capacity of the hippocampus is impaired by lesions. For example, it may be the case that hippocampal function is more sensitive to smaller lesions, as a result of the way this structure is functionally organized (Baxter and Murray, 2001). If this were true, and assuming behavioural changes were a direct result of hippocampal damage, more expansive hippocampal lesions would mimic the behavioural changes seen after smaller lesions (Baxter and Murray, 2001). Indeed, this seems to be reflected in our data.

It should also be noted that there exists a variant of the NVHL model, which involves reversible inactivation of the hippocampus by means of the Na\(^+\) channel blocker TTX on PD7 (Lipska and Weinberger, 2002). Though the use of TTX does not produce a physical lesion, it does recapitulate most of the deficits caused by the excitotoxic lesion, although to a lesser degree (e.g., less pronounced WM deficits) than that resulting from an excitotoxic lesion.
(Lipska and Weinberger, 2002). However, this suggests that even transient loss of VH activity during a developmentally sensitive time point is capable of causing permanent changes to the normal development of the neural pathways that mediate select dopamine- and NMDA-dependent behaviours (Lipska and Weinberger, 2002).

However, one must also acknowledge the possibility that lesion volume (i.e., ventricular enlargement) is not the primary determinant of developmentally delayed behavioural deficits seen in the NVHL model—a seemingly plausible scenario in light of the TTX lesion literature, presented above. For example, demyelination may be the primary determinant in the expression of cognitive deficits seen in our NVHL animals. Schneider and Koch (2005) showed that lesions of the mPFC using IBO in PD7 rats led to a significant loss of myelination in some efferent projections of the mPFC (e.g., hippocampus, thalamus, nucleus accumbens, and amygdala) in adulthood. These reductions in myelination were associated with impulsivity and pre-pulse inhibition deficits in the lesioned animals when they were tested as adults. Interestingly, findings of myelination reductions in schizophrenia patients are known to exist and are thought to result from a developmental disruption of oligodendrocyte function (Davis et al., 2003; Schneider and Koch, 2005). In clinical schizophrenia, oligodendrocyte and myelin dysfunction has been linked to cognitive symptoms with patients presenting with such dysfunction showing more severe cognitive dysfunction (Nestor et al., 2004; Fields, 2008; Cocci et al., 2009). Thus, future NVHL experiments may be aimed at employing the use of diffusion tensor imaging, along with structural MRI, in an effort to dissociate the contributions of ventricular enlargement and demyelination to the emergence of WM deficits.
Finally, it should be noted that a relationship between the volume of the AH (the human analog of the rat VH) and symptom severity in schizophrenia patients is unclear. For example, in patients with childhood onset schizophrenia, findings of bilateral inward deformation in AH correlated positively with the severity of positive symptoms (Johnson et al., 2013). Conversely, outward deformations in AH were positively correlated with measures of overall functioning (Johnston et al., 2013). Overlooked, however, is the relationship between AH shape or size abnormality and direct measures of WM. Unlike the findings of Johnson et al. (2013), Csernansky et al. (2002), despite noting significant abnormalities in AH shape and asymmetry, found no evidence of a relationship between the magnitude of AH abnormality and symptom severity or illness duration across 52 adult patients; whereas, Bilder et al. (1995) found a weak ($r = < 0.2$) yet significant correlation between AH volume and memory performance in patients. Considering the studies above, it becomes clear that the evidence linking AH structural abnormalities and cognitive symptoms, specifically WM deficits, in schizophrenia patients is inconclusive. Such inconclusive findings may result from the fact that, for the most part, research has been aimed at understanding the link between hippocampal abnormalities and positive or negative symptom severity, with significantly less effort directed at understanding the link between hippocampal abnormalities and cognitive symptom severity. Nonetheless, the fact that there exists no studies which show overwhelmingly convincing evidence of a clinically significant relationship between AH volume abnormalities and cognitive symptoms adds support to our previous hypothesis that WM deficits in schizophrenia are driven by factors other than reductions in hippocampal volume or ventricular enlargement.
4.3 Is delay-independent WM a feature of PFC damage?

Although the actual excitotoxic lesions were delivered to the hippocampus, it is believed that many of the cognitive deficits, such as those in WM, are actually the result of downstream changes in the PFC (Lipska, Al-Amin, and Weinberger, 1998; O’Donnel et al., 2002; Wood, Quirion, and Srivastava, 2003). For example, many of the behavioural changes that accompany NVH lesions are behaviours largely reliant on a properly functioning PFC, such as WM, cognitive flexibility, and amphetamine-induced hyperlocomotion. Indeed the delay-independent deficit on a WM task reported here, is also a feature of adult PFC lesions in isolation. For instance, following a bilateral NMDA-induced lesion of the dorsomedial prefrontal cortex (DMPFC), rats showed delay-independent performance deficits in a T-maze spatial WM paradigm (Sanchez-Santed et al., 1997). Similar delay-independent findings were also found in rats on a delayed non-match to position task, following excitotoxic lesions of the prelimbic cortex (Chudasama and Muir, 1997), as well as NMDA receptor blockade in the DMPFC (Juhana and Paavo, 1999). This same pattern of WM deficits was also observed in a delayed match to position task, following infusion of the cholinergic antagonist scopolamine into the mPFC (Herremans et al., 1996). Such delay-independent effects on WM performance following PFC lesions are also seen in non-spatial tasks. Following radiofrequency lesions to the mPFC, Porter, Burk, and Mair (2000) reported that adult rats exhibit delay-independent performance deficits in a retractable lever delayed match-to-sample task. Therefore, direct PFC damage can lead to a pattern of WM deficits that parallels the WM deficits found in the NVHL animals of the current dissertation.

The delay-independent effects discussed above for the rodent literature are also consistent with the results of parallel studies in non-human primates. In a nonspatial two-
object alternation task (for detailed description, see Petrides, 1995), monkeys with mid-dorsal lateral frontal cortex (LFC) lesions were found to perform significantly worse than control yet the deficit was delay-independent. Furthermore, lesioned animals eventually improved to the same level as control animals (Petrides, 1995). Petrides (1995; 1996) also had animals perform a self-ordered task in which they had to monitor two or three different stimuli, with a fixed delay length of 10 seconds. In this task, LFC animals performed significantly worse than control animals when they were required to monitor three different stimuli over the delay period. Thus, unlike delay length, the number of stimuli monitored within WM is a critical factor determining whether or not LFC lesions will result in an impairment of performance. These studies therefore are consistent with the claim that a delay-independent WM deficit is consistent with damage to the PFC.

4.4 From specific regions to pathways

While NVHL lesions produce a deficit similar to an adult PFC lesion, the same deficit is not produced by neonatal PFC lesions. In fact, neonatal PFC lesions do not result in a WM deficit whatsoever when animals are tested in adulthood. For example, de Brabander, de Bruin, and ven Eden (1990) lesioned the PFC of PD 6 rat pups and when tested on a spatial delayed-alternation WM task in adulthood, their performance was no different than the performance of control animals. Similarly, Schwabe et al. (2004) delivered excitotoxic lesions to the PFC of PD7 rat pups, and found that WM performance on a spatial delayed-alternation task was spared in adulthood. Finally, a study by Carter et al. (1995) reported while that rats with neonatal mPFC lesions displayed a delay-independent WM deficit when tested at PD 21 and 22, they performed no different from controls by PD 28.
The ability of neonatally PFC lesioned rats to ultimately show normal WM performance may be due to the ability of the developing PFC and its connections to reorganize and possibly regenerate neurons damaged early in life (Schwabe et al., 2004). This somewhat surprising conjecture is supported by studies showing normal morphology in the regions of remaining PFC directly outside the lesioned area of adult animals that received PFC lesions as neonates (de Brabander, de Bruin & van Eden, 1990; Kolb and Gibb, 1990; Kolb and Gibb, 1993; Schwabe et al., 2004). For example, PD10 PFC lesioned rats show no difference in dendritic arborization and spine density of layer II-III pyramidal cells at PD60 when compared to control animals (Kolb and Gibb, 1993). In contrast, no such recovery of morphology is observed in the adult VH following a NVH lesion, suggesting a hindered capacity of the developing VH, and its associated projections to other cortical regions, to recover from insult when compared to the capacity of the developing PFC. This suggests that the NVHL may produce enduring abnormalities in the VH-PFC circuitry that may ultimately prove more detrimental than having no PFC at all. Accordingly, postpubertal PFC lesions in NVH lesioned animals are actually able to restore behavioural (Lipska and Weinberger, 1998) and electrophysiological (Goto and O’Donnell, 2004) deficits resulting from the NVHL manipulation. Therefore, to the extent that the NVHL models the cognitive deficits in schizophrenia, it would seem that the cognitive deficits in these patients are not the direct result of damage to the hippocampus or PFC but rather dysregulation in the VH-PFC pathway.
4.5 The underlying nature of the WM deficit in the NVHL model and schizophrenia

As highlighted above, a central aspect of the WM impairment in NHVL rats was its delay-independence. Notably, delay-independence is also a characteristic of the WM deficit observed in patients with schizophrenia. For example, Gold et al. (2010), as well as an earlier meta-analysis by Lee and Park (2005), both reported an absence of a delay-dependence in the WM task performance of schizophrenia patients. Specifically, in a colour-matching task (for a complete task description, see Gold et al., 2010) of visual WM, schizophrenia patients have been shown to display a delay-independent impairment in task performance at multiple delay lengths. Further, it was noted that the standard deviation in patient performance (i.e., the error in patient-recalled colour, compared to the actual cued colour) was no different from that of control participants, which was taken to be suggestive of spared WM precision in patients (Gold et al., 2010). Consistent with the effects of PFC lesions reviewed above, a deficit in patient WM capacity was evidenced by a reduction in the number of items patients were able to maintain in WM (Gold et al., 2010). In general, WM capacity rather than delay-duration seem to be a key factor in the WM deficits of schizophrenia patients (e.g., Condray et al., 1996; Goldberg et al., 1998; Gold et al., 2003), consistent with what we have found in the NVHL animal model of schizophrenia.

What are the implications of a delay-independent WM deficit for mechanistic theories of schizophrenia? One popular view is that in schizophrenia WM representations may be characteristically unstable or noisy as a result of decreased stability of the attractor states that are thought to represent information with PFC networks (Lisman et al., 2008; Rolls et al., 2008; Durstewitz & Seamans 2008). If the attractor states that maintain WM information in
PFC were inherently unstable or susceptible to collapse due to noise or distraction in schizophrenia patients, then the likelihood of such collapse would be directly related to the delay interval. In other words, these theories predict the presence of a delay-dependent WM impairment. I would therefore argue that the delay-independent WM deficit in our NVHL animals is not likely a result of unstable WM maintenance in the PFC but rather impaired or noisy information encoding. An inability to initially form an internal representation of the WM task during the encoding stage would result in a WM deficit that is delay independent because, if the animals encoded the wrong stimulus or aspect of the vDNMS task, WM errors would occur irrespective of delay length. In accordance with this view, evidence of a slowed encoding process has been reported for schizophrenic patients by Hartman et al. (2002) who found that increasing the duration of stimulus presentation augmented patient performance in a visuospatial WM task. Additionally, Lee and Park (2002) found that increasing the attentional salience of target objects in a visuospatial WM task seemed to facilitate patient performance.

The underlying cause of the proposed aberrant encoding in our rats, just as in the human literature, is unclear; however, several explanations are plausible. In line with Hartman et al. (2002), it may be the case that our NVHL animals require a longer encoding period in order to form precise mental representations of the WM task. Another possibility is that poor encoding by our NVHL animals may be due to a reduced ability to select relevant information from, or efficiently direct their attention to, relevant features of the WM task (Adler et al., 1998; Braver, Barch, and Cohen, 1999; Lee and Park, 2005). Such deficits may be the cause of imprecise encoding, encoding of the wrong stimuli, or both, in our NVHL animals as well as in schizophrenic patients.
While encoding deficits are the most parsimonious explanation for a delay-independent WM deficit, it should be noted that even in experiments that allowed for optimized encoding, deficits in spatial WM were still observed in schizophrenia patients (Park, Swisher, and Knurek, 2001; Lee and Park, 2002). Therefore, the potential contributions of dysfunctional maintenance and/or retrieval processes cannot be excluded. Future studies are therefore required to disentangle the individual contributions of each WM element and their individual role in the cognitive deficits seen in schizophrenia patients.

4.6 Conclusion

The current dissertation provides insight into the contributions of developmental changes in neuro-physiological, -anatomical, and -chemical systems towards the expression of working deficits in schizophrenia. A major point of contention in Neuroscience is the ability to effectively translate the implications of basic research findings from the bench to the bedside. In order to bridge this translational gap, animal models must accurately reproduce behavioural endpoints that characterize patient illness. The current dissertation found that neonatal ventral hippocampal lesion volume did not correlate with performance deficits on a vDNMS task, suggesting that lesion volume alone cannot explain for the variance in working memory deficits in our NVHL animals. Additionally, it was found that NVHL animals display delay-independent deficits on a vDNMS task of working memory, which are consistent with deficits during the encoding stage of working memory. These two findings are consistent with those of the existing literature on schizophrenia patients.
Each trial was composed of a “forced” and “choice” run, in that order. Beginning with the “forced” run, animals started in the “start” location and would travel down the stem, towards the two perpendicular choice arms, where either the left or right choice door was closed. This forced the animals down the opposite arm, where they would then travel back to the start location, via the return arm. This commenced the end of the “forced” run. Upon returning to the “start” location, animals were contained there via closure of the return doors and stem door, for the specified delay time (10s, 20s, or 30s). Following the end of the delay period, the “choice” run would begin by opening the stem door and both choice arm doors, giving them free access to either choice arm. In order to receive a food reward, animals were required to travel back down the stem and into the arm opposite the one visited in the “forced” run.
Figure 2. Illustration of disadvantages in using histology for lesion volume calculations.

Images show a common issue encountered when using histology to determine lesion volume. Figures 2a) and 2b) are taken from two different animals, at roughly -3.5 mm and -3 mm from bregma, respectively. Red arrows indicate areas of ventricular enlargement and tissue loss. **Figure 2a** Lack of CSF in the ex-vivo brain (left) causes outlying tissue to collapse inward. Thus, calculation of lesion volume from histology can lead to an underestimate of true lesion volume obtained from MRI. **Figure 2b** Due to the delicate nature of lesioned tissue, histological processing often causes the damage to the brain, which is especially evident following mounting on a microscope slide. By contrast, the MRI image on the right shows an accurate picture of what the true lesion extent was in this animal. Thus, calculation of lesion volume from histology can also lead to an overestimate of the true lesion size obtained from MRI.
Figure 3. Comparative images of MRI and histology.

Images depict the MRI and histological views of sham lesioned animals, as well as examples of small and large NVH lesions. Arrows indicate areas of ventricular enlargement and tissue loss. Coronal slices were taken at roughly -4 mm and -6 mm relative to bregma.
Figure 4a-n. 3D models of NVHL lesion shapes and respective performance.

Figures a) through n) depict the MRI-generated 3D models of lesion volume of each NVHL animal, as well as each animals’ respective vDNMS performance across the five days of testing (bar graph). Figures are arranged in order of smallest (a) to largest (n) lesion size. I) Shows coronal view of lesion at most anterior end. II) Shows coronal view of lesion at most posterior end. III) Shows left-sagittal view of lesion. IV) Shows right-sagittal view of lesion. For figures III) and IV), “R” and “C” refer to rostral and caudal coordinates, respectively.
**Total lesion volume:** 16.52 mm$^3$
Total lesion volume: 19.37 mm³
Total lesion volume: 22.50 mm³
4c)

![Graph showing average performance (%) vs. vDNMS delay length (s).](image)

![Images of brain regions labeled I, II, III, and IV.](image)

Total lesion volume: 26.88 mm$^3$
4f)

Average performance (%)

Rostral

Caudal

I

II

III

Rostral

Caudal

IV

Caudal

Rostral

Total lesion volume: 29.39 mm³
I

II

III

IV

Total lesion volume: 30.53 mm$^3$
Total lesion volume: 35.80 mm³
Rostral
Caudal
I
II
III
IV

4i)

Average performance (%)

0 20 40 60 80 100

vDNMS delay length (s)

10 20 30

Total lesion volume: 45.47 mm³
Total lesion volume: 49.05 mm³
Total lesion volume: 50.47 mm³
Total lesion volume: 53.64 mm³
Total lesion volume: 55.40 mm³
Rostral
Caudal

Rostral
Caudal

Total lesion volume: 63.01 mm³
Figure 5. Comparison of sham and NVHL animals on days preceding criterion performance on the 1s DNMS task

Sham, compared to NVHL, animals performed significantly better on the 1s DNMS task across all three days required to reach criterion performance (defined as at least 80% correct trials over three consecutive days).
Figure 6. Daily performance of all animals in the vDNMS task.

NVHL rats performed significantly worse than control animals at all delay lengths and across all days of testing (* p < 0.05).
Figure 7. Relationship between lesion volume and vDMNS performance.

In the NVHL group, ventral hippocampal lesion volume showed no statistically significant relationship with performance deficit at any delay length.
Figure 8. Relationship between lesion volume and days to reach criterion performance on the 10s DNMS task.

In the NVHL group, lesion volume showed no statistically significant relationship with the number of days required to reach criterion performance (defined as <10% variability in performance) in order to advance to the vDNMS task.
Bibliography


Guidotti A., Auta J., Davis J.M., et al. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder. *Archives of General Psychiatry, 57*, 1061–1069.


Seeman P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse, 1*, 133-152.


