SPONTANEOUS RECOGNITION IN RATS: SYNAPTIC PLASTICITY AND NEURODEVELOPMENTAL CHALLENGE

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ABSTRACT

Disruptions in memory are a hallmark feature of several psychiatric diseases. These illnesses are often marred by an inability to recognize that a stimulus or event as been previously experienced, a phenomenon known as recognition memory. Previous study has demonstrated that cognitive disruptions reflect aberrant signaling, including disruptions in synaptic plasticity, in key regions of the brain, such as prefrontal cortex (PFC), hippocampus, and perirhinal cortex (PRh). However, in the case of recognition memory, how these disruptions arise and what specific plasticity mechanisms are involved is less clear. An understanding of the etiological factors underlying disruption and the synaptic processes involved in recognition will greatly advance the treatment and prevention of psychiatric disorders. As a result, the present thesis examined recognition memory in rodents in two experiments. In the first experiment, we blocked the endocytosis of AMPA receptors during the encoding, consolidation, or retrieval phase of object recognition memory using local PRh infusions of the cell membrane permeable Tat-GluA2$_{3Y}$ interference peptide. Tat-GluA2$_{3Y}$ infusion before the encoding and consolidation phases did not alter memory. In contrast, Tat-GluA2$_{3Y}$ infusion prior to the retrieval phase significantly disrupted memory. These results indicate a distinct role for AMPA receptor endocytosis during a specific phase (retrieval) of visual recognition memory. In the second experiment, pregnant dams were treated with PolyI:C (4mg/kg, i.v.) on gestational day (GD) 15, and both the male and female offspring of these rats were tested as young adults in three different recognition memory tests: spontaneous novel object recognition, novel object location recognition, and object-in-place recognition. Male, but not female, rats were impaired in an object-in-place memory test that depends on processing between medial temporal lobe and PFC. However, neither male nor female rats were impaired on tests of simpler discriminations dependent on the medial temporal

lobe. These findings support clinical studies demonstrating impaired object location binding in clinical populations and further demonstrate the plausibility of prenatal immune activation as an etiological factor in neurodevelopmental disease. Taken together, these results highlight the importance of a specific form of synaptic plasticity during the recognition of familiar stimuli and demonstrate that early life adversity can disrupt recognition memory processes.

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AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid
AP	anterior-posterior
Ca^{2+}	divalent calcium
DMS	delayed match to sample
DNMS	delayed non-match to sample
DR	discrimination ratio
DV	dorsal-ventral
Ent	entorhinal cortex
EtOH	ethanol
GD	gestational day
IL	interleukin
LPS	lipopolysaccharide
LTD	long-term depression
LTP	long-term potentiation
mAChR	muscarinic acetylcholine receptor
mGluR	metabotropic glutamate receptor
ML	medial-lateral
Na^{+}	monovalent sodium
NMDA	N-methyl-D-aspartate
PND	postnatal day
PolyI:C	polyriboinosinic-polyribocytidilic acid
PFC	prefrontal cortex

PRh	perirhinal cortex
Ser	serine
TLR	toll-like receptor
Tyr	tyrosine
VDCC	voltage gated Ca^{2+} channel
VOLT	visual object learning test

CHAPTER 1
GENERAL INTRODUCTION

1.1. Learning and Memory

The ability to remember is central to human experience. From birth to death, our memories shape what we know about ourselves, what we know about the world, and how we interact with our world. The importance of memory is further underscored by the consequences that emerge as a result of the memory loss observed in neurological pathology. Illnesses including Alzheimer's disease, Huntington's disease, and schizophrenia are characterized by enduring cognitive deficits, including changes in memory, that often result in profound frustration and devastation for disease sufferers and their families.

For several years, how and where the brain represents memory have been central questions in the field of neuroscience. Considerable evidence now suggests that memory is supported by the brain's capacity for plasticity (Eichenbaum 1996; Eichenbaum 1999; Martin et al. 2000; Roman et al. 1987; Whitlock et al. 2006). Neuronal firing patterns are not fixed; rather, they show incredible faculty for change based on experience, enabling the processing and storage of vast amounts of information (Collingridge et al. 2004; Hofer et al. 2009; Hubener and Bonhoeffer 2010; Tropea et al. 2009). At present, we are only beginning to understand the processes underlying this plasticity. Rigorous study of these mechanisms as they relate to memory will undoubtedly lead to an understanding of how the brain represents memory and inform better treatment and prevention of the disorders characterized by disruptions in memory.

1.2. Definition and Characteristics of Recognition Memory

Memory can be divided into two distinct classes: declarative memory, characterized by conscious memory for facts and events; and non-declarative memory, characterized by unconscious changes in skilled behaviour or motor performance often as a result of practice (Kandel 2009). Previous study has demonstrated that these distinct types of memory display considerable differences in brain regions of interest, susceptibility to disruption by competing information, degree of permanence, and presentation in animal species (Squire et al. 1993;

Tulving and Schacter 1990). Declarative and non-declarative memory can be further divided into several sub-classes including semantic and episodic memory in the declarative domain and non-associative learning, associative learning, procedural memory, and priming in the non-declarative domain (Squire and Kandel 2009). This thesis focuses on the mechanisms and disruptions of recognition memory, a particular type of declarative memory that plays a fundamental role in conscious memory for past experience.

Recognition memory describes the capacity to determine that a stimulus has been previously encountered. This broad definition encompasses memory for previously experienced objects, places, and events. Generally, recognition memory is divided into distinct phases: encoding, consolidation, retrieval, and reconsolidation. During encoding, information about a novel stimulus is acquired while during consolidation, this information is stored as memory. Upon reintroduction to a stimulus (retrieval phase), the memory is recalled. This retrieved memory exists in a particularly labile state at which time it is vulnerable to disruption or update by new information; this lability means each time the memory is recalled, it must be re-stored or reconsolidated to maintain permanence (Nader et al. 2000; Nader and Einarsson 2010). As with most forms of declarative memory, the strength of recognition memory differs with degree of encoding and delay between the acquisition and subsequent recall of the memory (Mumby et al. 2005; Ozawa et al. 2011a; Paul et al. 2005; Schacter and Wagner 1999). In general, with longer delays between the first and second exposure to a stimulus, the memory becomes weaker in strength while deeper, more meaningful encoding tends to increase the strength of the memory. Interestingly, short and long delays may require differential processing in distinct brain areas (Barker et al. 2006b; Hammond et al. 2004; Mumby et al. 2007).

Early experiments in humans suggested that the recognition of familiar stimuli was characterized by two distinct processes: familiarity (feeling), and recollection (knowing; Hanley 1984; Mandler 1981). Subsequent experimentation has cemented familiarity and recollection as distinct processes in a dual processing model of recognition with divisions made on the basis of attentional requirements, speed of processing, degree of encoding, and susceptibility to disruption (Eichenbaum et al. 2007; Gruppuso et al. 2007; Rugg and Yonelinas 2003; Yonelinas 1997; Yonelinas 2001). Specifically, recollection requires greater attention towards stimuli during encoding and retrieval, and recall of this sort tends to be slower than with familiarity discriminations. In contrast, familiarity judgments involve more superficial encoding and are

more susceptible to disruption as a result of perceptual changes between the encoding and retrieval of information (review (Eichenbaum et al. 2007). In this sense, familiarity describes the abstract and less confident sense of having experienced a stimulus before whereas recollection describes more conscious, detailed recall. Lesions of specific brain regions proposed to be involved in recognition disrupt recollection without disturbing familiarity (Aggleton et al. 2005; Holdstock et al. 2002; Vargha-Khadem et al. 1997; Yonelinas et al. 2002) while electroencephalogram (EEG) and functional magnetic resonance imaging (fMRI) studies provide strong evidence for a separation between familiarity and recollection (Brewer et al. 1998; Eldridge et al. 2000; Henson et al. 1999; Rugg et al. 1998).

Most studies of recognition memory have focused on visual recognition of discrete objects or words whereas previously encountered places and events have historically fallen under the spatial and episodic memory domains. In more recent years, study of recognition memory has grown to consider how previously encountered objects are represented in association with their particular location in space or in relation to other objects (Barker et al. 2007; Mumby et al. 2002; Oliva and Torralba 2007; Postma et al. 2008; Vargha-Khadem et al. 1997; Warburton and Brown 2010). These studies have provided novel insight regarding the different brain regions and neurobiological mechanisms involved in recognition memory when only simple object discriminations are required versus more complex discriminations requiring greater perceptual processing and interactions between several sensory modalities. Tests of this type have become particularly important in assessing the early cognitive deficits that arise in neurological and psychiatric pathology.

1.3. Animal Preparations of Recognition Memory

The susceptibility of recognition processes to disruption in both neurological and neuropsychiatric disorders necessitates a more thorough understanding of the processes involved in recognition. The type of invasive manipulation required to understand such processes is not possible in humans, and tests of recognition memory in both non-human primate species and rodents have emerged as suitable alternatives. In primates, the most commonly used tests of recognition memory are the delayed non match to sample (DNMS) test and the delayed match to sample (DMS) test (Eichenbaum et al. 2007; Winters et al. 2008). In both tests, monkeys are

tested in a sample phase, during which they encounter a novel object that can be displaced to receive food reward; and in a test phase, during which they encounter the sample object and a novel object. In DNMS, monkeys must displace the novel object to receive food while in DMS, they must displace the familiar object. This task is performed over several trials with many different object pairs to facilitate rule learning. The considerable amount of training required for D(N)MS tests in monkeys as well as the expense and ethical considerations of housing and experimenting with non-human primates has led to the development of objection recognition in rodents, particularly the spontaneous one trial novel object recognition paradigm (Dere et al. 2007). In this paradigm, similar to the DNMS, rodents are tested in a sample and test phase separated by a variable delay. During the sample phase, rodents freely explore two identical objects in an open field. During the test phase, rodents again explore one copy of the sample object and one novel object. Unlike D(N)MS, this recognition paradigm relies upon a rodent's innate preference for novelty (Ennaceur 2010; Ennaceur and Delacour 1988), so the task requires no training that may confound results (Winters et al. 2008). This lack of requirement for extensive pre-training as well as relative ease of implementation has made the spontaneous one trial object recognition paradigm a popular test for the examination of memories of prior occurrence, and it is through this model especially that the neurobiological correlates of recognition memory have been resolved to date. Further, this paradigm has been manipulated to impinge on more complex processing and incorporation of several brain regions working in conjunction (Barker et al. 2007; Dere et al. 2007; Eacott and Norman 2004; Hannesson et al. 2004; Mumby et al. 2002; Warburton and Brown 2010). Several laboratories now incorporate studies of object location memory, concerning memory for familiar objects in novel locations, and object-in-place memory, concerning memory for objects in relation to location and each other, in tests of recognition. These tests follow the aforementioned shift of the human literature to studies of object association, making them especially adept for modeling key cognitive deficits of psychiatric disease. It is for these reasons that the spontaneous object recognition memory paradigm in rats was chosen for study in the present thesis.

1.4. Mechanisms of Recognition Memory

Recent study in humans, non-human primates, and rodents has contributed significantly to our understanding of the neural circuitry and neurobiological mechanisms integral to the recognition of familiar stimuli. In particular, these studies have focused on distinct brain regions of the medial temporal lobe and the changes that occur in the connections between neurons as a result of activity and experience. While this literature is extensive, this thesis is primarily concerned with synaptic processes in a particular region of the brain, perirhinal cortex (PRh) and how early developmental adversity (prenatal infection) may disrupt these processes.

1.4.1. Brain Regions of Interest

In 1957, Brenda Milner and colleagues first described the extensive memory impairments of Henry Molaison, or H.M. Following bilateral resection of the medial temporal lobes to treat intractable epilepsy, H.M. was rendered unable to form new long-term memories but was able to retain memory for many of the events that occurred prior to his surgery (Scoville 1954; Scoville and Milner 2000). Subsequent study demonstrated that this impairment was restricted to declarative memories, including profound deficits in two tests of recognition memory, the paired association and DNMS tasks. Conversely, memories non-declarative in nature, including procedural and perceptual learning, were largely spared (Corkin et al. 1964; Milner 1965; Milner et al. 1968; Milner 1972; Milner and Taylor 1972; Sidman et al. 1968). This case study and others has highlighted the medial temporal lobe as fundamental in supporting declarative memories, including recognition memory.

Since then, considerable debate has surrounded which specific areas of the medial temporal lobe are necessary for the recognition of objects. Much of the debate has focused on the relative requirements of hippocampus, a major component of the limbic system, and PRh, a region of cerebral cortex that shares reciprocal connection with hippocampus (Broadbent et al. 2004; Brown and Aggleton 2001; Brown and Xiang 1998; Norman 2010; Warburton and Brown 2010; Yonelinas et al. 2010). Several lesion and functional magnetic resonance imaging (fMRI) studies as well as electrophysiological data have demonstrated a role for both (Clark et al. 2000; Holdstock et al. 2002; Mumby et al. 2005; Mumby and Pinel 1994; Pascalis et al. 2004; Winters

et al. 2010; Wood et al. 1993; Zola et al. 2000; Zola-Morgan and Squire 1985; Zola-Morgan and Squire 1986). However, more recent evidence suggests that hippocampus may only be required when the memory requires use of spatial or contextual information or significant association between objects (Eacott and Gaffan 2005; Winters et al. 2010). In cases requiring only object information, PRh is sufficient to support the memory and lesions of hippocampus have minimal effect (Bussey et al. 2000; Forwood et al. 2005; Jackson-Smith et al. 1993; Kesner et al. 1993; Mumby 2001; Rawlins et al. 1993; Winters et al. 2004). For this reason, the present thesis focuses on PRh as the primary region implicated in the simple discrimination of novel and familiar stimuli. As will become clear in Chapter 3, study was extended to consider how PRh interacts with both hippocampus and another brain region, prefrontal cortex (PFC) in paradigms involving more complicated discriminations.

1.4.2. Cortical Anatomy and Connections of PRh

The anatomical boundaries of PRh have seen considerable modification since first described by Brodmann (Brodmann 1909), and some differences arise between the delineation of PRh in the non-human primate and the rat. In monkeys, PRh consists of two cytoarchitecturally distinct areas: the agranular Area 35 and granular Area 36 (Suzuki 1996). Both these areas lie lateral to the rhinal sulcus, lining its entire length. Perirhinal cortex is bounded medially by entorhinal cortex (Ent) and laterally by temporal association areas (Te2 and Te3; (Suzuki 1996; Suzuki and Amaral 1994a; Suzuki and Amaral 1994b; Suzuki and Amaral 2003). Based on a meta-analysis of cytoarchitectural, chemoarchitectural, and connectivity data, Burwell and colleagues (1994) suggest that in rat, PRh also consists of Area 35 and 36, but it only lines the more caudal position of the rhinal sulcus (Figure 1.1). It is bounded ventrally by Ent, dorsally by multimodal and auditory association cortices, rostrally by insular cortex, and caudally by postrhinal cortex consisting of areas TH and TF (Burwell et al. 1995; Kealy and Commins 2011). Within Area 35 and 36, pyramidal neurons predominate; however, the number of pyramidal neurons in PRh is lower than in other areas of the cortex (Furtak et al. 2007). These pyramidal neurons display various spiking patterns including fast spiking, regular spiking, and burst firing (Beggs and Kairiss 1994; Faulkner and Brown 1999; McGann et al. 2001; Moyer, Jr. et al. 2002) with differential distribution of neuronal firing type in different cortical layers.

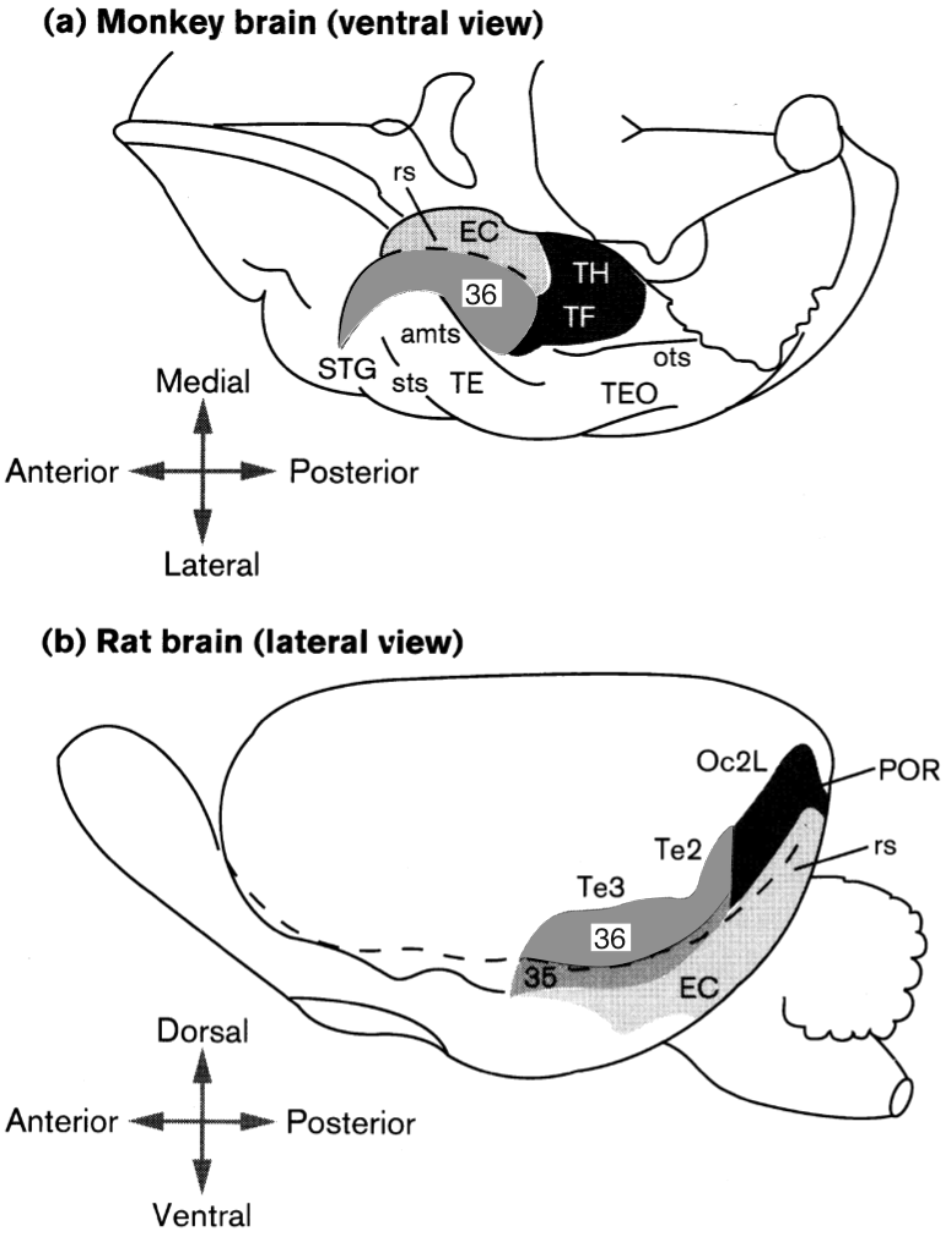

Figure 1.1. Perirhinal cortex of monkey and rat. Area 36 (35 not seen in ventral view) of monkey perirhinal cortex (A) and Area 35 and 36 of rat perirhinal cortex (B) are shown (dark grey shading). Perirhinal cortex in both monkeys and rats shares dense reciprocal connection with entorhinal cortex (EC; light grey shading). Also shown (black) are parahippocampal cortex in monkey (TH and TF) and the counterpart in rat, postrhinal cortex (POR). Figure reproduced with permission from Xiang and Brown 1998.

Perirhinal cortex has anatomical connections with several cortical and subcortical brain regions, including regions of the frontal and temporal lobes (Figure 1.2.; Burwell et al. 1995; Kealy and Commins 2011; Suzuki 1996). Inputs to PRh arise from unimodal and polymodal sensory areas; in monkey, these inputs are largely visual while in rat, inputs arise more evenly from somatosensory, auditory, olfactory, and visual areas (Suzuki 1996). Temporal lobe afferents of PRh include dense connections from unimodal visual cortices (TE and TEO) as well as afferents from polymodal association areas including parahippocampal regions (TF and TH) and superior temporal sulcus. While all of these areas project to Area 35 and 36, area TE has more dense projections to Area 35 than Area 36 while Area 36 receives more dense polymodal projections (Burwell et al. 1995).

A large amount of the debate between the necessity of PRh and hippocampus for recognition memory may arise from the dense reciprocal connections between PRh and hippocampus, equivocating lesion studies examining the general role of these structures in recognition. Two thirds of the cortical afferents to Ent, the major input structure of hippocampus, arise from PRh and parahippocampal cortex, with most PRh connections projecting laterally (Burwell and Amaral 1998; Suzuki 1996; Suzuki and Amaral 1994b). In turn, Ent densely projects back to PRh, largely from the rostral portion and terminating in all layers of PRh (Burwell and Amaral 1998). Perirhinal cortex further shares direct connection with hippocampus (Liu and Bilkey 1996a; Liu and Bilkey 1998; Segal and Landis 1974; Suzuki and Amaral 1990; Witter et al. 1989; Witter and Groenewegen 1984), projecting to dentate gyrus and distal CA1. Hippocampus, especially CA1, directly and indirectly (through subiculum) projects back to PRh terminating mostly in layers V and VI (Burwell and Amaral 1998; Deacon et al. 1983; Swanson and Cowan 1977; van and Wyss 1990).

Perirhinal cortex shares anatomical and functional connectivity with PFC. Reciprocal connections are found between medial precentral cortex, anterior cingulate cortex, prelimbic cortex, and infralimbic cortex (Deacon et al. 1983; Hoover and Vertes 2007; Sesack et al. 1989) Efferents to PFC largely originate in layer III/V or V/VI of PRh with the strongest connection terminating in dorsal anterior cingulate (Agster and Burwell 2009). Barker et al. (2007) suggest that in tests of object context or object association these connections become particularly important as lesions of both PFC and PRh significantly disrupt object association memory.

Finally, PRh has reciprocal connections with striatum and amygdala. Perirhinal cortex projects to lateral, basal, and accessory basal nuclei of amydgala in both monkey and rat, with Area 36 having more dense connectivity (Amaral and Price 1984; Burwell et al. 1995; Stefanacci et al. 1996; Suzuki 1996). Both Area 35 and Area 36 project to nucleus accumbens (Burwell et al. 1995; Suzuki 1996). Overall, PRh has connections with several cortical and subcortical structures (Figure 1.2). Through its connections with sensory association areas as well as a number of different structures implicated in memory, PRh is primed as a region for complex processing and a role in recognition.

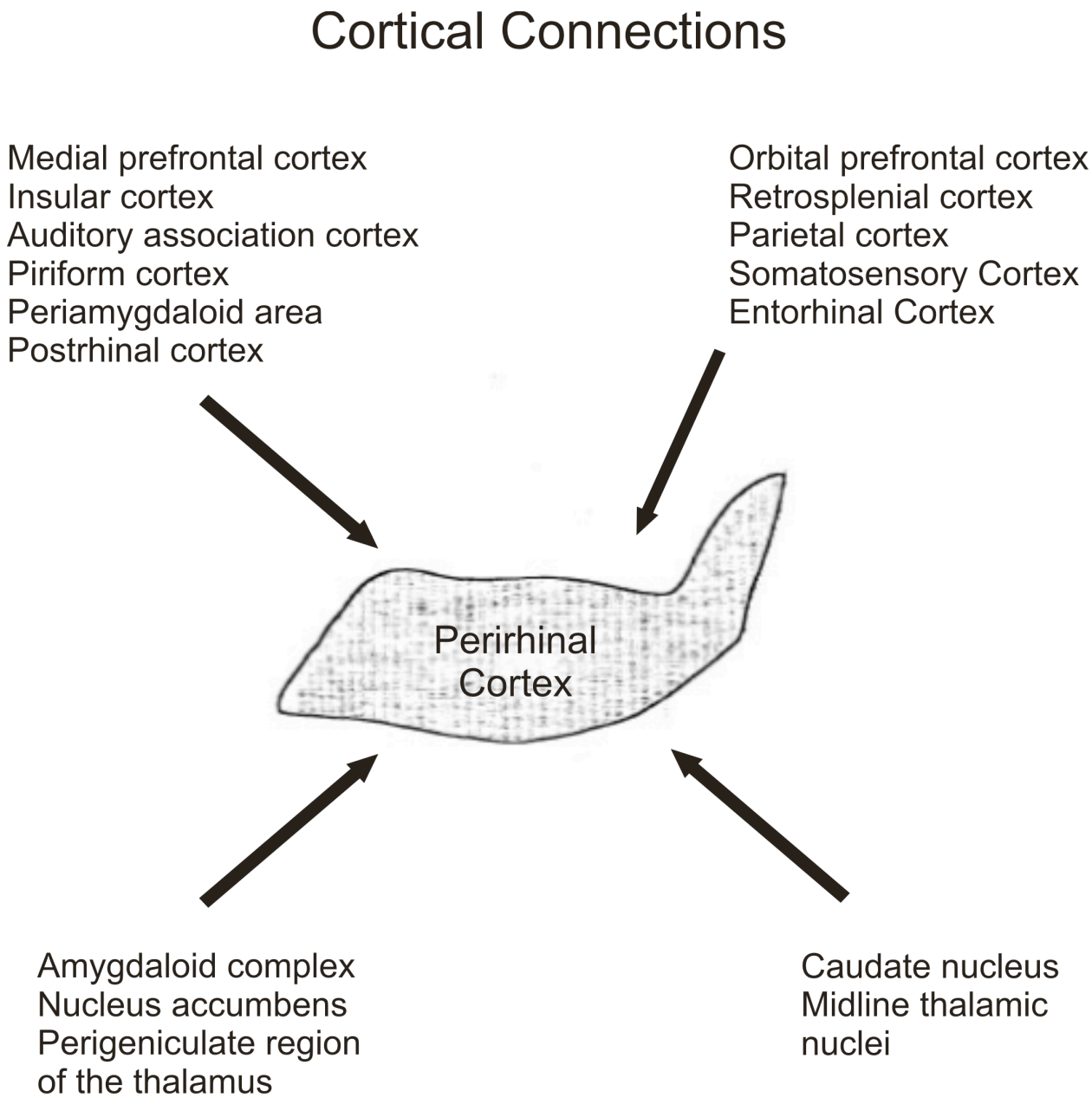

Figure 1.2. Anatomical connections of perirhinal cortex. Perirhinal cortex (PRh) shares connections with several regions of the brain, including dense connections with entorhinal cortex and hippocampus, prefrontal cortex, amygdala and striatum. Figure adapted from Burwell et al. 1995.

1.4.3. Neurobiological Substrates of Recognition Memory: Neurotransmitter Systems and Synaptic Plasticity

Much evidence now reinforces the notion that memories are supported by long-term activity dependent changes across the connections, or synapses, between neurons. Of the synaptic changes that occur in the brain, two have received particular focus: long-term potentation (LTP) and long-term depression (LTD). Bliss and Lomo (Bliss and Lomo 1973) first described LTP using electrophysiological slice recordings in rabbit brain whereby high frequency stimulation (HFS) to the perforant path input of the hippocampal dentate gyrus resulted in long lasting amplification of excitatory post synaptic potentials. In 1982, Ito and colleagues, in studies of the cerebellar Purkinje fibers, described a synaptic counterpart of LTP, LTD, whereby low frequency stimulation (LFS) produces long lasting attenuation in excitatory post synaptic potentials (Ito et al. 1982; Ito 1983; Ito and Kano 1982). Long-term potentiation and LTD have since been documented in several regions of the brain, in both excitatory and inhibitory neurons, arising through the coordinated activity of several different signaling cascades, most notably those involving glutamatergic signaling (Abraham and Bear 1996; Bear and Abraham 1996; Bliss and Collingridge 1993; Collingridge et al. 2004; Collingridge et al. 2010; Feldman 2009; Malenka and Bear 2004).

In both cortical and subcortical areas, glutamatergic N-methyl-D-aspartate (NMDA) receptor dependent LTP is the most well characterized and understood form of synaptic plasticity (Figure 1.3.). In this form of LTP, activation of NMDA receptors both through postsynaptic depolarization and presynaptically released glutamate leads to an increase in intracellular Ca^{2+} in postsynaptic neurons (Collingridge and Bliss 1995; Malenka and Bear 2004). This rise in intracellular Ca^{2+} results in the activation of several intracellular proteins, most notably α–calcium/calmodulin-dependent kinase-II (αCAMKII; (Bliss and Collingridge 1993). CAMKII, in turn, activates a series of intracellular signaling cascades resulting in trafficking and insertion of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid (AMPA) receptors in the postsynaptic membrane (Kauer et al. 1990; Luscher et al. 1999; Malenka and Bear 2004). This postsynaptic increase in the number of AMPA receptors results in an overall fast increase in excitatory current and an amplification in signal across the synapse (Bliss and Collingridge 1993; Collingridge and Bliss 1995; Kauer et al. 1990; Kauer and Malenka 2007; Malenka and Bear

2004). Further maintenance of this amplification is achieved through the production of new proteins and growth of dendritic spines (Hubener and Bonhoeffer 2010; Matsuzaki et al. 2004; Yuste and Bonhoeffer 2001). While NMDA receptors were first implicated in LTP, what has become clear over the last several decades is that this view of LTP is not complete in itself nor is NMDA receptor dependent LTP the exclusive form of LTP in the brain. Several studies now provide evidence for NMDA-independent LTP, dependent upon presynaptic increases in intracellular Ca^{2+} and an increase in the presynaptic release of glutamate (Castillo et al. 1997; Kauer and Malenka 2007; Malenka and Bear 2004; Nicoll and Malenka 1995; Nicoll and Schmitz 2005; Zalutsky and Nicoll 1990). Further increases in intracellular Ca^{2+} in the postsynaptic membrane may arise from sources other than NMDA receptors including voltage gated calcium channels (VDCC) and intracellular Ca^{2+} stores while several other neurotransmitter systems, including dopamine and acetylcholine, modulate the induction and maintenance of LTP (Goto et al. 2010; Kenney and Gould 2008; McKay et al. 2007).

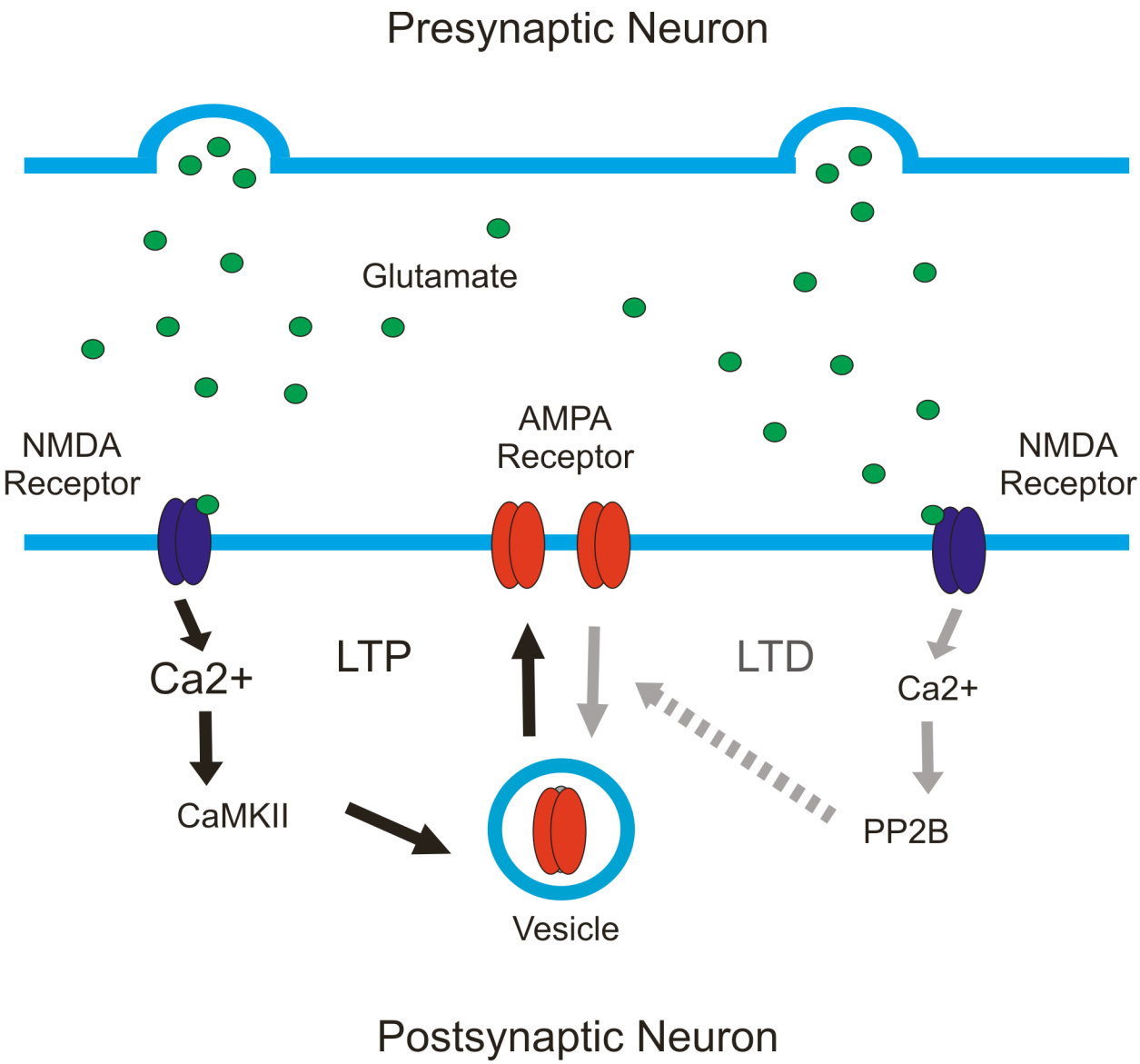

Figure 1.3. NMDA receptor dependent synaptic plasticity. Several forms of long-term potentiation (LTP) and long-term depression (LTD) have been described. The most common of these processes involves activation of NMDA receptors and either the insertion of AMPA receptors (LTP) or the endocytosis of AMPA receptors (LTD). Both processes involve several intracellular proteins in distinct calcium dependent signaling cascades. (AMPA = α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid; CAMKII = calcium/calmodulin dependent kinase II; NMDA = N-methyl-D-aspartate; PP2B = protein phosphatase 2B).

Like LTP, LTD has also been demonstrated to have NMDA receptor dependence (Figure 1.3.; Bear and Abraham 1996; Collingridge et al. 2010). Activation of NMDA receptors through low frequency stimulation (LFS) results in increases in postsynaptic Ca^{2+} triggering a number of different phosphatases, including protein phosphatase 2B (PP2B, or calcineurin), that in turn dephosphorylate several proteins including AMPA receptors. Dephosphorylation of AMPA receptors induces the endocytosis of these receptors from the dendritic membrane, resulting in a decrease in synaptic strength and attenuation of the excitatory potential (Collingridge et al. 2004; Collingridge et al. 2010; Malenka 2003; Malenka and Bear 2004). Several other forms of LTD have now been characterized, most notably those whose induction depends upon metabotropic glutamate receptors (mGluR) and endocannabinoids. Metabotropic glutamate receptors have several subunit types and different brain regions show subunit specific LTD (Collingridge et al. 2010). Like NMDA receptor dependent LTD, mGluR dependent LTD in at least some cases converges upon AMPA receptor endocytosis as a mechanism for LTD maintenance with this endocytosis activated in a cascade involving the IP3/DAG activation of protein kinase C (PKC). However, other mechanisms for the activation of AMPA receptor endocytosis as well as alternative mechanisms for LTD maintenance with mGluR induction, including glutamate receptor desensitization and decreases in presynaptic glutamate release (Kameyama et al. 1998; Kemp and Bashir 2001; Massey and Bashir 2007), have not been extensively explored.

Both LTP and LTD have been demonstrated electrophysiologically in PRh. In slice preparations, high frequency tetanic stimulation of 100 Hz induces potentiation in both PRh layer I and layer II/III; this LTP is inhibited by (2R)-amino-5-phosphonovaleric acid (APV) blockade of NMDA receptors, indicating NMDA dependence (Bilkey 1996); however, other receptor types including TrKB (Aicardi et al. 2004) and $GABA_A$ (Wan et al. 2004) also play a role. Low frequency stimulation using both 1 Hz and 5 Hz tetanic stimulation readily induces LTD in PRh (Aicardi et al. 2004). Both NMDA dependent and mGluR dependent LTD have been demonstrated, with particular dependence of mGluR LTD on group I, II, and III mGluRs (Cho et al. 2000; Cho et al. 2002; Griffiths et al. 2008; Ziakopoulos et al. 1999). Interestingly, unlike other areas of the brain where NMDA receptor and mGluR dependent LTD are observed independently from one another, NMDA and mGluR I dependent forms of LTD must act in concert to produce depression in PRh. Further, this PRh LTD also displays a voltage dependence as mGlu II receptors are further required when LTD is evoked at -70 mV but not at -40 mV (Cho

et al. 2000; Cho and Bashir 2002; Kealy and Commins 2011). This suggests that mGluRs and NMDA receptors modulate the activity of each other, allowing for more varied responses within PRh depending on prior activation. Blockade of voltage gated L-type Ca^{2+} channels, muscarinic acetylcholine receptors (mAChRs), and glutamatergic kainite receptors have all been shown to disrupt PRh LTD in slice, without concomitant disruptions in LTP, indicating a specific role for these receptors in PRh LTD (Massey et al. 2001; Park et al. 2006; Seoane et al. 2009; Warburton et al. 2003). At least some of these forms of LTD likely involve the removal of AMPA receptors from the synapse as blockade of AMPA receptor endocytosis disrupts LTD in slice.

Perirhinal long-term depression, in particular, has received much focus as a potential substrate for recognition memory. Many of the substances that block LTD, but not LTP, also disrupt recognition memory in vivo (Barker et al. 2006a; Griffiths et al. 2008; Seoane et al. 2009; Warburton et al. 2003) while electrophysiological recordings from both rats and monkeys demonstrate decreased responding in PRh neurons following reintroduction to a familiar stimulus (Fahy et al. 1993; Xiang and Brown 1998; Zhu and Brown 1995). However, many of these studies have not considered the distinct time points of memory in pharmacological manipulation, so understanding of the processes underlying encoding, consolidation, retrieval, and reconsolidation are incomplete. Further, to date, few pharmacological studies of synaptic plasticity in PRh have been conducted in electrophysiological recordings in vivo, likely due to the experimental difficulty of accurately positioning electrodes in this region. Whether the LTP and LTD phenomenon noted in slice recordings also translate to systems level recording remains an open question.

In addition to LTP and LTD within PRh, synaptic plasticity between PRh and other regions of the brain have also been described. High frequency and low frequency stimulation of PRh-hippocampal CA1 induces LTP and LTD respectively (Cousens and Otto 1998; Ivanco and Racine 2000; Kealy and Commins 2009; Kealy and Commins 2010; Liu and Bilkey 1996b; Naber et al. 1999). These connections are reciprocal and plasticity has been shown to depend upon NMDA receptor activation. As previously described, PRh shares dense connectivity with other regions of the brain, including PFC; however, studies of synaptic plasticity in these regions has not been examined.

In Chapter 2 of this thesis, I examine the role of a particular mechanism of PRh synaptic plasticity, AMPA receptor endocytosis (proposed to underlie LTD) in distinct phases of the

object recognition memory process. As will become clear in Chapter 2, few studies have provided evidence for phase specific effects of plasticity; this is surprising given evidence for long-term depressive like changes upon the second, and not the first, encounter of an object. AMPA receptors undergo both regular (constitutive) and activity dependent (regulated) internalization from the plasma membrane. This endocytosis is clathrin mediated and dependent upon adaptor complex 2 (AP2) interaction with the GluA2 subunit of the AMPA receptor, a process likely initiated by the activity of several upstream phosphatases and kinases (Collingridge et al. 2004). In particular, during regulated endocytosis, the phosphorylation of the Tyr876 residue on the C terminus of the GluA2 subunit (possibly by Src kinases) is required for endocytosis; disrupting this activity can disrupt endocytosis and LTD (Ahmadian et al. 2004; Collingridge et al. 2004). Using a novel interference peptide, the Tat-GluA2$_{3Y}$ that likely interferes with the phosphorylation of GluA2 at specific Tyr residues (Ahmadian, 2004), we can transiently disrupt regulated AMPA receptor endocytosis. This study has significant implications for the understanding of the how a specific form of synaptic plasticity, AMPA receptor endocytosis, regulates memory across different time points.

1.5. Implications of Studying Recognition Memory for Mental Illness

In addition to its utility as a tool in understanding the neural substrates of normal memory, study of recognition memory has large implications for patients with brain damage and neurological disease. In recent years, this utility has also extended to studies of psychiatric illness, with patients displaying significant deficits in cognitive capacity, including recognition memory. Understanding the neurobiological mechanisms that underlie memory disruption and identifying the etiological factors that contribute to these disruptions has significant implications for how we diagnose, treat, and prevent the emergence of these disorders.

1.5.1. Neurodevelopmental Disease and the Requirement for Appropriate Model Organisms.

A number of disorders are thought to originate early in life and persist into adulthood. These disorders, termed neurodevelopmental disorders, include illnesses like autism and schizophrenia; and are characterized by pervasive social, cognitive, and behavioural deficits. Further, these disorders likely arise from several etiological factors including genetic predisposition and exposure to environmental challenges that converge to disrupt normal brain development. However, the distinct etiological factors involved in neurodevelopmental disease and how these factors specifically impact brain developmental are not well understood. Epidemiological studies have highlighted adverse in utero environments, including obstetric complications and prenatal infection as salient risk factors for neurodevelopmental disease (Arndt et al. 2005; Ciaranello and Ciaranello 1995; McDonald and Murray 2000; Meyer et al. 2007; Opler and Susser 2005; Wong and Van Tol 2003).

As previously mentioned, ethical considerations prevent the study of many neural processes, including the neurobiological changes that arise in brain development as a result of early life stress. In humans, the invasive procedures and potentially lethal consequences of studying early life developmental prevent most studies of this type. Therefore, appropriate animal preparations that closely model the morphological and behavioural changes seen in human subjects must be developed to better address (1) what adverse factors in early life most contribute to neurodevelopmental disease (2) the neurotransmitter systems, neural circuits, and mechanisms of synaptic plasticity that are altered as a result of adverse early life conditions (Floresco et al. 2005; Lewis and Gonzalez-Burgos 2006).

The third chapter of this thesis is primarily concerned with the cognitive changes that arise from prenatal exposure to inflammation, and in particular, deficits in recognition memory. The consequences of prenatal infection and inflammation can be studied in rodent models using in utero administration of specific pathogens or bacterial and viral mimetics. While developmental changes may arise from an effect that is specific to a distinct pathogen (infection type model), effects may also be due to general maternal immune activation (inflammation type model; Meyer et al. 2009a; Meyer and Feldon 2011). To study this general immune insult, the bacterial endotoxin lipopolysaccharide (LPS), a pathogen that closely mimics gram-negative

bacteria infections, and the viral mimetic polyinosinic-polycytidylic acid (PolyI:C), a synthetic double stranded RNA recognized as a virus by the mammalian immune system, have been used in rodents. Both agents result in a robust immune response leading to a generalized inflammation and fever in pregnant dams. Further, unlike specific bacterial or viral pathogens, both LPS and PolyI:C are non-replicating agents, meaning their effects can be contained to a specific time point during pregnancy and subsequent effects can be attributed to general immune activation at these specific points (Meyer et al. 2009a). In this thesis, PolyI:C was chosen to mimic viral infection in utero. Responses to PolyI:C are achieved through activation of Toll-like receptors (TLR), specifically TLR3 (Alexopoulou et al. 2001; Meyer et al. 2009a; Takeuchi and Akira 2007). Toll-like receptor activation initiates the production of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Meyer et al. 2005; Meyer et al. 2009b; Meyer et al. 2009a).

Previous studies of prenatal immune activation using PolyI:C to mimic have shown that the immune activation and the subsequent inflammation can result in significant behavioural changes in offspring. Both mice and rats show deficits in measures of attention (pre-pulse inhibition), strategy shifts, spatial exploration, and working memory (Meyer et al. 2005; Meyer et al. 2009a; Ozawa et al. 2006; Shi et al. 2003). Many of these deficits are consistent with deficits seen in human patients diagnosed with schizophrenia (Meyer et al. 2009a). However, the possibly exists that these rodents display additional behavioural deficits relevant to neurodevelopmental disorders, including deficits in recognition memory.

1.5.2. Disrupted Synaptic Plasticity in Key Brain Regions

Given the considerable evidence supporting synaptic plasticity as a substrate of cognition, an important question is whether synaptic plasticity is disrupted in neurodevelopmental disease and what brain regions of interest show disruptions. Recent studies highlight aberrant glutamatergic signaling in the medial temporal lobe and PFC. In neonatal lesion models of schizophrenia, lesions of ventral hippocampus disrupt connectivity between the PFC and hippocampus (Gruber et al. 2010). Prenatal infection models in rodents also show disrupted hippocampal and PFC short and long-term plasticity (Escobar et al. 2011; Lante et al. 2008; Lowe et al. 2008; Oh-Nishi et al. 2010) as well as disrupted coherence between hippocampus

and PFC (Dickerson et al. 2010). Other manipulations during gestation, including protein malnutrition, synthetic corticosteroid treatment, chronic stress, and cannabinoid agonists, also consistently alter patterns of long-term synaptic plasticity, especially in PFC and hippocampus (Hernandez et al. 2008; Mereu et al. 2003; Noorlander et al. 2008; Yang et al. 2007). Whether these disruptions extend to PRh is unknown.

To study synaptic plasticity deficits as they relate to recognition memory, especially in vivo, thorough assessment of the behavioural deficits in rodents exposed to prenatal infection is required. An understanding of these deficits may highlight brain regions of interest to further examine for synaptic deficits. Chapter 3 of this thesis examines recognition memory in three different paradigms proposed to depend upon different brain regions, including PRh, hippocampus, and PFC. Given the documented disruptions in these regions in neurodevelopmental disease, this study examined the validity of both prenatal inflammation as an etiological factor in adult cognitive disruption and the use of recognition memory to assess memory deficits and possible deficits in synaptic plasticity as they relate to neurodevelopmental disease.

1.6. Study Design and Hypothesis

Several gaps in our understanding of recognition memory under normal and pathological states have been highlighted in this introduction. The goals of this thesis are to address two of these gaps using rodent models of recognition memory.

1.6.1. Experiment 1

In experiment 1, I examined the role of AMPA receptor endocytosis in three distinct phases of recognition memory: (1) encoding, the time during which information about a stimulus is acquired; (2) consolidation, the time during which information is stored as memory; and (3) retrieval, the time during which previously stored memories are accessed for use. Previous study has demonstrated an integral role for AMPA mediated excitatory transmission and AMPA receptor endocytosis in memory (Winters and Bussey 2005; Griffiths 2008). However, experimental limitations in these studies meant AMPA receptor endocytosis could not be

examined at any specific time during the memory process. I infused the interference peptide Tat-$GluA2_{3Y}$, a specific inhibitor of regulated AMPA receptor endocytosis (Ahmadian et al. 2004), in PRh of normal adult rats and examined how infusions at different time points during the memory process influenced discrimination between novel and familiar objects. Given the documented reduction in firing amplitude of PRh neurons upon reintroduction to familiar stimuli, I hypothesized that AMPA receptor endocytosis would be required at a specific time point (retrieval) during recognition.

1.6.2. Experiment 2

In experiment 2, I investigated the influence of prenatal immune activation on recognition memory in young adult offspring. Patients with schizophrenia demonstrate significant deficits on tests of recognition memory; however, these deficits have not been extensively studied in prenatal infection models. In this study, pregnant dams received intravenous injections of PolyI:C at gestational day (GD) 15. The female and male offspring of these dams were then assessed in three different tasks of recognition relevant to neurodevelopmental disease: (1) object recognition; (2) object location recognition; and (3) object-in-place recognition. I hypothesized that in utero exposure to PolyI:C would globally impair subjects on all three tasks of recognition; however, the more difficult recognition paradigm, object-in-place recognition would produce the largest cognitive deficits.

CHAPTER 2

EXPERIMENT 1: AMPA RECEPTOR ENDOCYOTSIS IN RAT PERIRHINAL CORTEX UNDERLIES RETRIEVAL OF OBJECT MEMORY[1]

2.1. Introduction

Memory for objects and events plays an integral role in how an organism experiences its environment. Such memories depend on processing in a number of brain areas, especially those in the medial temporal lobe (Squire et al. 2004). Several lines of evidence suggest that memory for previously encountered objects and the ability to discriminate between novel and familiar objects requires activity in PRh (Brown and Aggleton 2001; Eichenbaum et al. 2007; Hannesson et al. 2004; Pihlajamaki et al. 2004; Yonelinas 2001). For example, lesions of PRh in both primate and rodent species result in deficits in the discrimination between novel and familiar objects while lesions of other areas of the medial temporal lobe, including hippocampus, spare these discriminations (Abe et al. 2004; Ennaceur et al. 1996; Forwood et al. 2005; Hannesson et al. 2004; Meunier et al. 1993; Mumby and Pinel 1994; Nemanic et al. 2004; Winters and Bussey 2005b). As a result, understanding the neural processes in PRh mediating recognition memory, especially in regard to the different phases of the memory process, are of considerable interest.

Patterns of long-term synaptic plasticity have been proposed to underlie several forms of cognition, including recognition memory (Castro et al. 1989; Collingridge et al. 2010; Dalton et al. 2008; Davis et al. 1992; Griffiths et al. 2008; Morris et al. 1986; Whitlock et al. 2006); however, direct demonstrations of the role of synaptic plasticity in PRh dependent recognition memory are rare. In some studies, reduced responses of PRh neurons have been reported following repetitive exposure to visual stimuli (Brown and Bashir 2002; Fahy et al. 1993; Xiang and Brown 1998; Zhu and Brown 1995). These observations are consistent with the involvement of LTD, a form of synaptic plasticity, in recognition memory. Long-term depression has been consistently demonstrated in PRh slices where its induction depends on several different receptor types including NMDA receptors, mGluRs, mAChRs, and L-type VDCCs (Cho et al. 2000; Griffiths et al. 2008; Seoane et al. 2009; Warburton et al. 2003). Importantly, blockade of these

[1] This chapter has been accepted in revised form as a brief communication in *Learning and Memory.*

receptor types also disrupts object recognition memory using various intervals between the first and second presentation of a stimulus. In PRh, blockade of NMDA receptors prior to or immediately after the sample trial but not prior to the test trial disrupts object recognition memory while AMPA receptor blockade during any stage results in memory disruption (Abe et al. 2004; Barker et al. 2006b; Winters and Bussey 2005a). Blockade of mAChRs produces effects on novel object recognition that are isolated to the sample and consolidation phase, with no effect on retrieval (Warburton et al. 2003; Winters et al. 2006; Winters et al. 2007).

While several studies have examined the effect of receptor antagonism on object recognition memory, few studies have examined the role of the mechanisms underlying the expression of LTD in this memory. Recently, Griffiths and colleagues (2008) provided evidence for the involvement of AMPA receptor endocytosis in PRh dependent object memory using an interference peptide approach (Collingridge et al. 2010). However, as their method involved a viral-mediated expression system, AMPA receptor endocytosis was blocked during the entire recognition memory test, preventing an examination of the involvement of AMPA receptor endocytosis during specific memory time points.

Previous work has shown the utility of delivering interference peptides conjugated to the Tat protein either systemically or intracranially to examine the neural mechanisms underlying cognition (Collingridge et al. 2010). We chose to use the well characterized peptide Tat-GluA2$_{3Y}$ a specific inhibitor of the regulated (activity-dependent) endocytosis of GluA2-containing AMPA receptors (Ahmadian et al. 2004; Brebner et al. 2005; Dalton et al. 2008; Fox et al. 2007; Van den Oever et al. 2008; Wong et al. 2007). Regulated endocytosis of AMPA receptors requires the phosphorylation of critical Tyr residues on the carboxy tail of the GluA2 subunit (Ahmadian et al. 2004; Hayashi and Huganir 2004). The Tat-GluA2$_{3Y}$ peptide, a synthetic peptide composed of 9 amino acids (^{869}YKEGYNVYG^{877}), mimics this critical region of the carboxy tail likely producing a competitive inhibition of GluA2 subunit phosphorylation. Previous experiments in vitro have demonstrated that the peptide becomes phosphorylated with cell stimulation, and this phosphorylation correlates with blockade of AMPA receptor endocytosis (Ahmadian et al. 2004). This blockade does not occur when a control peptide, in which Tyr residues have been replaced by Ala, or when a scrambled peptide, in which the Tyr residues are out of sequence, is used. Importantly, the effects of the peptide are both highly selective and transient in both in vitro and in vivo studies (Ahmadian et al. 2004; Brebner et al.

2005; Dalton et al. 2008; Fox et al. 2007; Van den Oever et al. 2008; Wong et al. 2007). As such, we employed Tat-GluA2$_{3Y}$ to specifically block AMPA receptor endocytosis at discrete time points during a spontaneous recognition memory test (Figure 2.1). Surprisingly, we observed that direct infusion of the Tat-GluA2$_{3Y}$ peptide into PRh blocks retrieval of object recognition memory. No effects were observed following infusions before encoding or during the consolidation phase.

2.2. Materials and Methods

2.2.1. Subjects

Twenty-two adult male Long-Evans rats (Charles River Laboratories, Quebec, Canada) weighing 250 – 300 g before surgery were paired housed in plastic cages with food and water available ad libitum. All experimentation occurred during the light phase of a 12:12 h light/dark cycle (lights on at 0700 h). Experiments were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Care and Use Committee.

2.2.2. Surgery

While anesthetized (isofluorane) and secured in a stereotaxic apparatus, rats were bilaterally implanted with 13 mm 23 gauge stainless steel guide cannulae above PRh (AP -4.0 mm; ± ML 5.4 relative to bregma; (Hannesson et al. 2004; Paxinos and Watson 1997). Cannulae were lowered (DV -7.0 mm) on a 10^{o} angle dorsal to PRh and cemented in place using 4 jeweler's screws and dental acrylic. Obdurators (0.033 cm diameter stainless steel wire) were inserted into each cannula to prevent blockage by bedding or other debris and remained in place except for during infusion. Obdurators were monitored daily and replaced when required. Following surgery, subjects were allowed to recover for 7 d prior to behavioural testing.

2.2.3. Behavioural Testing

2.2.3.1. Apparatus. All behavioural testing took place in a white rectangular room containing an empty plastic water maze. Four floor lamps provided illumination. Object recognition was conducted in a square open field arena (60 cm X 60 cm X 60 cm) constructed of white corrugated plastic (Figure 2.1A). Between each trial, the floors and walls of the box were wiped with 40% ethanol (EtOH) and a damp sponge. Two objects constructed of plastic, porcelain, or glass were positioned at the back of the box 10 cm from both the back and side walls. Velcro mounted in a cross orientation was used to secure each object in place. Objects were previously tested for preference to ensure rats did not have an innate bias for an object prior to recognition testing.

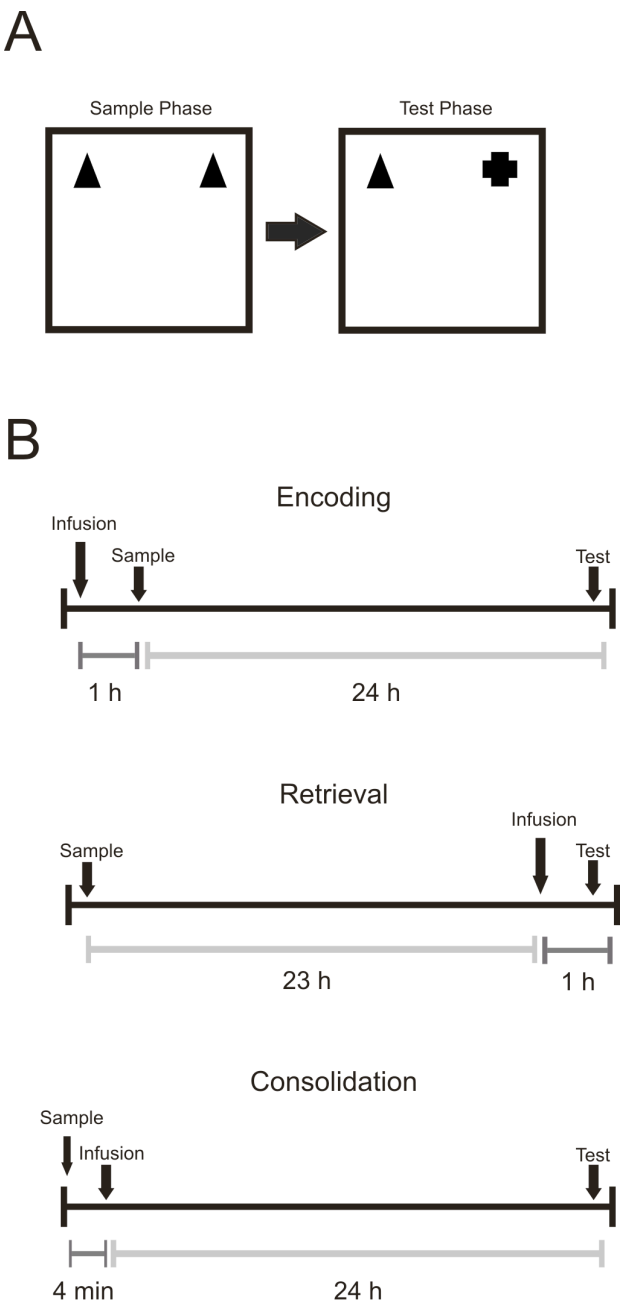

Figure 2.1. Object recognition paradigm. (A) Schematic depicting the object recognition memory test used in the present experiments. The figure depicts a bird's eye view of the open field and the arrangement of the objects for each phase. Duplicate copies of the same object were always used. **(B)** Outline of the infusion times relative to the phases of the object recognition memory test.

2.2.3.2. Habituation procedure. All testing was conducted between the hours of 0800 and 1200. Seven days after surgery, subjects were handled for 10 min per day over 3 days in the same procedure room used for object recognition testing. Following handling, rats were extensively habituated to the object recognition apparatus over 3 days in an attempt to reduce anxiety. On the first two days, rats were removed from the colony room in pairs and placed individually in an arena without objects for 10 min. On the third day, rats were removed individually from the colony room and again allowed to habituate for 10 min to the empty arena. On subsequent weeks, rats received one habituation session 24 h prior to the start of recognition memory testing. Over repeated habituation sessions, rats displayed increased levels of exploration in the centre of the arena (decreased stigmotaxis) and decreased fecal boli counts indicating an overall decrease in level of anxiety in the environment.

2.2.3.3. Novel object recognition paradigm. Behavioural testing consisted of a sample phase and a test phase. During the sample phase, subjects were placed in the arena and allowed to explore two identical objects (A1 and A2) for 4 min. Following the 4 min exploration period, subjects were removed from the arena and returned to their home cages. Between trials, objects were cleaned with 40% EtOH and paper towel to remove any confounding scent. Twenty-four h after the start of the sample phase, the rat was returned to the box for the test phase in which the rat explored an identical copy of the sample phase object (A3) and a novel object (B1). All trials were videotaped using a camera mounted to the ceiling above the arena and recorded using MPEG video recorder system. Subjects were counterbalanced for sample object and for the side of the box on which the novel object was placed. Testing was conducted over multiple weeks; different object pairs were used for each week, so that rats were never exposed more than once to a specific object pair.

2.2.4. PRh Infusion

2.2.4.1. General procedure. Subjects were separated into two distinct groups: the encoding/retrieval infusion group (n=16) and the consolidation infusion group (n=6). All rats were habituated for 2 days to the infusion procedure. The rats were brought to a separate procedure room individually, their obdurators removed, and a pair of short (5 mm) infusion

needles were placed in their cannulae. An infusion pump (Harvard Apparatus) was then run for 2 min (no infusion was made). The needles remained in place for an additional minute, and then animals were returned to their home cages. For infusion, a needle (30 gauge stainless steel; 14 mm length) connected via PE-50 tubing to the infusion pump was inserted into each cannula and 1 µl (0.5 µl/min; 40 ng/µl) of either Tat-GluA2$_{3Y}$ (YGRKKRRQRRR-^{869}YKEGYNVYG^{877}) or scrambled peptide (YGRKKRRQRRR -VYKYGGYNE) was delivered into the PRh. In sham rats, needles were inserted, but no peptide was delivered. The infusion needles were left in place for an additional minute following the infusion to allow for diffusion. Following the infusion, rats were returned to their home cages.

2.2.4.2. Experiment 1a: The role of AMPA receptor endocytosis in the encoding and retrieval of object recognition memory. In the encoding/retrieval group, infusions were conducted 1 h prior to either the sample phase or test phase (Figure 2.1B). Each subject was tested 4 times over 4 weeks (1 test/week). Each rat received two infusions of Tat-GluA2$_{3Y}$ (one prior to sample phase and one prior to test phase), one infusion of scrambled peptide (either prior to sample or test phase) and one sham infusion (either prior to sample or test phase).

2.2.4.3. Experiment 1b: The role of AMPA receptor endocytosis in the consolidation of object recognition memory. In the consolidation group, infusions were conducted immediately following the sample phase (Figure 2.1B). Subjects in the consolidation group were tested 2 times over 2 weeks. Each rat received either a sham infusion or a scrambled peptide infusion and one infusion of Tat-GluA2$_{3Y}$.

2.2.5. Histology

After behavioural testing, rats were deeply anesthetized and transcardially perfused with 30 ml of saline followed by 30 ml of 10% sucrose/10% formalin. Rats were then decapitated, and brains were stored in 10% sucrose/10% formalin for at least 3 days. Brains were sectioned into 60 µm sections using either a vibratome or microtome (siphoned CO_2), and cannulae placement in PRh was confirmed with the aid of a stereotaxic atlas (Paxinos and Watson 1997). Only rats with bilateral PRh cannulae placements were included in the study.

2.2.6. Data scoring and analysis

Data were scored according to measures previously described (Howland and Cazakoff 2010). Time spent exploring each of the two objects available on a given trial was scored from digital video files with stopwatches by an individual blind to the treatment status of the rat. A rat was judged to be actively exploring an object when its nose was directed within 2 cm of an object and either its head or vibrissae were moving. A rat was not considered to be exploring an object when the rat was standing on top of an object but not directing attention towards it. Previous experiments in our laboratory have shown that rats must obtain a total exploration time of at least 15 s over a 4 min exploration period in order to show reliable memory; thus, rats with less than 15 s of exploration time on either the sample or test phase were eliminated from the study (Howland and Cazakoff 2010). Total exploration times of the objects on a given trial and discrimination ratios (DR), calculated as ((time exploring the novel object – time exploring the familiar object)/total time exploring both objects) were quantified for each rat. Previous studies suggest that 2 min may be the ideal length for the test trial in a spontaneous object recognition memory paradigm as novel object preference is reduced after that time (Barker et al. 2007; Clark et al. 2000; Dix and Aggleton 1999). Given this, we also restricted our analysis to the first 2 min of the test phase.

Group means for total exploration in the sample phase, total exploration in the test phase, and DR of the test phase were analyzed. No significant differences were noted between the sham infused animals and scrambled peptide infused animals; as such data from these animals were pooled as a single control group. Previous study in our lab has shown that rats whose DR was greater than two standard deviations about or below the mean were often anxious (2 standard deviations below) or failed to explore one of the objects for a significant amount of time for reliable discrimination (2 standard deviations above the mean). As such, rats whose DR was greater than two standard deviations above or below the group mean were removed. In the consolidation experiment, the data from one control animal was removed from the group due to an extremely low DR (-0.46), and this data was replaced with the group mean. Analysis showed no order effects of Tat-GluA2$_{3Y}$ infusions or repeated testing (data not shown). Total exploration times for each test were analyzed using repeated measures ANOVA with Phase, Infusion Time, and Treatment as within subjects factors. For test phase data, the DR for each group was

analyzed using single group t-tests (with a comparison value of 0 indicating equal exploration of the two objects or chance performance) while between group comparisons were performed using two-way repeated measures ANOVA with Infusion Time and Treatment as within subjects factors. Post-hoc comparisons were made using paired t-tests with a Bonferroni correction (Hannesson et al. 2004). A p value of <0.05 was considered significant for all statistical tests.

2.3. Results

2.3.1. Histology

Representative needle placements for rats in both experiments are displayed in Figure 2.2 All rats included in the analysis had bilateral cannulae positioned slightly above or in anterior PRh 3.8-4.3 mm posterior to bregma. Needle tips were positioned 1 mm past the end of the cannulae and thus terminated in the PRh. A previous study examining the spread of lidocaine in cortical tissue suggests that infused volume conforms to a spread defined by $r = (3*V/4*\pi)^{1/3}$ (Hannesson et al. 2004; Seamans et al. 1995; Tehovnik and Sommer 1997) where r = effective radius and V = volume injected. Using this estimate, the infusion would be expected to affect tissue 0.8 to 1.3 mm from the infusion site which corresponds to Brodmann's area 35 and 36.

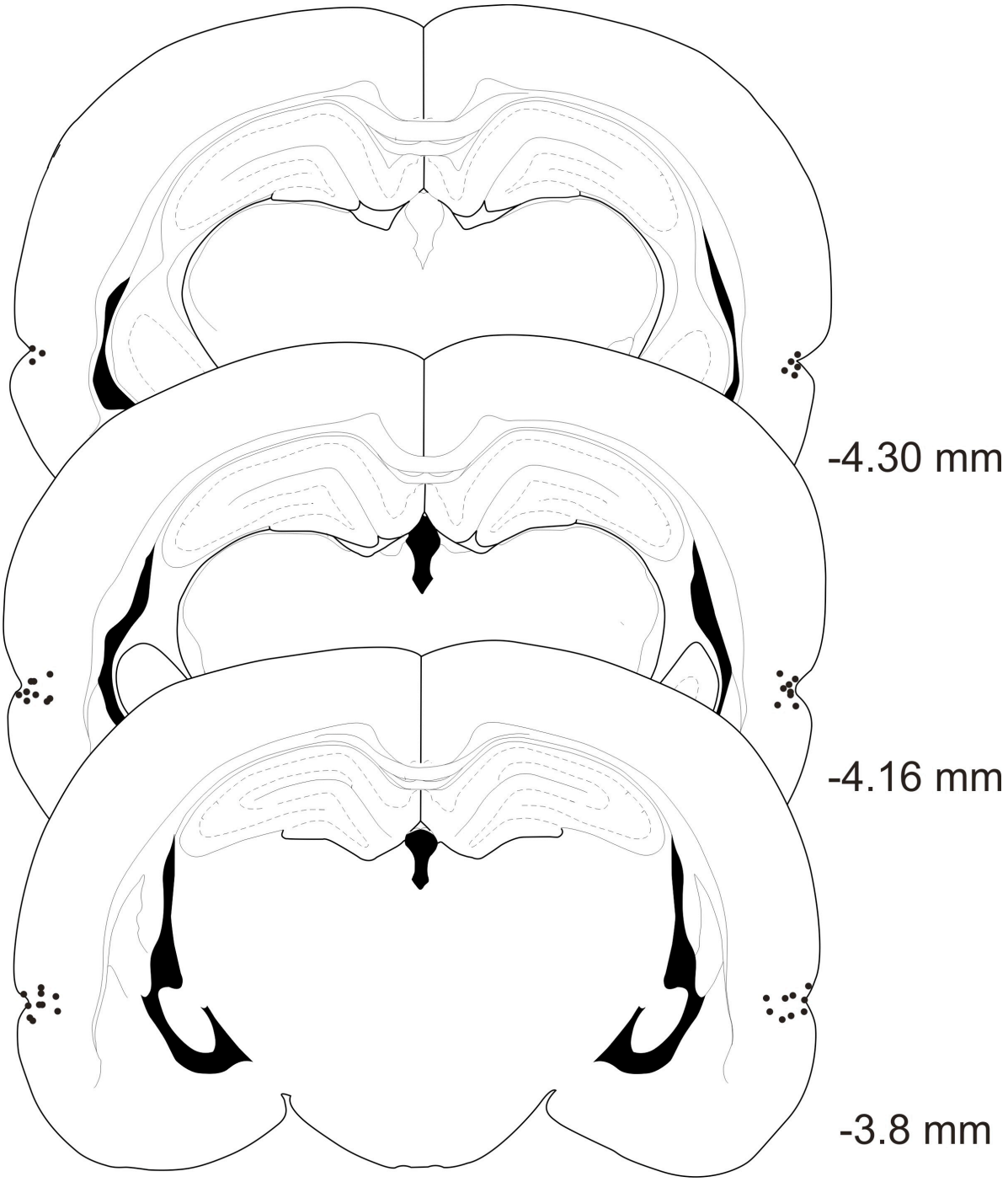

Figure 2.2. Perirhinal cortex infusion histology. Representative placements of the infusion needle tips in PRh. Only rats with needles bilaterally positioned in PRh were included in the study. Numbers denote the anterior-posterior position relative to bregma.

2.3.2. Experiment 1a: Role of AMPA receptor endocytosis in the encoding and retrieval of object recognition memory

2.3.2.1. Total exploration time. Total exploration times during the 4 min sample phase and test phases as well as the first 2 min of the test phase are summarized in Table 2.1 (see Methods for details). Over 4 min, rats explored both objects a total of 36.38 ± 3.60 s during the sample phase and 34.56 ± 3.02 s during the test phase. No significant difference between treatment groups was noted. A repeated measures ANOVA revealed no significant main effect of Exploration Phase ($F(1,15) = 0.63$, $p = 0.44$), Treatment ($F(1,15) = 0.08$, $p = 0.78$), or Infusion Time ($F(1,15) = 0.12$, $p = 0.73$). As well, there were no significant interactions between Phase and Treatment ($F(1,15) = 2.48$, $p = 0.14$), Phase and Infusion Time ($F(1,15) = 0.65$, $p = 0.43$), or Treatment and Infusion Time ($F(1,15) = 0.02$, $p = 0.88$) while the Phase X Treatment X Infusion Time interaction approached significance ($F(1,15) – 4.39$, $p = 0.053$). During the first 2 min of the test phase, rats explored objects for a total of 21.77 ± 1.04 s. A repeated measures ANOVA revealed no significant main effect of Phase ($F(1,15) = 1.11$, $p = 0.31$) or Treatement ($F(1,15) = 1.45$, $p = 0.25$) and no significant Phase X Treatment interaction ($F(1,15) = 0.048$, $p = 0.83$). Overall, subjects in all treatment groups explored the objects for a similar amount of time during both the sample and the test phases.

Table 2.1. Object exploration times in Tat-GluA2$_{3Y}$ or control infused rats. Total exploration time of both objects (s ± standard error of the mean) in the 4 min sample and 4 and 2 min test phases. Ctrl, sham and scrambled peptide rats combined; Pep, Tat-GluA2$_{3Y}$ peptide treated rats.

Treatment – Infusion Time	**Sample Phase (4 min)**	**Test Phase (4 min)**	**Test Phase (2 min)**
Ctrl Pre-sample	35.53 ± 3.24	35.43 ± 3.45	23.53 ± 2.08
Ctrl Pre-test	33.28 ± 4.81	36.30 ± 2.55	22.12 ± 2.33
Ctrl Post-sample	38.08 ± 3.64	30.26 ± 3.29	19.68 ± 2.24
Pep Pre-sample	36.48 ± 2.4	36.35 ± 3.70	21.84 ± 2.51
Pep Pre-test	40.21 ± 3.94	30.16 ± 2.37	19.62 ± 1.56
Pep Post-sample	39.99 ± 6.99	29.64 ± 2.77	16.33 ± 2.66

2.3.2.2. Effects of the Tat-GluA2$_{3Y}$ peptide on object recognition memory encoding and retrieval. A within subjects design was used to test the effects of peptide infusions on encoding and retrieval (Figure 2.1B). Figure 2.3A displays the DR calculated over 4 min of exploration for treatment groups infused before the sample phase (encoding) or before the test phase (retrieval). Animals receiving a sham or scrambled peptide infusion (control group) before either the sample or test phases showed robust recognition memory (pre-sample: DR = 0.28 ± 0.05; pre-test: DR = 0.31 ± 0.07). One sample t-tests confirmed this assertion (pre-sample: $t(15) = 5.75$, $p<0.001$; pre-test: $t(15) = 4.58$, $p<0.001$). When the rats were infused with the Tat-GluA2$_{3Y}$ prior to the sample phase, memory remained intact and at a level comparable to the control groups (DR = 0.28 ± 0.07; $t(15) = 4.21$, $p=0.001$). In dramatic contrast, Tat-GluA2$_{3Y}$ infusion prior to the retrieval phase disrupted memory for the previously encountered object as the rats did not show a preference for the novel object (DR = 0.06 ± 0.06; $t(15) = 0.97$, $p=0.39$). A repeated measures ANOVA revealed no significant main effect of Infusion Time ($F(1,15)=2.05$, $p=0.17$) or Treatment ($F(1,15)=2.99$, $p=0.10$) but a significant Infusion Time by Treatment interaction ($F(1,15)=5.71$, $p=0.03$). Post-hoc analysis revealed that the pre-retrieval Tat-GluA2$_{3Y}$ group displayed significantly lower discrimination than all other groups ($p < 0.05$).

Consistent with the results from the 4 min analysis, memory during the first 2 min of the test phase was intact for all animals except for those receiving Tat-GluA2$_{3Y}$ infusion at retrieval (Figure 2.3B). Subjects in the control sample infusion group (DR = 0.32 ± 0.05), the control retrieval infusion group (DR = 0.35 ± 0.07), and the Tat-GluA2$_{3Y}$ sample group (DR = 0.37 ± 0.08) displayed significant object recognition memory ($t(15) = 7.44$ $p<0.001$; $t(15) = 4.56$, $p<0.001$, $t(15) = 5.63$, $p<0.001$, respectively) while subjects in the Tat-GluA2$_{3Y}$ retrieval group failed to display significant memory for the familiar object (DR = 0.10 ± 0.06, $t(15) = 1.74$, $p=0.10$). A two-way repeated measures ANOVA revealed no significant main effect of Treatment ($F(1,15) = 1.29$, $p=0.27$), a close to significant effect of Infusion Time ($F(1,15) = 4.29$, $p=0.06$), and a significant Infusion Time by Treatment interaction ($F(1,15) = 10.65$, $p=0.005$). Post-hoc analysis revealed subjects that received Tat-GluA2$_{3Y}$ prior to retrieval displayed significantly poorer performance than all other groups ($p<0.05$).

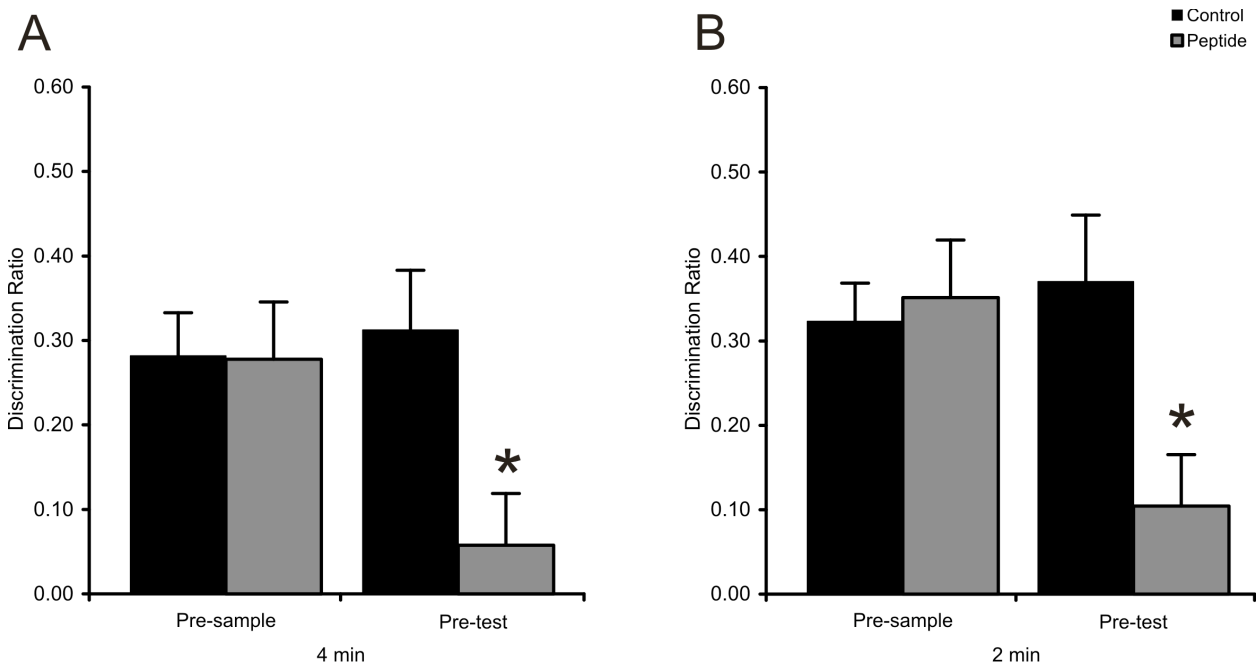

Figure 2.3. The effect of blocking AMPA receptor endocytosis on encoding and retrieval of object recognition memory. (A) Infusion of the Tat-GluA2$_{3Y}$ peptide prior to the test but not the sample phase disrupts memory during the 4 min test phase. **(B)** The retrieval dependent disruption persists when data are analyzed only during the first 2 min of test phase exploration. The mean discrimination ratio ± standard error of the mean is plotted for each group. Asterisks denote significant differences from all other groups.

2.3.3. Experiment 1b: Role of AMPA receptor endocytosis in the consolidation of object recognition memory

2.3.3.1. Total Exploration Time. Exploration times for the sample and test phases of the consolidation experiment are summarized in Table 2.1. Over 4 min, a repeated measures ANOVA revealed a significant effect of Phase ($F(1,5) = 8.096$, $p = 0.017$) but no significant Phase by Treatment interaction ($F(1,5) = 0.158$, $p=0.70$). Inspection of the data revealed that regardless of treatment, subjects explored the objects significantly less during the test phase (29.95 ± 2.14) than during the sample phase (39.03 ± 3.94). However, analysis of the first 2 min of the test phase using a paired t-test revealed no significant effect of Treatment for total exploration ($t(5) = 1.26$, $p = 0.26$).

2.3.3.2. Effects of the Tat-GluA2$_{3Y}$ peptide on object recognition memory consolidation. Object recognition memory in a separate cohort of subjects was examined with post sample infusions to examine the potential involvement of AMPA receptor endocytosis in object consolidation (Figure 2.1). Subjects were tested once in each of the treatment groups (post sample control and post sample Tat-GluA2$_{3Y}$). Tat-GluA2$_{3Y}$ infusions failed to significantly affect memory when either 4 min (Figure 2.4A; control DR = 0.21 ± 0.10; Tat-GluA2$_{3Y}$ DR = 0.20 ± 0.14; $t(5)=0.01$, $p = 0.91$) or 2 min (Figure 2.4B; control DR = 0.25 ± 0.12; Tat-GluA2$_{3Y}$ DR = 0.38 ± 0.11; $t(5)=1.68$, $p =0.25$) of the test phase was analyzed. One sample t-tests revealed that control rats showed DRs significantly above chance when 2 min (control: $t(5) = 2.44$, $p =0.029$) or 4 min (control: $t(5) = 2.56$, $p=0.025$) of the test trial was considered. The DR of Tat-GluA2$_{3Y}$ treated rats was significantly above chance during the first 2 min of the test phase ($t(5) = 3.84$, $p =0.012$) but not if all 4 min were considered ($t(5) = 1.54$, $p=0.18$).

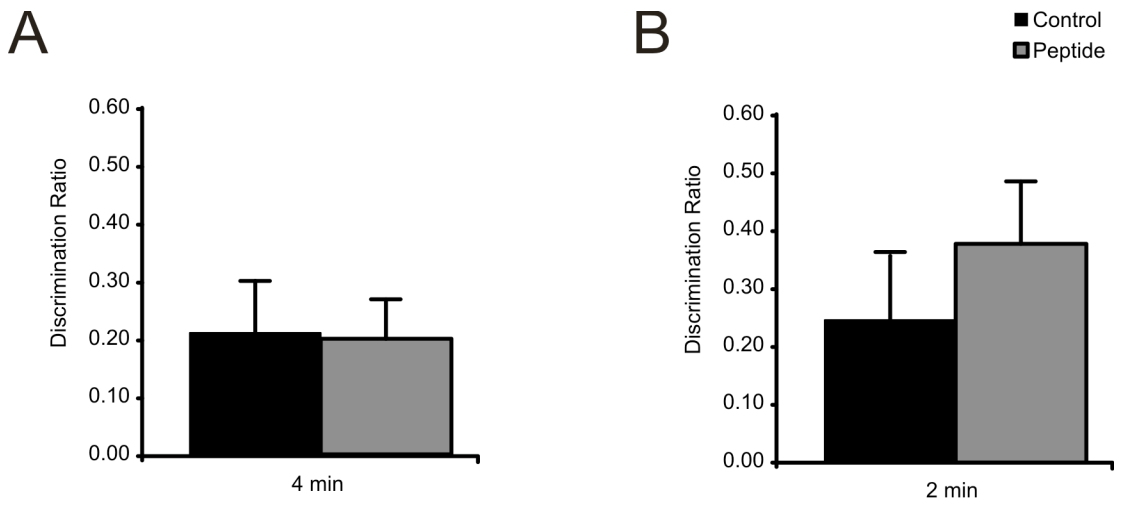

Figure 2.4. The effects of blocking AMPA receptor endocytosis during consolidation on object recognition memory.
(A) Infusion of the Tat-GluA3Y peptide immediately following the sample phase did not influence memory for the previously encountered object when either 4 min **(A)** or the first 2 min **(B)** of the test trial were considered. Data are displayed as the mean discrimination ratio ± the standard error of the mean.

2.4. Discussion

The present study examined the role of AMPA receptor endocytosis in the encoding, consolidation, and retrieval of object recognition memory. Using intra-PRh microinfusions of the Tat- $GluA2_{3Y}$ interference peptide, which disrupts the regulated endocytosis of GluA2-containing AMPA receptors, we transiently and specifically blocked AMPA receptor endocytosis during these three discrete phases of memory. The results indicate a distinct role for AMPA receptor endocytosis in PRh during the retrieval of memory (Figure 2.3), but not during the encoding (Figure 2.3) or early consolidation (Figure 2.4) of the memory.

The spontaneous object recognition memory test used in the present experiments is a well-established assay for examining recognition memory in rodents (Ennaceur and Delacour 1988). It is particularly suitable for examining the neural mechanisms of memory as confounds related to reward or extensive rule learning are minimized. In both experiments, a repeated measures design was implemented as is common in studies using the spontaneous object recognition memory test (Hannesson et al. 2004; Winters and Bussey 2005a). In experiment 1a, total exploration times for the sample and test phases did not differ among groups (Table 2.1). In the experiment 1b, rats in both treatment groups displayed lower total exploration times during the test phase than during the sample phase when all 4 min were considered but not when the first 2 min were considered. While it is not clear why total exploration was reduced during the second half of the test trial for these rats, it may be accounted for by short term habituation to the objects and recognition task over a test day (Howland and Cazakoff 2010). Importantly, lower exploration during the test trial was observed in both the control and Tat-$GluA2_{3Y}$ treated animals indicating that infusion of the peptide was not responsible for this effect. These data suggest that the observed disruption of memory retrieval following Tat-$GluA2_{3Y}$ infusion cannot be attributed to a non-specific effect of the Tat-$GluA2_{3Y}$ peptide on exploration or general motor activity.

To our knowledge, this is the first study demonstrating a pharmacological disruption of PRh dependent object recognition memory specific to the retrieval phase. Using a different interference peptide, Griffiths and colleagues (2008) provide direct evidence supporting the role of AMPA receptor endocytosis in object recognition memory. The peptide (ΔA843-Q853 or G2CT) is also derived from a short section of the carboxyl tail of the GluA2 subunit and blocks

the interaction of AMPA receptors with the clathrin adaptor protein AP2 (Collingridge et al. 2010; Lee et al. 2002). Intra-PRh expression of the ΔA843-Q853 peptide disrupted object recognition memory at delays of 5 min and 24 h. However, the lentiviral expression system used to express the peptide does not have the temporal specificity necessary to examine the effects of the peptide during the different phases of memory. Our present results support the assertion that GluA2-subunit containing AMPA receptor endocytosis in PRh is specifically required for the retrieval of object recognition memory.

The highly selective action of the Tat-GluA2$_{3Y}$ and ΔA843-Q853 peptides in blocking the regulated endocytosis of GluA2-containing AMPA receptors (Ahmadian et al. 2004; Collingridge et al. 2010; Lee et al. 2002) suggests that mechanisms consistent with synaptic weakening or LTD in PRh are involved in recognition memory retrieval. Long-term depression in PRh has been hypothesized to be critical for PRh-dependent object recognition memory (Brown and Bashir 2002; Collingridge et al. 2010; Massey et al. 2008; Massey and Bashir 2007) and several lines of evidence in rodents and primates support this hypothesis. Neurons in PRh slices demonstrate a propensity for LTD upon low frequency stimulation (Griffiths et al. 2008; Seoane et al. 2009; Warburton et al. 2003). In vivo electrophysiological studies have shown that upon the second but not first exposure to a stimulus, single neuron responses in PRh display attenuated responding in monkeys (Fahy et al. 1993; Xiang and Brown 1998) and anesthetized rats (Zhu and Brown 1995). Activation of PRh neurons, as assayed by c-fos expression, is also reduced upon repeated exposure to familiar pictures (Seoane et al. 2009; Zhu et al. 1995). The test trial used in the present study also involves presentation of one copy of the familiar object and a novel object (Figure 1). Thus, it is possible that re-exposure to a familiar stimulus may be the trigger for AMPA receptor endocytosis.

While it is tempting to speculate that the results of the Griffiths et al. study (2008) and the present experiments are congruent, some unresolved issues exist. Most importantly, the upstream regulators resulting in the endocytosis of AMPA receptors during the retrieval of object memory are unknown. Electrophysiological recordings from slices have shown that NMDA receptors, group I and II mGluRs, mAChRs, and L-type VDCC are required for LTD induction in PRh (Cho et al. 2000; Massey et al. 2004; Warburton et al. 2003). Both the Tat-GluA2$_{3Y}$ and ΔA843-Q853 peptides block NMDA receptor-dependent LTD in a number of brain areas including the PRh (Collingridge et al. 2010; Griffiths et al. 2008). However, blockade of NMDA receptors in

PRh prior to or immediately after encoding, but not prior to retrieval, disrupts object recognition (Abe et al. 2004; Winters and Bussey 2005a). Similarly, group I and II mGluR or cholinergic antagonists disrupt object memory when given prior to encoding but are without effect when delivered prior to retrieval (Barker et al. 2006a; Warburton et al. 2003).These findings provide correlational support for the role of LTD in the encoding of object recognition memory. However, in addition to their role in LTD, membrane receptors are involved in numerous excitatory and synaptic processes (Ferraguti and Shigemoto 2006; Nicoletti et al. 2011; Rebola et al. 2010). The possibility exists that blocking these receptors prior to the sample phase not only disrupts LTD but also other signaling functions of the receptors resulting in the observed memory deficits (Collingridge et al. 2010). Consistent with this assertion is data demonstrating that the blockade of AMPA receptors with CNQX during either encoding, consolidation, or retrieval impairs object recognition memory (Winters and Bussey 2005a) while L-type VDCC antagonists such as verapamil or diltiazem disrupt object recognition memory (24 h delay) when administered at either encoding or retrieval (Seoane et al. 2009). Given that none of the receptors discussed above (NMDA, AMPA, mGluR, acetylcholine, or L-type VDCC) have specific roles in only the retrieval of object recognition memory, future experiments will be necessary to determine the upstream regulators of AMPA receptor endocytosis. AMPA receptor endocytosis requires a complicated series of intracellular events that are specific to the induction trigger (Collingridge et al. 2004; Collingridge et al. 2010; Malenka 2003). Phosphorylation of the tyrosine residues located on the region of the GluA2 subunit mimicked by the $GluA2_{3Y}$ peptide is one event necessary for the regulated endocytosis of GluA2-containing AMPA receptors (Ahmadian et al. 2004; Hayashi and Huganir 2004) although it may not be sufficient (Hayashi and Huganir 2004). Examination of the role of kinases such as those from the SRC family (Hayashi and Huganir 2004) may clarify the upstream signaling events that cause GluA2-containing AMPA receptor endocytosis during object recognition memory retrieval.

2.5. Conclusion

In summary, these results further support the critical role of mechanisms consistent with LTD in PRh for object recognition memory. The present report demonstrates GluA2-containing AMPA receptor endocytosis is involved in the retrieval of object recognition memory but not in

the initial encoding or consolidation. Taken together, these results suggest that depressed neural responding in perirhinal neurons following reintroduction to familiar stimuli involves the removal of AMPA receptors from the synaptic cleft.

CHAPTER 3

EXPERIMENT 2: PROBING THE EFFECTS OF PRENATAL INFECTION ON RECOGNITION MEMORY[2]

3.1. Introduction

Disruptions in the uterine environment, including exposure to physiological and psychological stressors, have been proposed to impart significant risk for the development of psychiatric illness in the offspring. In particular, maternal inflammation has been suggested as an important etiological factor for schizophrenia with several lines of evidence reporting an increased risk of schizophrenia following in utero exposure to viral, bacterial, and parasitic infections. Exposure to respiratory infections during the second trimester has been proposed to account for 14 to 21 % of schizophrenia cases in the offspring (Brown et al. 2000; Brown and Derkits 2010; Ellman et al. 2009) while infection with pyelonephritis resulted in a five fold increased risk of schizophrenia in people with a family history of psychosis (Clarke et al. 2009). These population studies suggest that infection early in life may alter the normal development of the nervous system resulting in a number of different behavioural and cognitive abnormalities.

Patients with schizophrenia display a complex array of symptoms that have been grouped into negative, positive, and cognitive domains. Recently, cognitive symptoms, including deficits in learning and memory, have received particular attention given their demonstrated early pre-clinical emergence, ubiquitous presentation, and predictive value regarding patient outcome (Elvevag and Goldberg 2000; Keefe and Fenton 2007; Lewis and Gonzalez-Burgos 2006; Lewis and Gonzalez-Burgos 2008). Interestingly, deficits in cognitive ability are significantly worse in schizophrenia patients with confirmed exposure to influenza in utero than in those patients not exposed to prenatal infection (Brown et al. 2009). While this suggests a particularly salient link between prenatal infection and neurodevelopmental cognitive disruption, our current understanding of this potential link is limited. Furthermore, current antipsychotics produce limited improvement of cognitive symptoms (Lewis and Gonzalez-Burgos 2006). One strategy to assist in further understanding of the cognitive symptoms of schizophrenia and the development of novel therapeutics is the use of animal models. In rodents, both prenatal infection with

[2] This manuscript has been submitted along with other data to *Neuroscience*.

influenza and immune activation through the use of pathogen mimetics has been demonstrated to result in the disruption of several clinically important behaviours including alterations in pre-pulse inhibition, locomotor activity, latent inhibition, social interaction, and working memory (Meyer et al. 2009b; Shi et al. 2003; Wolff and Bilkey 2008; Wolff and Bilkey 2010; Zhang et al. 2011; Zuckerman et al. 2003; Zuckerman and Weiner 2003; Zuckerman and Weiner 2005). However, a more thorough examination of cognitive disruption is required.

Among the core cognitive deficits observed in patients with schizophrenia are disruptions in short and long term recognition memory as well as deficits in visuospatial processing. Patients show significant impairments in identifying previously viewed objects and faces following both short and long delays between learning and test trials in the Visual Object Learning Test (VOLT) and the Penn Face Memory Test (Calkins et al. 2005). Furthermore, pervasive deficits in both CANTAB paired associates learning and in tests of object location binding exist in patients with schizophrenia. Patients display marked decreases in their ability to recognize the association between objects and their locations compared to matched controls (Burglen et al. 2004; Chouinard et al. 2007; Salame et al. 2006; Wood et al. 2002) while deficits in these tasks correlate highly with daily functioning (Aubin et al. 2009). These deficits are noted in patients diagnosed with schizophrenia as well as those considered at increased risk of developing the disorder indicating that these recognition deficits arise early and are particularly enduring (Barnett et al. 2005; Bartok et al. 2005).

Several groups have examined the influence of prenatal immune activation on simple novel objection recognition tasks in rodent models, finding deficits in mice that are prenatally exposed to both LPS and PolyI:C (Bitanihirwe et al. 2010b; Coyle et al. 2009; Ibi et al. 2009; Ozawa et al. 2006). However, at present, the influence of prenatal immune activation on object location recognition and paired associations between objects and location has not been examined. Whether prenatal immune activation results in disruptions in recognition memory like those seen in patients with schizophrenia is unclear. Given this, I examined the influence of the viral mimetic PolyI:C on both simple recognition memory (object and object-place) and associative recognition memory in a paired object location association task developed for rats (Barker et al. 2007; Warburton and Brown 2010). We report here a deficit in object-in-place recognition memory in male rats as a result of prenatal PolyI:C induced inflammation. Other

forms of recognition memory were not disrupted. Both saline and PolyI:C females displayed deficits in the object-location and object-in-place paradigms compared to males.

3.2. Materials and Methods

3.2.1. Subjects

Timed pregnant Long-Evans rats (GD 7; Charles River Laboratories, Quebec, Canada) were singly housed in transparent plastic cages in a temperature controlled (21^{o}C) colony room with food (Purina Rat Chow) and water available ad libitum. All experimentation occurred during the light phase of the 12:12 h light dark cycle (lights on at 0700 h). All experiments were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Care and Use Program.

3.2.2. Prenatal Treatment

On GD 15, dams were individually transported to a separate room where weight and rectal temperature (Homeothermic Blanket System, Harvard Instruments, MA) were recorded. Rats were then anaesthetized with isoflurane (5% induction and 2.5% maintenance) and injected intravenously (tail vein) with either saline (n=18) or PolyI:C (4.0 mg/kg, High Molecular Weight, InVitroGen, San Diego, CA; n = 14). The injection procedure lasted ≈ 10 min, and care was taken to ensure that saline treated animals were anaesthetized for the same length of time as the PolyI:C treated animals. Weight and rectal temperature were again measured 8, 24, and 48 h after the injection. Following the last temperature and weight recording, rats were left undisturbed in the colony room, except for weekly cage changing, until the day after parturition (postnatal day (PND) 1) when the pups were culled to include 10 per litter (6 males, 4 females where possible). Pups remained with their mother until PND 21 when they were weaned and housed in same-sex cages of 3 to 4 animals. Where possible, a maximum of two male and two female offspring from each dam were tested in recognition memory experiments to control for litter effects. Recognition memory testing began after PND 56. This age, considered young adulthood in rats, was chosen to (1) coincide with the mature development of cortical brain

regions including hippocampus and PFC (Piontkewitz et al. 2011) and to (2) reflect the time point (adolescence and young adulthood) at which cognitive disruptions emerge in humans (Boksa 2010; Meyer and Feldon 2010). Previously, some animals used in this experiment were also tested in a paired-pulse inhibition task and MK-801 induced locomotor activity assay at PND 35 and PND 56.

3.2.3. Behavioural Testing

3.2.3.1. Apparatus. Behavioural testing took place in a white rectangular room containing an empty plastic water maze. Four floor lamps provided background illumination. Recognition memory testing was conducted in a square open field arena (60 cm X 60 cm X 60 cm) constructed of white corrugated plastic (Figure 3.1). Between each trial, the floors and walls of the box were wiped with 40% EtOH and a damp sponge. For the object-in-place task, an additional black wall was inserted along the back wall of the box (Figure 3.1C).

3.2.3.2. Habituation. Prior to testing, subjects were handled for three days (5 min/day) in the room in which behavioural testing took place. Following this, animals were extensively habituated to the open field arena to decrease the influence of anxiety and stress on memory. Subjects received three habituation sessions prior to the first recognition test (the novel object recognition test). During the first two habituations, subjects were brought in pairs into the testing room and placed individually in separate arenas for 10 min. On the last day, subjects were brought individually in the room and again spent 10 min in an arena. Following habituation, subjects were returned to the colony room. The last habituation occurred 24-48 h before the first testing session. For subsequent tests (object location and object-in-place tests), subjects received only one habituation session, in pairs, 24-48 h prior to the new test.

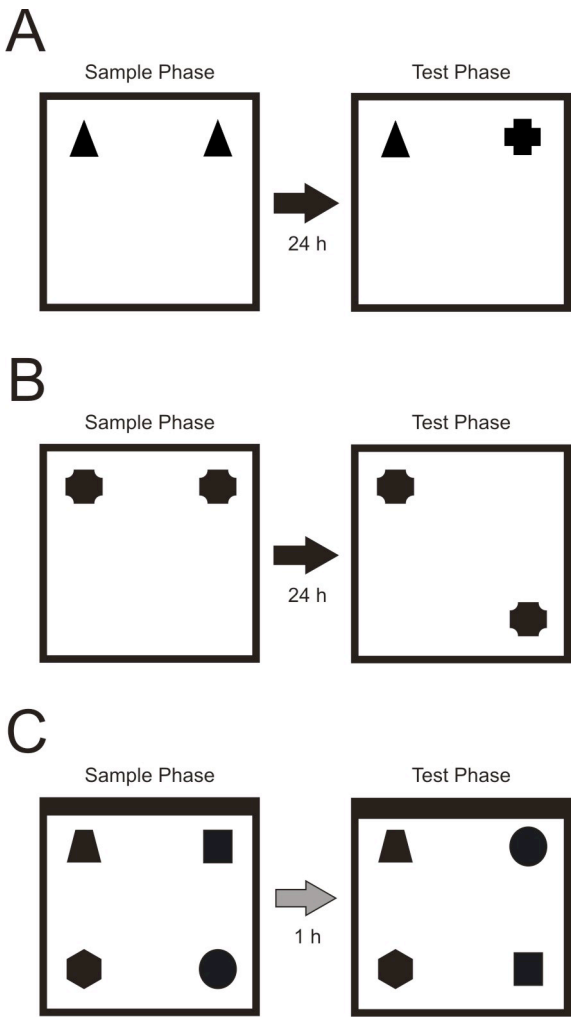

Figure 3.1. Recognition memory paradigms. **(A)** Object recognition. Rats explored two identical objects during a sample phase followed 24 h later by a test phase in which rats explored one copy of the sample object and one novel object. **(B)** Object location recognition. Rats explored two identical objects during the sample phase. Following a 24 h delay, they explored two objects identical to the sample phase objects but with one object in a new location. **(C)** Object-in-place recognition. During the sample phase, rats explored 4 different objects located in the corners of the box. One h after the sample phase, rats again explored copies of the same 4 sample objects, but with two objects in displaced positions

3.2.3.3. Experiment 2a. Novel Object Recognition Paradigm. The object recognition paradigm as well as the object location and object-in-place paradigms consisted of two phases: a sample phase and a test phase. During the sample phase of novel object recognition, subjects explored two identical objects (A1 and A2) for 4 min (Figure 3.1A). Objects were constructed of glass, plastic, or porcelain and were all similar in size (~ 10 cm in height and length). Following the 4 min of exploration, rats were then returned to the colony room. Twenty-four h following the sample phase, memory for the previously encountered objects was tested during the 4 min test phase in which subjects explored a copy of the sample object (A3) and a novel object (B1; Figure 3.1A). Novel objects were counterbalanced for the left and right side of the arena to eliminate the effect of any side preference. For both the sample and test phase, objects were located in the corners of the arena 10 cm from each of the nearest walls, while subjects were placed in the arena facing the wall opposite the objects. Between each rat, objects and the arena were wiped with 40% EtOH.

3.2.3.4. Experiment 2b. Object Location Recognition Paradigm. One week following the novel object recognition test, subjects were tested in the object location paradigm. The sample phase was identical to the novel object recognition sample phase in which subjects explored two identical objects (C1 and C2) located at the back of the arena 10 cm from each of the nearest walls (Figure 3.1B). Twenty-four hours later, subjects received a test phase in which they explored two identical copies of the sample objects (C3 and C4) but with one object moved to a corner location at the front of the box and the other object in the original sample phase location (Figure 3.1B). Object displacement was counterbalanced to eliminate any effect of side preference. Novel objects were used in the object location paradigm.

3.2.3.5. Experiment 2c. Object-in-Place Recognition Paradigm. One week following object location testing, subjects were tested in the object-in-place paradigm. During the sample phase, subjects explored four different objects (D1, E1, F1, G1) for 5 min. Objects were located in the four corners of the arena 10 cm from each of the nearest walls (Figure 3.1C). Following a 1 h delay (spent in colony room home cage), subjects were placed back in the arena and exposed to four additional copies of the objects (D2, E2, F2, G2). However, during the test phase, the positions of two of the objects were switched (Figure 3.1C). Only the two objects on the left side

of the arena or the right side of the arena were switched (i.e., front object becomes the back object and vice versa). Object-in-place memory is inferred when rats spend more time exploring the pair of objects that switch locations than the objects that remain in their sample positions. In addition to the subjects that underwent repeated testing of object recognition, object location recognition and object-in-place recognition, a separate cohort of animals (12 male, 12 female) were only tested in the object-in-place recognition paradigm. Results of these rats were identical to those seen in the repeated measures designed; as a result, data from these two groups of subjects were pooled.

3.2.4. Estrous Cycle Measurements

Vaginal samples were collected in a manner similar to that previously described (Zhang et al. 2011). Briefly, beginning on ~ PND 54, vaginal smears were collected daily from the female offspring (n= 44). Each morning between 0800 and 1000 h, a vaginal sample was collected by inserting a pipette tip containing 20 uL of 0.9% saline in to the vaginal cavity (2.5 to 5 mm deep). The saline was ejected, immediately reloaded, and then expelled onto a clean glass slide. Wet samples were viewed under a light microscope and estrous cycle was determined using established cytological methods (Devall et al. 2009; Goldman et al. 2007; Marcondes et al. 2002). Following viewing of vaginal smears, samples were preserved with Cytoprep spray (Fisher Scientific). All rats displayed normal alterations in estrous cycle. For behavioural experiments, no effort was made to test female subjects during a specific phase of the estrous cycle. Instead, performance was correlated post hoc with the naturally occurring estrous cycle phase (Point biserial correlation $p<0.05$ considered significant).

3.2.5. Data Scoring and Analysis

3.2.5.1. Experiment 2a and 2b: Object recognition and object location recognition paradigms. Data were scored according to measures previously described (Howland and Cazakoff 2010). Time spent exploring each of the objects available on a given trial was scored from digital video files with stopwatches by an individual blind to the treatment status of the rat. A rat was judged to be actively exploring an object when its nose was directed within 2 cm of an

object and either its head or vibrissae were moving. A rat was not considered to be exploring an object when the rat was standing on top of an object but not directing attention towards it. Previous experiments in our laboratory have shown that rats must explore both objects for at least 15 s in both the sample and test phases in order to show reliable memory. As a result, rats with less than 15 s of exploration on either the sample or test phase were eliminated from the study. Both total exploration times of the objects on a given trial and DR, calculated as ((time exploring the novel object/object in novel location – time exploring the familiar object/object in familiar location)/total time exploring both objects) were quantified for each rat, and group means for total exploration in the sample phase, total exploration in the test phase, and DR of the test phase were analyzed. Rats whose DR was greater than two standard deviations above or below the group mean were removed.

3.2.5.2. Experiment 2c: Object-in-place paradigm. Data scoring and analysis were conducted in a manner similar to that described previously (Barker et al. 2007; Barker and Warburton 2008). Exploration on all 4 objects was measured according to the same criteria used in the object recognition and object location recognition paradigms, and exploration times in the sample and test phase as well as DR in the test phase were measured. Previous research has shown that 10 s of exploration during the test phase of this paradigm is a sufficient amount of time for subjects to display reliable memory. Any rats that did not explore at > 15 s during the sample phase and > 10 s during the test phase were thus removed from the study. The DR in the test phase was calculated as ((time spent exploring objects in displaced positions – time spent exploring objects in familiar positions)/total time spent exploring objects).

Given that spontaneous object recognition memory tests have been reported to be most sensitive in the first 2 min of the test trial while object location and object in place recognition memories are most sensitive during the first min of the task (Barker et al. 2007; Clark et al. 2000; Dix and Aggleton 1999), reported here are the DR calculated from the first 2 min of the test phase in all recognition tests. Total exploration times for each test were analyzed using a two-way ANOVA with Treatment and Sex as between subject factors where appropriate. For test phase data, the DR for each group was analyzed using single group t-tests (with a comparison value of 0 indicating equal exploration of the two objects or chance performance) while between group comparisons were performed using two-way ANOVA with Treatment and Sex as between

subjects factors. Post hoc analysis was completed using Newman-Keuls test. Correlations between estrous cycle phase and DR on all tests were made using a point biserial correlation. A p value of <0.05 was considered significant for all statistical tests.

3.3. Results

3.3.1. Maternal PolyI:C Treatment

Pups from PolyI:C and saline treated dams were used in multiple experiments in our lab including for examinations of set-shifting, paired pulse inhibition, locomotor activity, and fear conditioning. The data from the mothers of all of these pups are reported here. Weight and rectal temperature of all dams was taken 0, 8, 24, 48 h following PolyI:C injection. Statistical analysis of dam weights revealed a significant main effects of Time ($F(3, 87) = 45.72$, $p < 0.001$), Treatment ($F(1,29) = 5.11$, $p = 0.031$), and a significant Treatment by Time interaction ($F(3, 87) = 17.28$, $p < 0.001$). Inspection of the data revealed that dam weights were significantly lower for the saline than PolyI:C-treated group (at 0 h: 345.72±8 g vs. 387.38±10 g). Further analysis of the weight data revealed that while dams in both groups lost weight in response to being anesthetized, PolyI:C-treated animals lost more weight and gained significantly less weight than the saline-treated animals over the subsequent 48 h relative to initial weight at 0 h (main effect of Treatment: $F(1,29)=68.73$, $p<0.001$; Treatment by Time interaction: $F(2,58)=5.11$, $p=0.009$). At 8, 24, and 48 h after treatment, saline animals weighed -6.0±1.6, +4.72±1.4, and +15.83±1.3 g compared to their weights at 0 h. PolyI:C- treated dams lost 17.92±2.1, 15.77±2.0, and 3.69±4.1 g relative to their initial weight 8, 24, and 48 h later. Analysis of temperature data with ANOVA showed a significant main effect of Time ($F(3, 75)=10.24$, $p<0.001$) without significant main effects of Treatment ($F(1, 25) = 0.49$, $p = 0.49$) or Time by Treatment interaction ($F(3,75) = 1.71$, $p=0.17$). Post-hoc analyses indicated that both treatment groups showed a significant increase in temperature at 8 h relative to all other time points. Subsequent analyses with paired samples t-tests revealed that the dams treated with PolyI:C showed a significant increase in temperature at 8 h (0.67 °C; $t(10)=-6.91$, $p<0.001$) whereas the dams treated with saline did not (0.29 °C; $t(15)=-1.88$, $p=0.080$). An average of 13.06±0.89 (6.86±0.15 g/pup) and 12.21±0.92 (6.62±0.07 g/pup) pups were born to the saline-treated and PolyI:C-treated dams, respectively.

No significant effect was noted for prenatal treatment regarding the number ($t(30) = 0.65$, $p=0.521$) or weight of the pups on PND 1, 8, 14, or 21 (data not shown). Collectively, these results are consistent with a significant infection like state and fever in the PolyI:C treated dams but not the saline treated dams.

3.3.2. Behaviour

The numbers of animals tested in all recognition tests were as follows: (1) Object recognition paradigm: saline treated males n = 15, saline treated females n = 14, PolyI:C treated males n = 15, PolyI:C treated females n = 16 (2) Object location paradigm: saline treated males n = 15, saline treated females n = 13, PolyI:C treated males n = 16 , and PolyI:C treated females n = 17 (3) Object-in-place paradigm: saline treated males n = 16, saline treated females n = 17, PolyI:C treated males n = 14; PolyI:C treated females n =16. For females, no correlation between estrous cycle phase and object exploration time nor estrous cycle phase and DR was noted ($p>0.05$) for any of the recognition tests.

3.3.2.1. Experiment 2a: The effects of prenatal PolyI:C treatment on novel object recognition memory

3.3.2.1.1. Exploration times. Total exploration times for all rats in both the sample and test phase are summarized in Table 3.1. Analysis of the sample phase exploration with a two-way ANOVA revealed a significant main effect of Sex ($F(1,56) = 20.39$, $p < 0.001$), with no effect of Treatment ($F(1,56) = 2.55$, $p = 0.12$) or Sex by Treatment interaction ($F(1,56) = 0.62$, $p=0.44$). Post-hoc analysis revealed that, during the sample phase, females explored the objects more (88.91 ± 2.49 s) than the males (65.94 ± 2.49 s) regardless of treatment. Analysis of exploration during the 2 min test phase revealed both a significant main effect of Sex ($F(1,56) = 12.93$, $p < 0.001$) and Treatment ($F(1,56) = 9.50$, $p = 0.003$); however, there was no significant Sex by Treatment interaction ($F(1,56) = 0.45$, $p = 0.51$). Post hoc analysis revealed that females (46.26 ± 2.05 s) explored objects for a greater amount of time than males (37.39 ± 1.58 s) regardless of Treatment while PolyI:C treated rats (45.51 ± 1.73 s) explored the objects for a greater amount of time than the Saline treated rats (37.88 ± 2.03 s) regardless of Sex.

3.3.2.1.2. Effects of prenatal Poly I:C treatment on object recognition memory: Poly I:C treatment failed to produce any significant effect on memory for previously viewed objects (Figure 3.3). Both male (D2 = 0.38 ± 0.05) and female (D2 = 0.31 ± 0.05) PolyI:C treated rats performed at levels comparable to their prenatal saline treated counterparts (male D2 = 0.30 ± 0.04; female D2 = 0.27± 0.07). All groups showed memory that was significantly different from zero (Male Saline $t(14) = 7.37$, $p < 0.001$; Male PolyI:C $t(14) = 7.38$, $p < 0.001$; Female Saline $t(13) = 4.82$, $p < 0.001$; Female PolyI:C $t(15) = 6.63$, $p < 0.001$). A two-way ANOVA revealed no effect of Treatment ($F(1,56) = 0.65$, $p = 0.42$), Sex ($F(1,56) = 1.02$, $p = 0.32$), and no Sex by Treatment interaction ($F(1,56) = 0.43$, $p = 0.52$).

Table 3.1. Object recognition, object location, and object-in-place exploration times for saline and PolyI:C treated male and female rats. Total exploration time of objects (s ± standard error of the mean) during the sample and test phases of the object recognition, object location, and object-in-place paradigms. The total time for the entire 4 min of is presented for the sample phases while the time over the first 2 min is presented for the test phases.

	Object Recognition		**Object Location**		**Object-in-Place**	
Treatment	**Sample Phase (Total)**	**Test Phase (1st 2 min)**	**Sample Phase (Total)**	**Test Phase (1st 2 min)**	**Sample Phase (Total)**	**Test Phase (1st 2 min)**
Male Saline	63.90 ± 3.55	34.51 ± 2.29	52.56 ± 5.56	31.99 ± 2.60	64.70 ± 3.41	26.13 ± 1.70
Male PolyI:C	67.98 ± 3.65	40.26 ± 2.03	66.34 ± 3.52	35.33 ± 2.51	83.28 ± 7.82	41.40 ± 4.25
Female Saline	82.57 ± 5.10	41.49 ± 3.30	60.65 ± 5.21	35.38 ± 3.96	74.90 ± 5.54	36.31 ± 3.46
Female PolyI:C	94.46 ± 7.14	50.43 ± 2.21	58.18 ± 4.48	36.89 ± 2.39	81.70 ± 6.09	40.81 ± 3.12

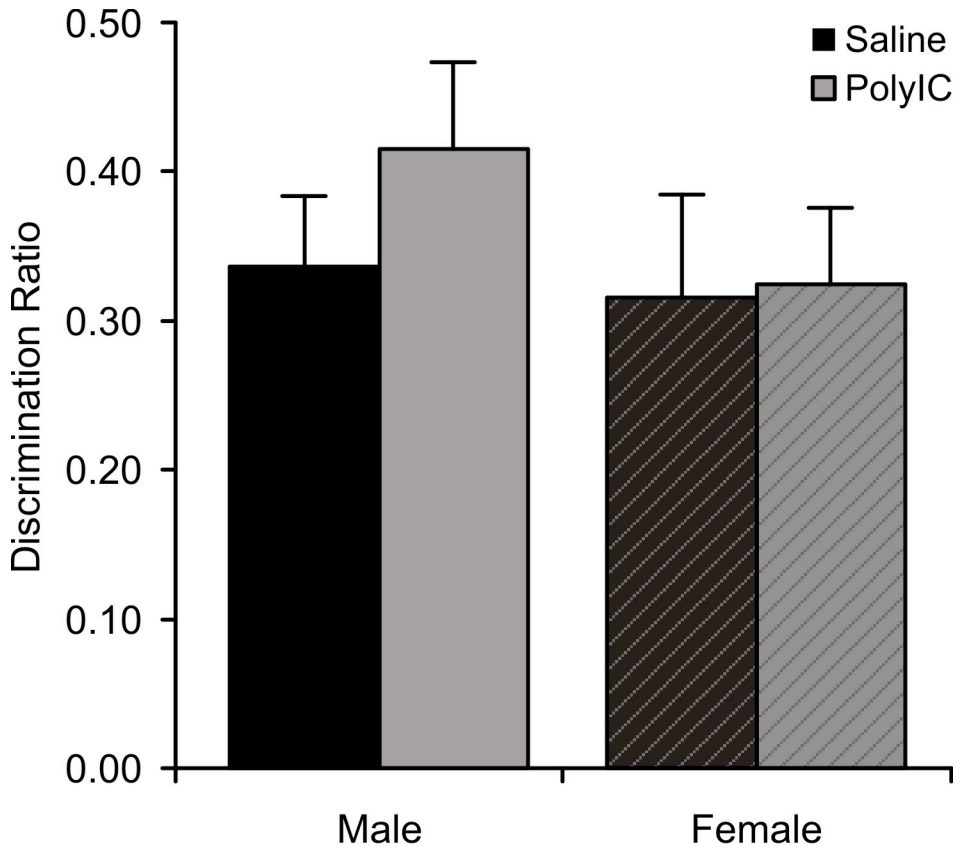

Figure 3.3. Effect of PolyI:C on object recognition memory. Prenatal PolyI:C treatment did not influence spontaneous novel object recognition in male or female rats. Saline treated animals are shown in black while PolyI:C treated animals are shown in grey. Solid colours indicate males while diagonal lines indicate females. Data are displayed as DR ± standard error of the mean (SEM) for the first 2 min of the testing period.

3.3.2.2. Experiment 2b: The effects of prenatal PolyI:C treatment on object location recognition memory

3.3.2.2.1. Exploration times. A summary of exploration times for all rats in the object location recognition paradigm is displayed in Table 3.1. In the sample phase, rats across all groups explored the objects for similar amounts of time. ANOVA revealed no effect of Sex ($F(1,57) = .00$, $p = 0.99$), Treatment ($F(1,57) = 1.49$, $P = 0.23$), or a Sex by Treatment interaction ($F(1,57) = 3.07$, $p = 0.09$). During the test phase, rats showed no significant differences in the time spent exploring the objects. Analysis of the first 2 min of exploration revealed no significant effects of Sex ($F(1,57) = 0.77$, $p = 0.38$) or Treatment ($F(1,57) = 0.74$, $p = 0.39$) and no significant Sex by Treatment interaction ($F(1,57) = 0.11$, $p = 0.75$).

3.3.2.2.2. Effects of prenatal PolyI:C treatment on object location recognition memory. Similar to object recognition memory, both PolyI:C treated male (DR = 0.23 ± 0.06) and female rats (DR = 0.12 ± 0.05) showed significant object location memory that was similar to that of male (DR = 0.24 ± 0.05) and female saline treated rats (DR = 0.11 ± 0.06; Figure 3.4). One sample t-tests revealed that all treatment groups showed memory that was significantly different from zero (Male Saline $t(14) = 5.048$, $p < 0.001$; Male PolyI:C $t(15) = 3.72$, $p = 0.002$; Female Saline $t(12) = 2.01$, $p = 0.03$ – one tail; Female PolyI:C $t(16) = 2.2$, $p = 0.04$). Analysis with a two-way ANOVA revealed a significant main effect of Sex ($F(1,57) = 4.77$, $p = 0.03$), no effect of Treatment ($F(1,57) = 0.00$, $p = .98$), and no significant Sex by Treatment interaction ($F(1,57) = 0.03$, $p = 0.87$). Inspection of the data revealed that females showed a significantly reduced preference for the object that was moved (DR = 0.11 ± 0.04) than males (DR = 0.23 ± 0.04) regardless of treatment.

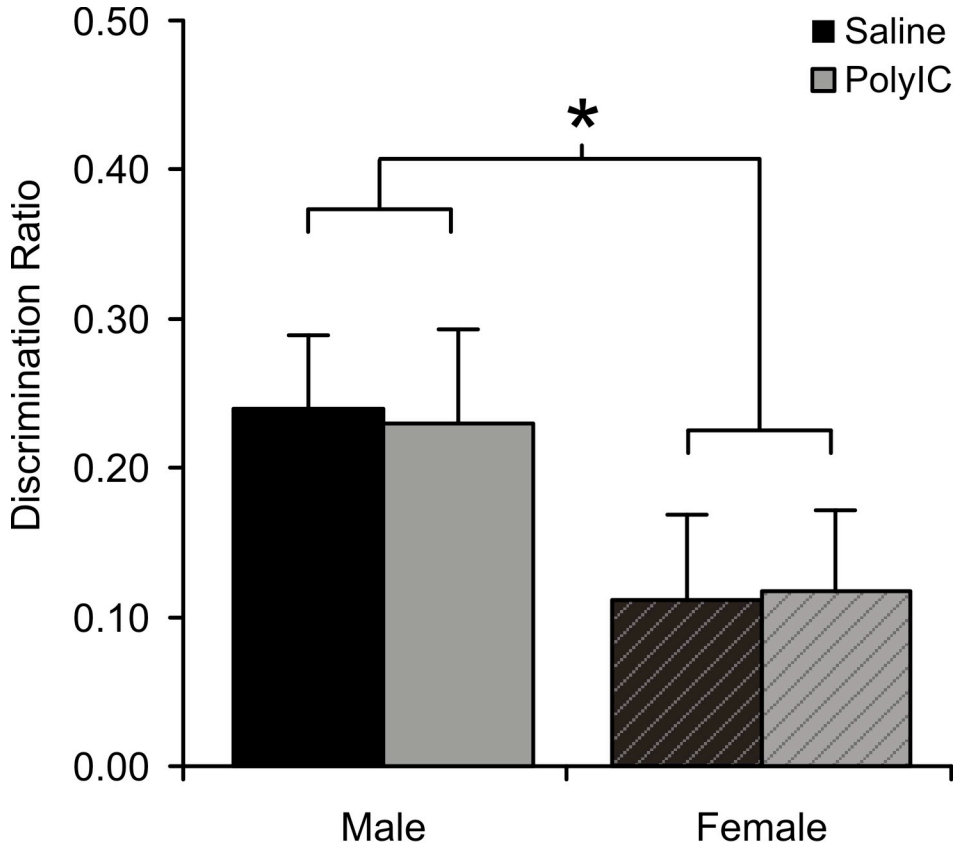

Figure 3.4. Effect of PolyI:C on object location recognition memory. Prenatal PolyI:C treatement did not influence object location recognition memory. Over the 2 min period, no difference was found between non-treated (black solid bar) and treated males (grey solid bar) or non-treated (black patterned bar) and treated females (grey patterned bar). Female rats did have significantly worse memory overall with no effect of treatment. Data are displayed as the DR ± SEM.

3.3.2.3. Experiment 2c. The effects of prenatal PolyI:C treatment on object-in-place recognition memory

3.3.2.3.1. Exploration times. Table 3.1 displays the mean exploration times for all rats in both the sample and test phases of the object-in-place test. During the sample phase, analysis with two-way ANOVA showed no significant effect of Sex ($F(1,59) = 0.67$, $p = 0.42$) and no significant Sex by Treatment interaction ($F(1,59) = 0.78$, $p = 0.38$). However, there was a significant main effect of Treatment ($F(1,59) = 5.16$, $p = 0.03$). Post hoc analysis revealed that rats in the PolyI:C treated group displayed more exploration during the sample phase. Analysis of the test phase revealed a significant main effect of Sex ($F(1,59) = 15.29$, $p < 0.001$) but no significant effect of Treatment ($F(1,59) = 2.23$, $p = 0.14$) and no significant Sex by Treatment interaction ($F(1,59) = 0.17$, $p = 0.68$). Post hoc analysis showed that PolyI:C treated females spent more time exploring the objects than saline treated males.

3.3.2.3.2. Effects of prenatal PolyI:C treatment on object-in-place recognition memory. During the object in place recognition memory task, rats with significant memory for the novel object/location association will spend more time exploring the objects that have switched locations as opposed to the two objects that remain in the same location as the sample phase. Analysis of the DR during the object-in-place test phase revealed a significant difference between the PolyI:C treated and saline treated male rats (Figure 3.5). While saline treated male rats showed intact object-in-place memory ($D2 = 0.26 \pm 0.08$; $t(15) = 3.14$, $p < 0.01$), PolyI:C treated rats failed to show memory for the object-in-place conjunction ($D2 = 0.01 \pm 0.04$; $t(13) = 0.23$, $p = 0.83$). By comparison, female rats in either treatment group failed to show intact object-in-place memory (saline-treated: $DR = 0.06 \pm 0.08$; $t(16) = 0.76$, $p = 0.46$; PolyI:C treated: $DR = 0.13 \pm 0.07$; $t(15) = 1.82$, $p = 0.09$). A two-way ANOVA revealed no significant effect of Sex ($F(1,59) = 0.31$, $p = 0.58$) or Treatment ($F(1,59) = 1.59$, $p = 0.21$. However, there was a significant Sex by Treatment interaction ($F(1,59) = 4.73$, $p = 0.03$). Post-hoc analysis revealed that the PolyI:C treated males differed significantly from the saline treated males.

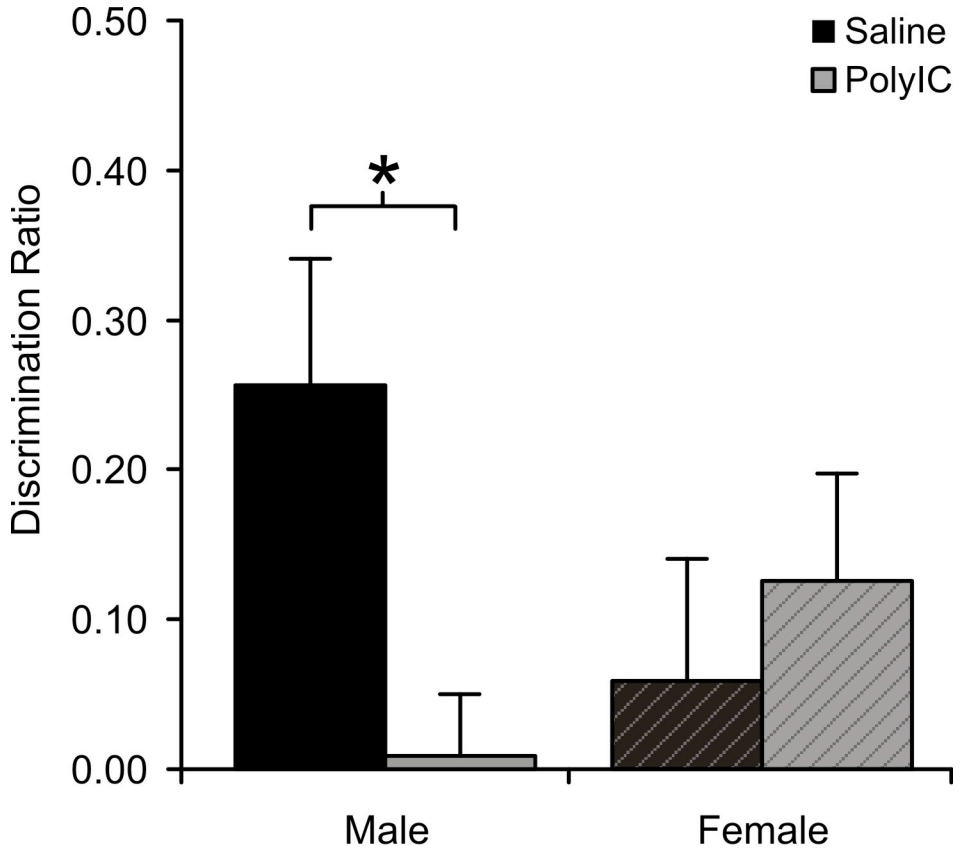

Figure 3.5. Effect of PolyI:C on object-in-place recognition memory. Prenatal PolyI:C treatment significantly disrupted object-in-place memory in male rats. Male saline treated (black solid bars) rats displayed significant memory while male treated rats (grey solid bars) failed to discriminate between novel objects in novel locations. Female treated (grey patterned bars) and non-treated rats (black patterned bars) failed to show reliable memory.

3.4. Discussion

In the present study, we examined the influence of prenatal treatment with PolyI:C on the capacity for recognition memory in young adult male and female rats using three spontaneous tests: novel object recognition, object location, and object-in-place. Our results yielded a number of important findings. While recognition memory was not disrupted in tests of novel object recognition or novel object location recognition, PolyI:C male rats displayed significantly poorer performance in a test of associative object-in-place memory. In contrast, female PolyI:C treated rats failed to show significant disruptions compared to their saline treated female counterparts; however, object-in-place memory was poor in both the saline- or PolyI:C-treated females. Taken together, these results suggest that in male rats, prenatal immune activation disrupts the capacity for associations between object and place while leaving more simple discrimination ability intact.

To our knowledge, this is the first demonstration of a disruption of object-in-place associations as a result of prenatal insult. The apparent lack of memory was not likely due to alterations in exploratory behaviour or attentional processing as memory was normal in the simple recognition tests and total exploration did not differ between animals during the test phase. During the sample phase, exploration times for PolyI:C treated animals were significantly different from saline treated animals. However, both PolyI:C treated males and females demonstrated significantly more exploration than saline treated males and female leading us to believe that treated animals had adequate time to acquire memory for the object place associations. Previously, prenatal infection has been implicated in deficits in working memory, strategy set-shifting, and reversal learning (Boksa 2010; Meyer et al. 2008; Zhang et al. 2011; Zuckerman and Weiner 2005). The present results further support the notion that prenatal immune challenge results in significant changes in the cognitive processes later in life. Importantly, our findings are consistent with studies of object association learning in patient populations. Patients with schizophrenia display more difficulty in combined object location tasks than in the object or location tasks alone (Burglen et al. 2004; Leiderman and Strejilevich 2004; Salame et al. 2006). Interestingly, deficits in paired association learning arise early in patient populations and increase with further disease progression, so it would be of interest to test

animals at both early and later time points in this task (Barnett et al. 2005; Bartok et al. 2005; Wood et al. 2002).

We did not find disruptions in spontaneous novel object recognition or object location recognition in either male or female PolyI:C treated rats. Previous studies have demonstrated novel object recognition deficits in mice prenatally exposed to PolyI:C and LPS (Coyle et al. 2009; Ibi et al. 2009; Ozawa et al. 2006) while one study has demonstrated no change in object location memory and an improvement in object recognition memory (Ito et al. 2010). In rodents prenatally exposed to maternal stress, stress results in varying effects (Bowman et al. 2004; Schulz et al. 2011). Prenatal infection has also been demonstrated to disrupt performance in spatial memory tasks including deficits in water maze and radial arm maze reference memory (Hao et al. 2010; Lante et al. 2008; Meyer et al. 2005; Samuelsson et al. 2006). The present results contrast these previous reports as, in both male and females, we failed to find disruptions in tests of more simple discriminations between novel and familiar objects or locations. The discrepancy between our results and others may reflect differences in both task choice and animal model. Previous studies were completed in mice or different strains of rat that display demonstrable deficits in cognitive performance when compared to their rat counterparts (Andrews 1996). Furthermore, many of the spatial memory tasks in which deficits are observed involve considerably more training and components of stress (i.e., food deprivation in the radial arm maze or water exposure in the water maze) that could complicate task performance. Across studies, the recognition tasks were also implemented with variable delays between the sample and test phases. In previous studies, changes have been observed with delays of 15 min, 1 h, and 4 h between the sample and test phase (Coyle et al. 2009; Ibi et al. 2009; Ito et al. 2010; Ozawa et al. 2006) while in our study, no deficit was observed in the novel object recognition test using a 24 h delay. With no deficit at 24 h, it might be expected that no deficit would either be found at a shorter delay that is presumably easier and less taxing on long term memory. However, considerable dissociation exists between shorter term and longer term recognition memory with reports of different glutamatergic plasticity mechanisms distinctly involved in memory over both a rapid and long time scale. Specifically, in novel object recognition, antagonism of kainite receptors produces deficits in recognition memory with a short (20 min), but not a long (24 h), delay between the sample and test phase, while antagonism of NMDA receptors produces deficits at long, but not short, delays (Barker et al. 2006b). Disruptions in glutamatergic signaling

have been proposed as a putative mechanism for the observed deficits in patients with schizophrenia with several studies reporting altered glutamate receptor binding, NMDA receptor malfunction, and genetic glutamate polymorphisms in both patients with schizophrenia and animal models (Javitt 2007; Javitt 2010; Lewis and Gonzalez-Burgos 2006; Lewis and Gonzalez-Burgos 2008; Moghaddam 2003; Moghaddam and Jackson 2003; Stone et al. 2007). Whether prenatal infection results in differential changes in NMDA and kainite receptors leading to the observed cognitive deficits remains to be examined.

The effects of prenatal PolyI:C treatment observed in the present study were restricted to male rats. Few sex differences in the effect of prenatal infection on cognition in rodents have been reported although male specific deficits in hyperlocomotion, strategy set shifts, and fear conditioning have been observed in rodents (Bitanihirwe et al. 2010a; Bitanihirwe et al. 2011; Schwendener et al. 2009; Zhang et al. 2011) while male specific deficits in working memory and set-shifting have been reported in humans (Goldstein et al. 1998; Lecardeur et al. 2010). Neonatal and adult females display altered levels of dopamine and glutamate neurotransmitters and receptors compared to their male counterparts (Honack and Loscher 1993; McCarthy et al. 1997; Staiti et al. 2011). Many of these alterations are found in key regions of the brain implicated in schizophrenia and as a result, differences in glutamatergic processing may contribute to the lack of deficit observed in this study. At present, neuronal processing differences or compensatory mechanisms employed by females in cognitive tasks have not been extensively explored. Interestingly, while there were no differences between female treated and female non treated rats in the object location and object-in-place experiments, both female saline and female PolyI:C displayed deficits when compared to male control animals. The literature concerning visual and spatial memory differences between male and female subjects is extensive with competing effects reported in both humans and rodents contingent on task employed. Previous reports suggest that estrus cycle phase can influence novel object location recognition in rodents with higher levels of estrogen and progesterone inhibiting performance (Frye and Sturgis 1995; Sutcliffe et al. 2007). In our study, there was no correlation between estrous cycle phase and memory performance, ruling out an effect of cycle on poor memory. By comparison, in humans, males generally perform better in tests of spatial reference and working memory (Driscoll et al. 2005; Faraji et al. 2010; Lejbak et al. 2011; Piper et al. 2011; Sandstrom et al. 1998) while females consistently perform better on tests of object location memory (Barnfield

1999; De and Postma 2008; Hassan and Rahman 2007; Lejbak et al. 2009; Saucier et al. 2007; Silverman et al. 2007; Sutcliffe et al. 2007). It is not clear why our results contrast with the human literature although difference in task parameters may contribute.

Aberrant processing in several brain regions has been suggested to underlie the development and expression of schizophrenia. In particular, the frontal and medial temporal lobes have received emphasis as dysfunction of hippocampus, perirhinal and entorhinal cortices, and PFC exists in both human patients and rodent models (Goldman and Mitchell 2004; Keri 2008; Swerdlow 2010; Tamminga et al. 2010; Volk and Lewis 2010). Both lesion and pharmacological studies demonstrate a crucial role for PRh and hippocampus in novel object and novel object location recognition, with PRh playing a particular role in the recognition of previously viewed objects (Broadbent et al. 2004; Warburton and Brown 2010; Winters and Bussey 2005b; Wixted and Squire 2004). In the present study, the lack of effect seen in both the object and object location tasks suggests that processing in the medial temporal lobe regions alone is not profoundly disturbed by prenatal infection. In contrast, the object-in-place recognition task relies upon processing in both the medial temporal lobe and PFC with the integration of objects and locations purported to depend substantially on PFC (Barker et al. 2007; Barker and Warburton 2008; Barker and Warburton 2009). Our observed results suggest that prenatal immune activation disrupts neural processing in PFC of the offspring or in the connections between the PFC and other cortical regions. Aberrant functional connectivity between the PFC and medial temporal lobe structures has been reported following prenatal infection (Dickerson et al. 2010) and in other developmental models of schizophrenia (Gruber et al. 2010; Saunders et al. 1998). Further, the disruption of object-in-place memory is in accordance with the disconnectivity hypothesis of schizophrenia characterized by dysfunction in the interactions of the PFC with other cortical areas. At least some of this dysfunction could be mediated by altered glutamatergic plasticity in prefrontal or prefrontal-medial temporal lobe circuits (glutamatergic hypothesis) with object-in-place memory proposed to depend upon both NMDA and AMPA receptor signaling (Barker and Warburton 2008). However, further study is required to confirm this hypothesis.

3.5. Conclusion

The present experiments show that prenatal immune activation results in cognitive disruptions in rodents similar to those reported in schizophrenia patients. The demonstrated clinical relevance of this task and its relative ease in implementation make it suitable for understanding the pathophysiological mechanisms underlying cognitive disruption as a result of infection and for the testing of new therapeutics for psychiatric disorders.

CHAPTER 4
GENERAL DISCUSSION

4.1. Summary of Main Findings

In the present thesis, two studies were completed in which object recognition memory in rodents was examined. In Chapter 2, AMPA receptor endocytosis was demonstrated to be necessary for the retrieval of object memory but not for the initial encoding or storage of this memory. Subjects that received infusions of the Tat-GluA2$_{3Y}$ peptide either 1 h prior to the sample phase or immediately following the sample phase displayed significant memory for familiar objects and discriminated between novel and familiar objects in a manner similar to control subjects. In contrast, animals that received infusions of the Tat-GluA2$_{3Y}$ peptide 1 h prior to the test phase failed to show significant memory for familiar objects. In Chapter 3, object memory in three different paradigms, object recognition, object location, and object-in-place recognition, was examined in both female and male rats prenatally exposed to PolyI:C. A specific disruption of object-in-place memory in male rats without concomitant disruptions in object or object location memory was demonstrated. Furthermore, PolyI:C treatment in female rats did not disrupt memory in any of the paradigms; however, female rats in both the control and PolyI:C treated groups failed to show significant memory in the object-in-place paradigm while object location memory was significantly worse in both groups compared to male controls.

4.2. Synaptic Plasticity and Recognition Memory

In the first study of this thesis, I examined the time dependent requirement of AMPA receptor endocytosis in object memory, hypothesizing that AMPA receptor endocytosis would only be required during distinct time points. As already highlighted, the retrieval specific requirement of AMPA receptor endocytosis documented in this thesis is the first study to demonstrate a retrieval only effect. Previously, both AMPA receptors and AMPA receptor endocytosis have been shown to be involved in recognition memory.

Winters and Bussey (2005) demonstrated that CNQX infusions into PRh prior to encoding, during consolidation, or prior to retrieval disrupt object recognition. Further, Griffiths

and colleagues (2008) demonstrated that blockade of AMPA receptor endocytosis using an interference peptide expressed with a viral vector throughout the memory process disrupts recognition; further, this disruption of memory correlates with a disruption in LTD. The discrepancies between these studies and the results presented in Chapter 2 may be due to several factors. First, CNQX disrupts all AMPA receptor dependent excitatory activity. The possibility exists that all time points require some fast synaptic transmission mediated by AMPA receptors, but only retrieval requires the endocytosis of such receptors and a reduction in synaptic response. It is not implausible to suggest that some component of recognition memory requires LTP like activity; blocking AMPA receptors with CNQX would block LTP and also block behaviours dependent upon LTP.

Critically, AMPA receptor endocytosis and LTD as substrates for familiarity are in agreement with electrophysiological recordings in behaving animals. Reductions in neuronal responsiveness have been demonstrated in both rodents and monkeys between the first and second exposure to visual stimuli (Brown and Bashir 2002; Fahy et al. 1993; Xiang and Brown 1998; Zhu and Brown 1995). These response reductions are noted even when competing stimuli are presented in the interval between the first and second response (Brown and Bashir 2002) indicating that these reductions are not merely perceptual in nature but signal a remembrance of previous stimuli. What was not clear before this study was whether response reductions occurred immediately following the first presentation of the stimulus, so PRh circuits "held" the memory throughout the delay period and into the second presentation of the stimulus, or if response reduction occurred upon second presentation of the stimulus. While modeling studies suggest the former (Bogacz et al. 2001; Bogacz and Brown 2003), the present results suggest the second scenario may be more likely. Upon reintroduction to a stimulus, there is a short delay (90 ms) following stimulus presentation (longer than required for visual processing) before response reductions in both PRh and Area TE are noted, indicating that reintroduction is necessary (Fahy et al. 1993). It is not surprising then to find a retrieval specific effect of the endocytosis of AMPA receptors, and the possibility exists that such endocytosis may mediate this change in neuronal responding. Interestingly, the previous tasks employing neuronal recording did not present novel and familiar stimuli together in a trial; rather, repetitions of pictures of novel or familiar pictures and objects were presented in succession. Whether a difference in PRh activity

exists when discriminations are made concomitantly or in relation to previous trials remains an open question.

4.3. Recognition Memory and Neurodevelopmental Illness

In the second experiment, I examined how prenatal PolyI:C treatment altered recognition memory function in both male and female young adult offspring, hypothesizing that in utero exposure to PolyI:C would significantly alter the normal development of neural circuitry and result in memory deficits. Male rats only showed deficits on the object-in-place task but not on more simple discrimination tasks.

Previous experiments have demonstrated both disruptions (Bitanihirwe et al. 2010b; Ibi et al. 2009; Ozawa et al. 2011b) and improvements (Ito et al. 2010) of simple object recognition as a result of prenatal infection; the current thesis did not replicate these findings. One of the major differences between this study and others may be in choice of animal model. Previous studies used mice in tests of recognition. While mice offer many advantages, especially in the use of transgenics, mice are comparatively less able in tests of cognition (Andrews 1996; Cressant et al. 2007; Whishaw and Tomie 1996). In the novel object paradigms used by these studies, several of the control mice were performing only at chance levels (50% of time with novel object; 50% of time with familiar object), without clear preference for the novel object in the test phase (Bitanihirwe et al. 2010b; Ozawa et al. 2006). Further, many of the studies treated pregnant dams at different gestational days (Bitanihirwe et al. 2010b; Ito et al. 2010) or with more prolonged PolyI:C treatment (Ibi et al. 2009; Ozawa et al. 2006) which may account for the differences.

Several studies have suggested that medial PFC, PRh, and hippocampus are necessary for object-in-place memory (Barker et al. 2007; Browning et al. 2005; Bussey et al. 2001). In particular, the medial PFC is required for the association of an object with a location, but not for memory of only an object or a location and for intact object in place memory, PFC and PRh must interact (Barker et al. 2007). Further, lesions of the fimbria-fornix impair object-in-place memory implicating hippocampus in this paradigm (Bussey et al. 2000; Gaffan and Harrison 1988). The failure to find an impairment of PolyI:C treated male rats in either object recognition or object location recognition then suggests that there is a disruption in PFC function in these animals or a disruption in the connections between PFC and the medial temporal lobe. Previous

behavioural studies using prenatal infection have also demonstrated impaired connectivity between PFC and the medial temporal lobe. Rodents exposed to infection in utero demonstrate significant deficits in prefrontal dependent working memory and executive function (Bitanhirwe et al 2010b; Meyer and Feldon 2009; Zhang et al 2011). Further neonatal lesion and genetic models of schizophrenia in mice demonstrate altered connectivity, neurotransmitter release, and theta phase locking between medial PFC and hippocampus (Gruber et al. 2010; Saunders et al. 1998; Sigurdsson et al. 2010). Finally, humans with schizophrenia show disrupted PFC medial temporal lobe activity during memory tasks (Meyer-Lindenberg et al. 2005; Wolf et al. 2009). Taken together, these findings along with the results from the present study suggest altered prefrontal and medial temporal lobe connectivity underlies pathological disruption of cognitive function, including recognition memory in neurodevelopmental disease.

4.4. Disruptions in Synaptic Plasticity as the Foundation of Cognitive Disruptions in Neurodevelopmental Disease

Having demonstrated the involvement of a specific form of synaptic plasticity in novel object recognition and a disruption of object-in-place recognition memory following prenatal infection, a logical question arises as to whether disruptions in synaptic plasticity underlie the observed disruptions in recognition.

It is not implausible to suggest that aberrant synaptic plasticity, including dysfunction in glutamatergic signaling, underlies the disruptions in recognition memory observed in neurodevelopmental disease. While classic studies of schizophrenia first implicated aberrant dopamine signaling in the disease, largely as a result of the effectiveness of some therapeutic agents, recent focus has shifted to dysfunction in glutamate as the substrate for several neurodevelopmental diseases, including schizophrenia (Stone et al. 2007; Stone 2009; Stone and Pilowsky 2007). Antagonists of NMDA receptors induce psychotic symptoms in humans consistent with schizophrenia while studies in both human patients and rodent models have provided evidence for altered glutamate release, altered NMDA subunit composition, and decreased AMPA receptor density and binding as factors in schizophrenia (Balu and Coyle 2011; Goff and Coyle 2001). Further, as previously mentioned, these disruptions are seen in key brain

regions necessary for memory including PFC and hippocampus (Gruber et al. 2010; Sigurdsson et al. 2010).

The studies reported offer two important insights to the global study of dysfunctional synaptic plasticity in neurodevelopmental diseases, particularly schizophrenia. First, they demonstrate the involvement of a novel mechanism during a specific time point of object recognition; it is possible that AMPA receptor endocytosis is also involved in object-in-place memory retrieval and is further disrupted in pathological states. Second, they demonstrate that a specific prenatal insult results in significant cognitive impairment in a memory paradigm that depends critically upon intercommunication between PFC and the medial temporal lobe, in particular PRh. Previously, dysfunction in PRh-PFC circuits had not been examined with most focus on hippocampus-PFC circuits instead; these studies provide impetus to examine pathologies in PRh circuits in addition to others. As well, the paradigm used here is easy to implement and produces robust results, making it especially suitable for future studies of the dysconnectivity proposed to underlie neurodevelopmental disease. Further study based upon the results demonstrated here will deepen our understanding of how neural circuits are disrupted in pathological states and how these can be treated or prevented with the use of novel therapeutics.

4.5. Future Directions

4.5.1. Experiment 1

In Chapter 2, all experimentation was conducted using pharmacological manipulation in rats. No electrophysiological recordings were completed either in vitro or in vivo to solidify a blockade of activity dependent LTD by Tat-GluA2$_{3Y}$. As a result, I cannot be certain that the blockade of AMPA receptor endocytosis found in my study results in a concomitant disruption of LTD. Previous study using the peptide has shown both blockade of AMPA receptor endocytosis and LTD in different brain regions, including hippocampus, PFC, and nucleus accumbens (Ahmadian et al. 2004; Brebner et al. 2005; Wong et al. 2007). This leads us to believe that, with electrophysiological recordings, a disruption of LTD would also be found in PRh using the peptide.

In my study, no effort was made to discriminate between the Area 35 and 36 in needle placement. Rather, in agreement with other experiments of this type (Barker et al. 2007; Warburton et al. 2003; Winters and Bussey 2005a), if needle tips terminated in either area, the placement was considered correctly positioned. As previously mentioned, Area 36 receives more dense input from visual association areas than Area 35 (Burwell et al. 1995; Kealy and Commins 2011; Suzuki 1996). The possibility exists then that in the visual object recognition paradigm chosen for study, the endocytosis of AMPA receptors is primarily required in Area 36, separate from Area 35. Previous electrophysiological recordings from PRh slices suggest that LTD can be evoked from both areas and blocked by a number of pharmacological agents. However, there is some indication that many of the slice recordings were conducted in Area 35 (Cho et al. 2000). Future study should consider the relative contribution of each Area to object memory and how synaptic plasticity may differ between these areas.

While this thesis considered the role of AMPA receptor endocytosis during the encoding, consolidation, and retrieval phases of object recognition memory, a future study should also consider the role of AMPA receptor endocytosis during reconsolidation. Established memories are susceptible to disruption and re-update upon retrieval; with each recall, they must be reconsolidated (Nader et al. 2000). To date, the role of glutamatergic synaptic plasticity has not been examined in the reconsolidation of object memory. However, there is good reason to believe that synaptic plasticity would underlie this phase of memory as well. Previous studies demonstrate a requirement for reconsolidation in object memory (Kelly et al. 2003) while studies of other types of memory demonstrate that synaptic plasticity, including AMPA receptor activity, plays an integral role in reconsolidation (Nader et al. 2000; Nader and Einarsson 2010).

Given the results observed in Chapter 2 of this thesis, one of the biggest questions that remains is what processes lie upstream of AMPA receptor endocytosis. That is, what are the enzymes and signaling cascades that are activated in order to initiate the removal of AMPA receptors from the membrane. Correlative evidence suggests that classic upstream activators of LTD including NMDA receptors, mGluRs I II and III, and mAChRs are not involved as they fail to disrupt object memory retrieval. Future study might consider alternative signaling mechanisms including VDCCs, which have a demonstrated role in retrieval, and Src kinases which have been implicated in Tyr876 phosphorylation. Recently, transient receptor potential vanilloid 1 (TRPV1) receptors have been shown to modulate LTD in hippocampus. My own preliminary studies

suggest that TRPV1 receptors are not involved; however, these studies are by no means extensive, and future experimentation should examine this further.

4.5.2. Experiment 2

In line with previous experiments (Barker et al. 2007; Barker and Warburton 2008; Griffiths et al. 2008; Warburton and Brown 2010), this study examined object recognition and object location memory over a 24 h delay period and object-in-place recognition over a 1 h delay period. Rats, like humans, will maintain memory for novel objects over much shorter and longer delays; differences in the synaptic processes and brain regions involved at these different delays have been demonstrated (Barker et al. 2007; Winters and Bussey 2005). An interesting question is whether prenatal infection disrupts all of these mechanisms or whether some are spared in a delay dependent manner. Examining the bounds of recognition memory in PolyI:C treated rats can give insight into the specific mechanisms disrupted by prenatal insult.

An alternative explanation for the spared memory in the object and object location paradigms but a deficit in the object-in-place paradigm is that the object-in-place paradigm reflects an increased cognitive load on neuronal processing. With fours objects in the arena, subjects must process much more sensory information and make several discriminations as opposed to the two required in the object and object location paradigms. Examinations of object and object location recognition memory using more difficult versions of these paradigms should be conducted. This may be most easily done using four objects in the object recognition paradigm as opposed to two (Figure 4.1). Importantly, this may reveal deficits in object recognition memory not seen when only two objects are used and lead to important insights on the effects of prenatal PolyI:C treatment on adult memory under increased cognitive load.

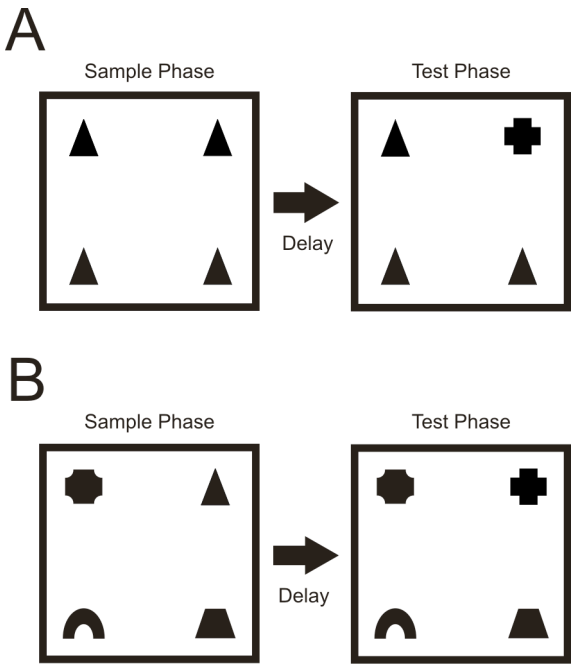

Figure 4.1. Recognition memory paradigms: increased cognitive load. (A) Schematic depicting the object recognition memory test using 4 objects instead of 2. In the paradigm, four copies of one object are used during the sample phase, while one object is replaced with a novel object during the test phase. **(B)** In a more difficult version of the paradigm, four different objects can be used during the sample phase, with one sample object replaced with a novel object during the test phase. Rats must attend to the different features of four objects instead of two, increasing the processing load. Different delays should be tested to determine at which delay optimal memory is displayed.

This experiment did not find significant object-in-place memory for either untreated or treated female rats. While gender differences in object and location memory have been reported, these reports are often in the opposite direction to what was observed in the present experiments. Generally, females perform better on tests of object-in-place recognition than males. However, several of these studies were performed in humans over very short delays (s to min). In our test, we examined recognition memory after 1 h. The possibility exists that, at a shorter delay, the female rats would display significant recognition. Current study in our lab is examining this possibility; given reliable recognition in the females at a short delay, we will then examine the influence of prenatal infection on object-in-place recognition using these parameters.

In this study, I only examined how prenatal PolyI:C treatment at GD 15 influenced recognition memory in young adult rats. This was primarily done in conjunction with another study (Zhang et al. 2011) and the GD was chosen to replicate previous results from other groups. More recent data suggests that, in humans, infection in the first trimester confers the greatest risk for neurodevelopmental disorders like schizophrenia (Brown et al. 2004). In rats, this would correspond to an earlier time point (~GD 9). While this does not negate the validity of the cognitive disruption seen (as cognitive disruption is also seen with infection in the second trimester), the possibility exists that different behavioural disruptions or disruptions of different magnitude would be observed at the various time points of infection. This avenue should be explored in future experiments.

As previously mentioned, the findings here necessarily lead to a plethora of studies concerning the molecular and synaptic disruptions that underlie a disruption in object-in-place memory. To date, no studies of the influence of prenatal infection on PFC-PRh or PFC-hippocampus electrophysiology have been conducted despite the hypothesis of the dysconnectivity between these circuits underlying infection induced cognitive impairment. Further, the molecular correlates underlying the disruption of recognition memory by prenatal infection remains an open question.

CHAPTER 5
CONCLUSION

This thesis presented novel findings related to the processes underlying the normal retrieval of object memory and how environmental insult can disrupt specific forms of recognition relevant to disease states. In the first study, AMPA receptor endocytosis was demonstrated to be necessary for the retrieval but not encoding or consolidation of object recognition memory. In the second experiment, prenatal immune activation at GD 15 using PolyI:C disrupted object-in-place recognition memory in males but did not affect object recognition or object location recognition. Both of these studies contribute significant findings to our understanding of recognition memory during normal and disrupted states and will spur further study into the synaptic processes underlying recognition during pathology.

CHAPTER 6

REFERENCES

Abe H, Ishida Y, Iwasaki T. 2004. Perirhinal N-methyl-D-aspartate and muscarinic systems participate in object recognition in rats. *Neurosci Lett* **356**: 191-194.

Abraham WC and Bear MF. 1996. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* **19**: 126-130.

Aggleton JP, Vann SD, Denby C, Dix S, Mayes AR, Roberts N, Yonelinas AP. 2005. Sparing of the familiarity component of recognition memory in a patient with hippocampal pathology. *Neuropsychologia* **43**: 1810-1823.

Agster KL and Burwell RD. 2009. Cortical efferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *Hippocampus* **19**: 1159-1186.

Ahmadian G, Ju W, Liu L, Wyszynski M, Lee SH, Dunah AW, Taghibiglou C, Wang Y, Lu J, Wong TP, Sheng M, Wang YT. 2004. Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. *EMBO J* **23**: 1040-1050.

Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, Canossa M. 2004. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A* **101**: 15788-15792.

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* **413**: 732-738.

Amaral DG and Price JL. 1984. Amygdalo-cortical projections in the monkey (Macaca fascicularis). *J Comp Neurol* **230**: 465-496.

Andrews JS. 1996. Possible confounding influence of strain, age and gender on cognitive performance in rats. *Brain Res Cogn Brain Res* **3**: 251-267.

Arndt TL, Stodgell CJ, Rodier PM. 2005. The teratology of autism. *Int J Dev Neurosci* **23**: 189-199.

Aubin G, Stip E, Gelinas I, Rainville C, Chapparo C. 2009. Daily functioning and information-processing skills among persons with schizophrenia. *Psychiatr Serv* **60**: 817-822.

Balu DT and Coyle JT. 2011. Neuroplasticity signaling pathways linked to the pathophysiology of schizophrenia. *Neurosci Biobehav Rev* **35**: 848-870.

Barker GR, Bashir ZI, Brown MW, Warburton EC. 2006a. A temporally distinct role for group I and group II metabotropic glutamate receptors in object recognition memory. *Learn Mem* **13**: 178-186.

Barker GR, Bird F, Alexander V, Warburton EC. 2007. Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J Neurosci* **27**: 2948-2957.

Barker GR and Warburton EC. 2008. NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. *J Neurosci* **28**: 2837-2844.

Barker GR and Warburton EC. 2009. Critical role of the cholinergic system for object-in-place associative recognition memory. *Learn Mem* **16**: 8-11.

Barker GR, Warburton EC, Koder T, Dolman NP, More JC, Aggleton JP, Bashir ZI, Auberson YP, Jane DE, Brown MW. 2006b. The different effects on recognition memory of perirhinal kainate and NMDA glutamate receptor antagonism: implications for underlying plasticity mechanisms. *J Neurosci* **26**: 3561-3566.

Barnett JH, Sahakian BJ, Werners U, Hill KE, Brazil R, Gallagher O, Bullmore ET, Jones PB. 2005. Visuospatial learning and executive function are independently impaired in first-episode psychosis. *Psychol Med* **35**: 1031-1041.

Barnfield AM. 1999. Development of sex differences in spatial memory. *Percept Mot Skills* **89**: 339-350.

Bartok E, Berecz R, Glaub T, Degrell I. 2005. Cognitive functions in prepsychotic patients. *Prog Neuropsychopharmacol Biol Psychiatry* **29**: 621-625.

Bear MF and Abraham WC. 1996. Long-term depression in hippocampus. *Annu Rev Neurosci* **19**: 437-462.

Beggs JM and Kairiss EW. 1994. Electrophysiology and morphology of neurons in rat perirhinal cortex. *Brain Res* **665**: 18-32.

Bilkey DK. 1996. Long-term potentiation in the in vitro perirhinal cortex displays associative properties. *Brain Res* **733**: 297-300.

Bitanihirwe BK, Dubroqua S, Singer P, Feldon J, Yee BK. 2011. Sensorimotor gating and vigilance-dependent choice accuracy: a within-subject correlative analysis in wild-type C57BL/6 mice. *Behav Brain Res* **217**: 178-187.

Bitanihirwe BK, Peleg-Raibstein D, Mouttet F, Feldon J, Meyer U. 2010a. Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology* **35**: 2462-2478.

Bitanihirwe BK, Weber L, Feldon J, Meyer U. 2010b. Cognitive impairment following prenatal immune challenge in mice correlates with prefrontal cortical AKT1 deficiency. *Int J Neuropsychopharmacol* **13**: 981-996.

Bliss TV and Collingridge GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**: 31-39.

Bliss TV and Lomo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* **232**: 331-356.

Bogacz R and Brown MW. 2003. Comparison of computational models of familiarity discrimination in the perirhinal cortex. *Hippocampus* **13**: 494-524.

Bogacz R, Brown MW, Giraud-Carrier C. 2001. Model of familiarity discrimination in the perirhinal cortex. *J Comput Neurosci* **10**: 5-23.

Boksa P. 2010. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. *Brain Behav Immun* **24**: 881-897.

Bowman RE, Maclusky NJ, Sarmiento Y, Frankfurt M, Gordon M, Luine VN. 2004. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. *Endocrinology* **145**: 3778-3787.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, Phelps L, Phillips AG, Wang YT. 2005. Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science* **310**: 1340-1343.

Brewer JB, Zhao Z, Desmond JE, Glover GH, Gabrieli JD. 1998. Making memories: brain activity that predicts how well visual experience will be remembered. *Science* **281**: 1185-1187.

Broadbent NJ, Squire LR, Clark RE. 2004. Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A* **101**: 14515-14520.

Brodmann K. 1909. Localisation in the Cerebral Cortex. Laurence Garey (ed.). Smith-Gordon, London.

Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, Susser ES. 2004. Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Arch Gen Psychiatry* **61**: 774-780.

Brown AS and Derkits EJ. 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry* **167**: 261-280.

Brown AS, Schaefer CA, Wyatt RJ, Goetz R, Begg MD, Gorman JM, Susser ES. 2000. Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. *Schizophr Bull* **26**: 287-295.

Brown AS, Vinogradov S, Kremen WS, Poole JH, Deicken RF, Penner JD, McKeague IW, Kochetkova A, Kern D, Schaefer CA. 2009. Prenatal exposure to maternal infection and executive dysfunction in adult schizophrenia. *Am J Psychiatry* **166**: 683-690.

Brown MW and Aggleton JP. 2001. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* **2**: 51-61.

Brown MW and Bashir ZI. 2002. Evidence concerning how neurons of the perirhinal cortex may effect familiarity discrimination. *Philos Trans R Soc Lond B Biol Sci* **357**: 1083-1095.

Brown MW and Xiang JZ. 1998. Recognition memory: neuronal substrates of the judgement of prior occurrence. *Prog Neurobiol* **55**: 149-189.

Browning PG, Easton A, Buckley MJ, Gaffan D. 2005. The role of prefrontal cortex in object-in-place learning in monkeys. *Eur J Neurosci* **22**: 3281-3291.

Burglen F, Marczewski P, Mitchell KJ, van der LM, Johnson MK, Danion JM, Salame P. 2004. Impaired performance in a working memory binding task in patients with schizophrenia. *Psychiatry Res* **125**: 247-255.

Burwell RD and Amaral DG. 1998. Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *J Comp Neurol* **391**: 293-321.

Burwell RD, Witter MP, Amaral DG. 1995. Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus* **5**: 390-408.

Bussey TJ, Dias R, Amin E, Muir JL, Aggleton JP. 2001. Perirhinal cortex and place-object conditional learning in the rat. *Behav Neurosci* **115**: 776-785.

Bussey TJ, Duck J, Muir JL, Aggleton JP. 2000. Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. *Behav Brain Res* **111**: 187-202.

Calkins ME, Gur RC, Ragland JD, Gur RE. 2005. Face recognition memory deficits and visual object memory performance in patients with schizophrenia and their relatives. *Am J Psychiatry* **162**: 1963-1966.

Castillo PE, Janz R, Sudhof TC, Tzounopoulos T, Malenka RC, Nicoll RA. 1997. Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature* **388**: 590-593.

Castro CA, Silbert LH, McNaughton BL, Barnes CA. 1989. Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. *Nature* **342**: 545-548.

Cho K and Bashir ZI. 2002. Cooperation between mglu receptors: a depressing mechanism? *Trends Neurosci* **25**: 405-411.

Cho K, Brown MW, Bashir ZI. 2002. Mechanisms and physiological role of enhancement of mGlu5 receptor function by group II mGlu receptor activation in rat perirhinal cortex. *J Physiol* **540**: 895-906.

Cho K, Kemp N, Noel J, Aggleton JP, Brown MW, Bashir ZI. 2000. A new form of long-term depression in the perirhinal cortex. *Nat Neurosci* **3**: 150-156.

Chouinard S, Stip E, Poulin J, Melun JP, Godbout R, Guillem F, Cohen H. 2007. Rivastigmine treatment as an add-on to antipsychotics in patients with schizophrenia and cognitive deficits. *Curr Med Res Opin* **23**: 575-583.

Ciaranello AL and Ciaranello RD. 1995. The neurobiology of infantile autism. *Annu Rev Neurosci* **18**: 101-128.

Clark RE, Zola SM, Squire LR. 2000. Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* **20**: 8853-8860.

Clarke MC, Tanskanen A, Huttunen M, Whittaker JC, Cannon M. 2009. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. *Am J Psychiatry* **166**: 1025-1030.

Collingridge GL and Bliss TV. 1995. Memories of NMDA receptors and LTP. *Trends Neurosci* **18**: 54-56.

Collingridge GL, Isaac JT, Wang YT. 2004. Receptor trafficking and synaptic plasticity. *Nat Rev Neurosci* **5**: 952-962.

Collingridge GL, Peineau S, Howland JG, Wang YT. 2010. Long-term depression in the CNS. *Nat Rev Neurosci* **11**: 459-473.

Corkin S, Milner B, Rasmussen T. 1964. Effects of different cortical excisions on sensory thresholds in man. *Trans Am Neurol Assoc* **89**: 112-116.

Cousens G and Otto TA. 1998. Induction and transient suppression of long-term potentiation in the peri- and postrhinal cortices following theta-related stimulation of hippocampal field CA1. *Brain Res* **780**: 95-101.

Coyle P, Tran N, Fung JN, Summers BL, Rofe AM. 2009. Maternal dietary zinc supplementation prevents aberrant behaviour in an object recognition task in mice offspring exposed to LPS in early pregnancy. *Behav Brain Res* **197**: 210-218.

Cressant A, Besson M, Suarez S, Cormier A, Granon S. 2007. Spatial learning in Long-Evans Hooded rats and C57BL/6J mice: different strategies for different performance. *Behav Brain Res* **177**: 22-29.

Dalton GL, Wang YT, Floresco SB, Phillips AG. 2008. Disruption of AMPA receptor endocytosis impairs the extinction, but not acquisition of learned fear. *Neuropsychopharmacology* **33**: 2416-2426.

Davis S, Butcher SP, Morris RG. 1992. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J Neurosci* **12**: 21-34.

De GM and Postma A. 2008. Gender differences in memory for objects and their locations: a study on automatic versus controlled encoding and retrieval contexts. *Brain Cogn* **66**: 232-242.

Deacon TW, Eichenbaum H, Rosenberg P, Eckmann KW. 1983. Afferent connections of the perirhinal cortex in the rat. *J Comp Neurol* **220**: 168-190.

Dere E, Huston JP, de Souza Silva MA. 2007. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev* **31**: 673-704.

Devall AJ, Liu ZW, Lovick TA. 2009. Hyperalgesia in the setting of anxiety: sex differences and effects of the oestrous cycle in Wistar rats. *Psychoneuroendocrinology* **34**: 587-596.

Dickerson DD, Wolff AR, Bilkey DK. 2010. Abnormal long-range neural synchrony in a maternal immune activation animal model of schizophrenia. *J Neurosci* **30**: 12424-12431.

Dix SL and Aggleton JP. 1999. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res* **99**: 191-200.

Driscoll I, Hamilton DA, Yeo RA, Brooks WM, Sutherland RJ. 2005. Virtual navigation in humans: the impact of age, sex, and hormones on place learning. *Horm Behav* **47**: 326-335.

Eacott MJ and Gaffan EA. 2005. The roles of perirhinal cortex, postrhinal cortex, and the fornix in memory for objects, contexts, and events in the rat. *Q J Exp Psychol B* **58**: 202-217.

Eacott MJ and Norman G. 2004. Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *J Neurosci* **24**: 1948-1953.

Eichenbaum H. 1996. Learning from LTP: a comment on recent attempts to identify cellular and molecular mechanisms of memory. *Learn Mem* **3**: 61-73.

Eichenbaum H. 1999. The hippocampus and mechanisms of declarative memory. *Behav Brain Res* **103**: 123-133.

Eichenbaum H, Yonelinas AP, Ranganath C. 2007. The medial temporal lobe and recognition memory. *Annu Rev Neurosci* **30**: 123-152.

Eldridge LL, Knowlton BJ, Furmanski CS, Bookheimer SY, Engel SA. 2000. Remembering episodes: a selective role for the hippocampus during retrieval. *Nat Neurosci* **3**: 1149-1152.

Ellman LM, Yolken RH, Buka SL, Torrey EF, Cannon TD. 2009. Cognitive functioning prior to the onset of psychosis: the role of fetal exposure to serologically determined influenza infection. *Biol Psychiatry* **65**: 1040-1047.

Elvevag B and Goldberg TE. 2000. Cognitive impairment in schizophrenia is the core of the disorder. *Crit Rev Neurobiol* **14**: 1-21.

Ennaceur A. 2010. One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* **215**: 244-254.

Ennaceur A and Delacour J. 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* **31**: 47-59.

Ennaceur A, Neave N, Aggleton JP. 1996. Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behav Brain Res* **80**: 9-25.

Escobar M, Crouzin N, Cavalier M, Quentin J, Roussel J, Lante F, Batista-Novais AR, Cohen-Solal C, De Jesus Ferreira MC, Guiramand J, Barbanel G, Vignes M. 2011. Early, Time-Dependent Disturbances of Hippocampal Synaptic Transmission and Plasticity After In Utero Immune Challenge. *Biol Psychiatry*

Fahy FL, Riches IP, Brown MW. 1993. Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Exp Brain Res* **96**: 457-472.

Faraji J, Metz GA, Sutherland RJ. 2010. Characterization of spatial performance in male and female Long-Evans rats by means of the Morris water task and the ziggurat task. *Brain Res Bull* **81**: 164-172.

Faulkner B and Brown TH. 1999. Morphology and physiology of neurons in the rat perirhinal-lateral amygdala area. *J Comp Neurol* **411**: 613-642.

Feldman DE. 2009. Synaptic mechanisms for plasticity in neocortex. *Annu Rev Neurosci* **32**: 33-55.

Ferraguti F and Shigemoto R. 2006. Metabotropic glutamate receptors. *Cell Tissue Res* **326**: 483-504.

Floresco SB, Geyer MA, Gold LH, Grace AA. 2005. Developing predictive animal models and establishing a preclinical trials network for assessing treatment effects on cognition in schizophrenia. *Schizophr Bull* **31**: 888-894.

Forwood SE, Winters BD, Bussey TJ. 2005. Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. *Hippocampus* **15**: 347-355.

Fox CJ, Russell K, Titterness AK, Wang YT, Christie BR. 2007. Tyrosine phosphorylation of the GluR2 subunit is required for long-term depression of synaptic efficacy in young animals in vivo. *Hippocampus* **17**: 600-605.

Frye CA and Sturgis JD. 1995. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem* **64**: 83-96.

Furtak SC, Wei SM, Agster KL, Burwell RD. 2007. Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus* **17**: 709-722.

Gaffan D and Harrison S. 1988. Inferotemporal-frontal disconnection and fornix transection in visuomotor conditional learning by monkeys. *Behav Brain Res* **31**: 149-163.

Goff DC and Coyle JT. 2001. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry* **158**: 1367-1377.

Goldman JM, Murr AS, Cooper RL. 2007. The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res B Dev Reprod Toxicol* **80**: 84-97.

Goldman MB and Mitchell CP. 2004. What is the functional significance of hippocampal pathology in schizophrenia? *Schizophr Bull* **30**: 367-392.

Goldstein JM, Seidman LJ, Goodman JM, Koren D, Lee H, Weintraub S, Tsuang MT. 1998. Are there sex differences in neuropsychological functions among patients with schizophrenia? *Am J Psychiatry* **155**: 1358-1364.

Goto Y, Yang CR, Otani S. 2010. Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. *Biol Psychiatry* **67**: 199-207.

Griffiths S, Scott H, Glover C, Bienemann A, Ghorbel MT, Uney J, Brown MW, Warburton EC, Bashir ZI. 2008. Expression of long-term depression underlies visual recognition memory. *Neuron* **58**: 186-194.

Gruber AJ, Calhoon GG, Shusterman I, Schoenbaum G, Roesch MR, O'Donnell P. 2010. More is less: a disinhibited prefrontal cortex impairs cognitive flexibility. *J Neurosci* **30**: 17102-17110.

Gruppuso V, Lindsay DS, Masson ME. 2007. I'd know that face anywhere! *Psychon Bull Rev* **14**: 1085-1089.

Hammond RS, Tull LE, Stackman RW. 2004. On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiol Learn Mem* **82**: 26-34.

Hanley JR. 1984. Dual processes in recognition and in recognition failure. *Mem Cognit* **12**: 575-580.

Hannesson DK, Howland JG, Phillips AG. 2004. Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *J Neurosci* **24**: 4596-4604.

Hao LY, Hao XQ, Li SH, Li XH. 2010. Prenatal exposure to lipopolysaccharide results in cognitive deficits in age-increasing offspring rats. *Neuroscience* **166**: 763-770.

Hassan B and Rahman Q. 2007. Selective sexual orientation-related differences in object location memory. *Behav Neurosci* **121**: 625-633.

Hayashi T and Huganir RL. 2004. Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. *J Neurosci* **24**: 6152-6160.

Henson RN, Rugg MD, Shallice T, Josephs O, Dolan RJ. 1999. Recollection and familiarity in recognition memory: an event-related functional magnetic resonance imaging study. *J Neurosci* **19**: 3962-3972.

Hernandez A, Burgos H, Mondaca M, Barra R, Nunez H, Perez H, Soto-Moyano R, Sierralta W, Fernandez V, Olivares R, Valladares L. 2008. Effect of prenatal protein malnutrition on long-term potentiation and BDNF protein expression in the rat entorhinal cortex after neocortical and hippocampal tetanization. *Neural Plast* **2008**: 646919-

Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hubener M. 2009. Experience leaves a lasting structural trace in cortical circuits. *Nature* **457**: 313-317.

Holdstock JS, Mayes AR, Roberts N, Cezayirli E, Isaac CL, O'Reilly RC, Norman KA. 2002. Under what conditions is recognition spared relative to recall after selective hippocampal damage in humans? *Hippocampus* **12**: 341-351.

Honack D and Loscher W. 1993. Sex differences in NMDA receptor mediated responses in rats. *Brain Res* **620**: 167-170.

Hoover WB and Vertes RP. 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct Funct* **212**: 149-179.

Howland JG and Cazakoff BN. 2010. Effects of acute stress and GluN2B-containing NMDA receptor antagonism on object and object-place recognition memory. *Neurobiol Learn Mem* **93**: 261-267.

Hubener M and Bonhoeffer T. 2010. Searching for engrams. *Neuron* **67**: 363-371.

Ibi D, Nagai T, Kitahara Y, Mizoguchi H, Koike H, Shiraki A, Takuma K, Kamei H, Noda Y, Nitta A, Nabeshima T, Yoneda Y, Yamada K. 2009. Neonatal polyI:C treatment in mice results in schizophrenia-like behavioral and neurochemical abnormalities in adulthood. *Neurosci Res* **64**: 297-305.

Ito HT, Smith SE, Hsiao E, Patterson PH. 2010. Maternal immune activation alters nonspatial information processing in the hippocampus of the adult offspring. *Brain Behav Immun* **24**: 930-941.

Ito M. 1983. Evidence for synaptic plasticity in the cerebellar cortex. *Acta Morphol Hung* **31**: 213-218.

Ito M and Kano M. 1982. Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. *Neurosci Lett* **33**: 253-258.

Ito M, Sakurai M, Tongroach P. 1982. Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *J Physiol* **324**: 113-134.

Ivanco TL and Racine RJ. 2000. Long-term potentiation in the reciprocal corticohippocampal and corticocortical pathways in the chronically implanted, freely moving rat. *Hippocampus* **10**: 143-152.

Jackson-Smith P, Kesner RP, Chiba AA. 1993. Continuous recognition of spatial and nonspatial stimuli in hippocampal-lesioned rats. *Behav Neural Biol* **59**: 107-119.

Javitt DC. 2007. Glutamate and schizophrenia: phencyclidine, N-methyl-D-aspartate receptors, and dopamine-glutamate interactions. *Int Rev Neurobiol* **78**: 69-108.

Javitt DC. 2010. Glutamatergic theories of schizophrenia. *Isr J Psychiatry Relat Sci* **47**: 4-16.

Kameyama K, Lee HK, Bear MF, Huganir RL. 1998. Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. *Neuron* **21**: 1163-1175.

Kandel ER. 2009. The biology of memory: a forty-year perspective. *J Neurosci* **29**: 12748-12756.

Kauer JA and Malenka RC. 2007. Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**: 844-858.

Kauer JA, Malenka RC, Perkel DJ, Nicoll RA. 1990. Postsynaptic mechanisms involved in long-term potentiation. *Adv Exp Med Biol* **268**: 291-299.

Kealy J and Commins S. 2009. Antagonism of glutamate receptors in the CA1 to perirhinal cortex projection prevents long-term potentiation and attenuates levels of brain-derived neurotrophic factor. *Brain Res* **1265**: 53-64.

Kealy J and Commins S. 2010. Frequency-dependent changes in synaptic plasticity and brain-derived neurotrophic factor (BDNF) expression in the CA1 to perirhinal cortex projection. *Brain Res* **1326**: 51-61.

Kealy J and Commins S. 2011. The rat perirhinal cortex: A review of anatomy, physiology, plasticity, and function. *Prog Neurobiol* **93**: 522-548.

Keefe RS and Fenton WS. 2007. How should DSM-V criteria for schizophrenia include cognitive impairment? *Schizophr Bull* **33**: 912-920.

Kelly A, Laroche S, Davis S. 2003. Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. *J Neurosci* **23**: 5354-5360.

Kemp N and Bashir ZI. 2001. Long-term depression: a cascade of induction and expression mechanisms. *Prog Neurobiol* **65**: 339-365.

Kenney JW and Gould TJ. 2008. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol Neurobiol* **38**: 101-121.

Keri S. 2008. Interactive memory systems and category learning in schizophrenia. *Neurosci Biobehav Rev* **32**: 206-218.

Kesner RP, Bolland BL, Dakis M. 1993. Memory for spatial locations, motor responses, and objects: triple dissociation among the hippocampus, caudate nucleus, and extrastriate visual cortex. *Exp Brain Res* **93**: 462-470.

Lante F, Meunier J, Guiramand J, De Jesus Ferreira MC, Cambonie G, Aimar R, Cohen-Solal C, Maurice T, Vignes M, Barbanel G. 2008. Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. *Hippocampus* **18**: 602-609.

Lecardeur L, Mendrek A, Stip E. 2010. Sexual dimorphism of spatial working memory profiles in schizophrenia. *Cogn Neuropsychiatry* **15**: 397-405.

Lee SH, Liu L, Wang YT, Sheng M. 2002. Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. *Neuron* **36**: 661-674.

Leiderman EA and Strejilevich SA. 2004. Visuospatial deficits in schizophrenia: central executive and memory subsystems impairments. *Schizophr Res* **68**: 217-223.

Lejbak L, Crossley M, Vrbancic M. 2011. A male advantage for spatial and object but not verbal working memory using the n-back task. *Brain Cogn* **76**: 191-196.

Lejbak L, Vrbancic M, Crossley M. 2009. The female advantage in object location memory is robust to verbalizability and mode of presentation of test stimuli. *Brain Cogn* **69**: 148-153.

Lewis DA and Gonzalez-Burgos G. 2006. Pathophysiologically based treatment interventions in schizophrenia. *Nat Med* **12**: 1016-1022.

Lewis DA and Gonzalez-Burgos G. 2008. Neuroplasticity of neocortical circuits in schizophrenia. *Neuropsychopharmacology* **33**: 141-165.

Liu P and Bilkey DK. 1996a. Direct connection between perirhinal cortex and hippocampus is a major constituent of the lateral perforant path. *Hippocampus* **6**: 125-135.

Liu P and Bilkey DK. 1996b. Long-term potentiation in the perirhinal-hippocampal pathway is NMDA dependent. *Neuroreport* **7**: 1241-1244.

Liu P and Bilkey DK. 1998. Is there a direct projection from perirhinal cortex to the hippocampus? *Hippocampus* **8**: 424-425.

Lowe GC, Luheshi GN, Williams S. 2008. Maternal infection and fever during late gestation are associated with altered synaptic transmission in the hippocampus of juvenile offspring rats. *Am J Physiol Regul Integr Comp Physiol* **295**: R1563-R1571.

Luscher C, Xia H, Beattie EC, Carroll RC, von ZM, Malenka RC, Nicoll RA. 1999. Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* **24**: 649-658.

Malenka RC. 2003. Synaptic plasticity and AMPA receptor trafficking. *Ann N Y Acad Sci* **1003**: 1-11.

Malenka RC and Bear MF. 2004. LTP and LTD: an embarrassment of riches. *Neuron* **44**: 5-21.

Mandler G. 1981. The recognition of previous encounters. *Am Sci* **69**: 211-218.

Marcondes FK, Bianchi FJ, Tanno AP. 2002. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz J Biol* **62**: 609-614.

Martin SJ, Grimwood PD, Morris RG. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* **23**: 649-711.

Massey PV and Bashir ZI. 2007. Long-term depression: multiple forms and implications for brain function. *Trends Neurosci* **30**: 176-184.

Massey PV, Bhabra G, Cho K, Brown MW, Bashir ZI. 2001. Activation of muscarinic receptors induces protein synthesis-dependent long-lasting depression in the perirhinal cortex. *Eur J Neurosci* **14**: 145-152.

Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI. 2004. Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J Neurosci* **24**: 7821-7828.

Massey PV, Phythian D, Narduzzo K, Warburton EC, Brown MW, Bashir ZI. 2008. Learning-specific changes in long-term depression in adult perirhinal cortex. *J Neurosci* **28**: 7548-7554.

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. 2004. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**: 761-766.

McCarthy MM, Davis AM, Mong JA. 1997. Excitatory neurotransmission and sexual differentiation of the brain. *Brain Res Bull* **44**: 487-495.

McDonald C and Murray RM. 2000. Early and late environmental risk factors for schizophrenia. *Brain Res Brain Res Rev* **31**: 130-137.

McGann JP, Moyer JR, Jr., Brown TH. 2001. Predominance of late-spiking neurons in layer VI of rat perirhinal cortex. *J Neurosci* **21**: 4969-4976.

McKay BE, Placzek AN, Dani JA. 2007. Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochem Pharmacol* **74**: 1120-1133.

Mereu G, Fa M, Ferraro L, Cagiano R, Antonelli T, Tattoli M, Ghiglieri V, Tanganelli S, Gessa GL, Cuomo V. 2003. Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release. *Proc Natl Acad Sci U S A* **100**: 4915-4920.

Meunier M, Bachevalier J, Mishkin M, Murray EA. 1993. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J Neurosci* **13**: 5418-5432.

Meyer U and Feldon J. 2011. To poly(I:C) or not to poly(I:C): Advancing preclinical schizophrenia research through the use of prenatal immune activation models. *Neuropharmacology.* Epub ahead of print. **PMID**: 21238465

Meyer U and Feldon J. 2010. Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Prog. Neurobiol.* **90:** 285-326.

Meyer U, Feldon J, Fatemi SH. 2009a. In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neurosci Biobehav Rev* **33**: 1061-1079.

Meyer U, Feldon J, Schedlowski M, Yee BK. 2005. Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci Biobehav Rev* **29**: 913-947.

Meyer U, Feldon J, Yee BK. 2009b. A review of the fetal brain cytokine imbalance hypothesis of schizophrenia. *Schizophr Bull* **35**: 959-972.

Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. 2008. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. *Brain Behav Immun* **22**: 469-486.

Meyer U, Yee BK, Feldon J. 2007. The neurodevelopmental impact of prenatal infections at different times of pregnancy: the earlier the worse? *Neuroscientist* **13**: 241-256.

Meyer-Lindenberg AS, Olsen RK, Kohn PD, Brown T, Egan MF, Weinberger DR, Berman KF. 2005. Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Arch Gen Psychiatry* **62**: 379-386.

Milner B. 1965. Visually guided maze learning in man: effects of bilateral hippocampal, bilateral frontal, and unilateral cerebral lesions. *Neuropsychologia* **3**: 317-338.

Milner B. 1972. Disorders of learning and memory after temporal lobe lesions in man. *Clin Neurosurg* **19**: 421-446.

Milner B, Corkin S, Teuber H. 1968. Further analysis of the hippocampal amnesic syndrome. *Neuropsychologia* **6**: 215-234.

Milner B and Taylor L. 1972. Right-hemisphere superiority in tactile pattern-recognition after cerebral commissurotomy: evidence for nonverbal memory. *Neuropsychologia* **10**: 1-15.

Moghaddam B. 2003. Bringing order to the glutamate chaos in schizophrenia. *Neuron* **40**: 881-884.

Moghaddam B and Jackson ME. 2003. Glutamatergic animal models of schizophrenia. *Ann N Y Acad Sci* **1003**: 131-137.

Morris RG, Anderson E, Lynch GS, Baudry M. 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* **319**: 774-776.

Moyer JR, Jr., McNay EC, Brown TH. 2002. Three classes of pyramidal neurons in layer V of rat perirhinal cortex. *Hippocampus* **12**: 218-234.

Mumby DG. 2001. Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav Brain Res* **127**: 159-181.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. 2002. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem* **9**: 49-57.

Mumby DG and Pinel JP. 1994. Rhinal cortex lesions and object recognition in rats. *Behav Neurosci* **108**: 11-18.

Mumby DG, Piterkin P, Lecluse V, Lehmann H. 2007. Perirhinal cortex damage and anterograde object-recognition in rats after long retention intervals. *Behav Brain Res* **185**: 82-87.

Mumby DG, Tremblay A, Lecluse V, Lehmann H. 2005. Hippocampal damage and anterograde object-recognition in rats after long retention intervals. *Hippocampus* **15**: 1050-1056.

Naber PA, Witter MP, Lopez da Silva FH. 1999. Perirhinal cortex input to the hippocampus in the rat: evidence for parallel pathways, both direct and indirect. A combined physiological and anatomical study. *Eur J Neurosci* **11**: 4119-4133.

Nader K and Einarsson EO. 2010. Memory reconsolidation: an update. *Ann N Y Acad Sci* **1191**: 27-41.

Nader K, Schafe GE, LeDoux JE. 2000. The labile nature of consolidation theory. *Nat Rev Neurosci* **1**: 216-219.

Nemanic S, Alvarado MC, Bachevalier J. 2004. The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. *J Neurosci* **24**: 2013-2026.

Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP. 2011. Metabotropic glutamate receptors: From the workbench to the bedside. *Neuropharmacology* **60**: 1017-1041.

Nicoll RA and Malenka RC. 1995. Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* **377**: 115-118.

Nicoll RA and Schmitz D. 2005. Synaptic plasticity at hippocampal mossy fibre synapses. *Nat Rev Neurosci* **6**: 863-876.

Noorlander CW, Visser GH, Ramakers GM, Nikkels PG, de Graan PN. 2008. Prenatal corticosteroid exposure affects hippocampal plasticity and reduces lifespan. *Dev Neurobiol* **68**: 237-246.

Norman KA. 2010. How hippocampus and cortex contribute to recognition memory: revisiting the complementary learning systems model. *Hippocampus* **20**: 1217-1227.

Oh-Nishi A, Obayashi S, Sugihara I, Minamimoto T, Suhara T. 2010. Maternal immune activation by polyriboinosinic-polyribocytidilic acid injection produces synaptic dysfunction but not neuronal loss in the hippocampus of juvenile rat offspring. *Brain Res* **1363**: 170-179.

Oliva A and Torralba A. 2007. The role of context in object recognition. *Trends Cogn Sci* **11**: 520-527.

Opler MG and Susser ES. 2005. Fetal environment and schizophrenia. *Environ Health Perspect* **113**: 1239-1242.

Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M. 2006. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol Psychiatry* **59**: 546-554.

Ozawa T, Yamada K, Ichitani Y. 2011b. Long-term object location memory in rats: Effects of sample phase and delay length in spontaneous place recognition test. *Neurosci Lett* **497**: 37-41.

Ozawa T, Yamada K, Ichitani Y. 2011a. Long-term object location memory in rats: Effects of sample phase and delay length in spontaneous place recognition test. *Neurosci Lett* **497**: 37-41.

Park Y, Jo J, Isaac JT, Cho K. 2006. Long-term depression of kainate receptor-mediated synaptic transmission. *Neuron* **49**: 95-106.

Pascalis O, Hunkin NM, Holdstock JS, Isaac CL, Mayes AR. 2004. Visual paired comparison performance is impaired in a patient with selective hippocampal lesions and relatively intact item recognition. *Neuropsychologia* **42**: 1293-1300.

Paul BM, Elvevag B, Bokat CE, Weinberger DR, Goldberg TE. 2005. Levels of processing effects on recognition memory in patients with schizophrenia. *Schizophr Res* **74**: 101-110.

Paxinos G,Watson C. 1997. The rat brain in stereotaxic coordinates. Elsevier Academic Press, Amsterdam.

Pihlajamaki M, Tanila H, Kononen M, Hanninen T, Hamalainen A, Soininen H, Aronen HJ. 2004. Visual presentation of novel objects and new spatial arrangements of objects differentially activates the medial temporal lobe subareas in humans. *Eur J Neurosci* **19**: 1939-1949.

Piontkewitz Y, Arad M, Weiner I. 2011. Tracing the development of psychosis and its prevention: What can be learned from animal models. *Neuropharmacology.* Epud ahead of print. **PMID**: 21703648

Piper BJ, Acevedo SF, Edwards KR, Curtiss AB, McGinnis GJ, Raber J. 2011. Age, sex, and handedness differentially contribute to neurospatial function on the Memory Island and Novel-Image Novel-Location tests. *Physiol Behav* **103**: 513-522.

Postma A, Kessels RP, van AM. 2008. How the brain remembers and forgets where things are: the neurocognition of object-location memory. *Neurosci Biobehav Rev* **32**: 1339-1345.

Rawlins JN, Lyford GL, Seferiades A, Deacon RM, Cassaday HJ. 1993. Critical determinants of nonspatial working memory deficits in rats with conventional lesions of the hippocampus or fornix. *Behav Neurosci* **107**: 420-433.

Rebola N, Srikumar BN, Mulle C. 2010. Activity-dependent synaptic plasticity of NMDA receptors. *J Physiol* **588**: 93-99.

Roman F, Staubli U, Lynch G. 1987. Evidence for synaptic potentiation in a cortical network during learning. *Brain Res* **418**: 221-226.

Rugg MD, Mark RE, Walla P, Schloerscheidt AM, Birch CS, Allan K. 1998. Dissociation of the neural correlates of implicit and explicit memory. *Nature* **392**: 595-598.

Rugg MD and Yonelinas AP. 2003. Human recognition memory: a cognitive neuroscience perspective. *Trends Cogn Sci* **7**: 313-319.

Salame P, Burglen F, Danion JM. 2006. Differential disruptions of working memory components in schizophrenia in an object-location binding task using the suppression paradigm. *J Int Neuropsychol Soc* **12**: 510-518.

Samuelsson AM, Jennische E, Hansson HA, Holmang A. 2006. Prenatal exposure to interleukin-6 results in inflammatory neurodegeneration in hippocampus with NMDA/GABA(A) dysregulation and impaired spatial learning. *Am J Physiol Regul Integr Comp Physiol* **290**: R1345-R1356.

Sandstrom NJ, Kaufman J, Huettel SA. 1998. Males and females use different distal cues in a virtual environment navigation task. *Brain Res Cogn Brain Res* **6**: 351-360.

Saucier D, Lisoway A, Green S, Elias L. 2007. Female advantage for object location memory in peripersonal but not extrapersonal space. *J Int Neuropsychol Soc* **13**: 683-686.

Saunders RC, Kolachana BS, Bachevalier J, Weinberger DR. 1998. Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. *Nature* **393**: 169-171.

Schacter DL and Wagner AD. 1999. Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus* **9**: 7-24.

Schulz KM, Pearson JN, Neeley EW, Berger R, Leonard S, Adams CE, Stevens KE. 2011. Maternal stress during pregnancy causes sex-specific alterations in offspring memory performance, social interactions, indices of anxiety, and body mass. *Physiol Behav*

Schwendener S, Meyer U, Feldon J. 2009. Deficient maternal care resulting from immunological stress during pregnancy is associated with a sex-dependent enhancement of conditioned fear in the offspring. *J Neurodev Disord* **1**: 15-32.

Scoville WB. 1954. The limbic lobe in man. *J Neurosurg* **11**: 64-66.

Scoville WB and Milner B. 2000. Loss of recent memory after bilateral hippocampal lesions. 1957. *J Neuropsychiatry Clin Neurosci* **12**: 103-113.

Segal M and Landis S. 1974. Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res* **78**: 1-15.

Seoane A, Massey PV, Keen H, Bashir ZI, Brown MW. 2009. L-type voltage-dependent calcium channel antagonists impair perirhinal long-term recognition memory and plasticity processes. *J Neurosci* **29**: 9534-9544.

Sesack SR, Deutch AY, Roth RH, Bunney BS. 1989. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol* **290**: 213-242.

Shi L, Fatemi SH, Sidwell RW, Patterson PH. 2003. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J Neurosci* **23**: 297-302.

Sidman M, Stoddard LT, Mohr JP. 1968. Some additional quanititative observations of immediate memory in a patient with bilateral hippocampal lesions. *Neuropsychologia* **6**: 254-

Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA. 2010. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. *Nature* **464**: 763-767.

Silverman I, Choi J, Peters M. 2007. The hunter-gatherer theory of sex differences in spatial abilities: data from 40 countries. *Arch Sex Behav* **36**: 261-268.

Squire LR,Kandel ER. 2009. Memory: From Mind to Molecules. Roberts and Company Publishers, Greenwood Village, CO.

Squire LR, Knowlton B, Musen G. 1993. The structure and organization of memory. *Annu Rev Psychol* **44**: 453-495.

Squire LR, Stark CE, Clark RE. 2004. The medial temporal lobe. *Annu Rev Neurosci* **27**: 279-306.

Staiti AM, Morgane PJ, Galler JR, Grivetti JY, Bass DC, Mokler DJ. 2011. A microdialysis study of the medial prefrontal cortex of adolescent and adult rats. *Neuropharmacology*

Stefanacci L, Suzuki WA, Amaral DG. 1996. Organization of connections between the amygdaloid complex and the perirhinal and parahippocampal cortices in macaque monkeys. *J Comp Neurol* **375**: 552-582.

Stone JM. 2009. Imaging the glutamate system in humans: relevance to drug discovery for schizophrenia. *Curr Pharm Des* **15**: 2594-2602.

Stone JM, Morrison PD, Pilowsky LS. 2007. Glutamate and dopamine dysregulation in schizophrenia--a synthesis and selective review. *J Psychopharmacol* **21**: 440-452.

Stone JM and Pilowsky LS. 2007. Novel targets for drugs in schizophrenia. *CNS Neurol Disord Drug Targets* **6**: 265-272.

Sutcliffe JS, Marshall KM, Neill JC. 2007. Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behav Brain Res* **177**: 117-125.

Suzuki WA. 1996. The anatomy, physiology and functions of the perirhinal cortex. *Curr Opin Neurobiol* **6**: 179-186.

Suzuki WA and Amaral DG. 1990. Cortical inputs to the CA1 field of the monkey hippocampus originate from the perirhinal and parahippocampal cortex but not from area TE. *Neurosci Lett* **115**: 43-48.

Suzuki WA and Amaral DG. 1994a. Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. *J Comp Neurol* **350**: 497-533.

Suzuki WA and Amaral DG. 1994b. Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *J Neurosci* **14**: 1856-1877.

Suzuki WA and Amaral DG. 2003. Perirhinal and parahippocampal cortices of the macaque monkey: cytoarchitectonic and chemoarchitectonic organization. *J Comp Neurol* **463**: 67-91.

Swanson LW and Cowan WM. 1977. An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J Comp Neurol* **172**: 49-84.

Swerdlow NR. 2010. Integrative circuit models and their implications for the pathophysiologies and treatments of the schizophrenias. *Curr Top Behav Neurosci* **4**: 555-583.

Takeuchi O and Akira S. 2007. Recognition of viruses by innate immunity. *Immunol Rev* **220**: 214-224.

Tamminga CA, Stan AD, Wagner AD. 2010. The hippocampal formation in schizophrenia. *Am J Psychiatry* **167**: 1178-1193.

Tropea D, Van WA, Sur M. 2009. Molecular mechanisms of experience-dependent plasticity in visual cortex. *Philos Trans R Soc Lond B Biol Sci* **364**: 341-355.

Tulving E and Schacter DL. 1990. Priming and human memory systems. *Science* **247**: 301-306.

Van den Oever MC, Goriounova NA, Li KW, Van der Schors RC, Binnekade R, Schoffelmeer AN, Mansvelder HD, Smit AB, Spijker S, De Vries TJ. 2008. Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. *Nat Neurosci* **11**: 1053-1058.

van GT and Wyss JM. 1990. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J Comp Neurol* **302**: 515-528.

Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van PW, Mishkin M. 1997. Differential effects of early hippocampal pathology on episodic and semantic memory. *Science* **277**: 376-380.

Volk DW and Lewis DA. 2010. Prefrontal cortical circuits in schizophrenia. *Curr Top Behav Neurosci* **4**: 485-508.

Wan H, Warburton EC, Zhu XO, Koder TJ, Park Y, Aggleton JP, Cho K, Bashir ZI, Brown MW. 2004. Benzodiazepine impairment of perirhinal cortical plasticity and recognition memory. *Eur J Neurosci* **20**: 2214-2224.

Warburton EC and Brown MW. 2010. Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia* **48**: 2262-2272.

Warburton EC, Koder T, Cho K, Massey PV, Duguid G, Barker GR, Aggleton JP, Bashir ZI, Brown MW. 2003. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron* **38**: 987-996.

Whishaw IQ and Tomie J. 1996. Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiol Behav* **60**: 1191-1197.

Whitlock JR, Heynen AJ, Shuler MG, Bear MF. 2006. Learning induces long-term potentiation in the hippocampus. *Science* **313**: 1093-1097.

Winters BD, Bartko SJ, Saksida LM, Bussey TJ. 2007. Scopolamine infused into perirhinal cortex improves object recognition memory by blocking the acquisition of interfering object information. *Learn Mem* **14**: 590-596.

Winters BD and Bussey TJ. 2005a. Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* **25**: 4243-4251.

Winters BD and Bussey TJ. 2005b. Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *J Neurosci* **25**: 52-61.

Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ. 2004. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci* **24**: 5901-5908.

Winters BD, Saksida LM, Bussey TJ. 2006. Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: implications for cholinergic system function. *J Neurosci* **26**: 9520-9529.

Winters BD, Saksida LM, Bussey TJ. 2008. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci Biobehav Rev* **32**: 1055-1070.

Winters BD, Saksida LM, Bussey TJ. 2010. Implications of animal object memory research for human amnesia. *Neuropsychologia* **48**: 2251-2261.

Witter MP and Groenewegen HJ. 1984. Laminar origin and septotemporal distribution of entorhinal and perirhinal projections to the hippocampus in the cat. *J Comp Neurol* **224**: 371-385.

Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AH. 1989. Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. *Prog Neurobiol* **33**: 161-253.

Wixted JT and Squire LR. 2004. Recall and recognition are equally impaired in patients with selective hippocampal damage. *Cogn Affect Behav Neurosci* **4**: 58-66.

Wolf RC, Vasic N, Sambataro F, Hose A, Frasch K, Schmid M, Walter H. 2009. Temporally anticorrelated brain networks during working memory performance reveal aberrant prefrontal and hippocampal connectivity in patients with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* **33**: 1464-1473.

Wolff AR and Bilkey DK. 2008. Immune activation during mid-gestation disrupts sensorimotor gating in rat offspring. *Behav Brain Res* **190**: 156-159.

Wolff AR and Bilkey DK. 2010. The maternal immune activation (MIA) model of schizophrenia produces pre-pulse inhibition (PPI) deficits in both juvenile and adult rats but these effects are not associated with maternal weight loss. *Behav Brain Res* **213**: 323-327.

Wong AH and Van Tol HH. 2003. Schizophrenia: from phenomenology to neurobiology. *Neurosci Biobehav Rev* **27**: 269-306.

Wong TP, Howland JG, Robillard JM, Ge Y, Yu W, Titterness AK, Brebner K, Liu L, Weinberg J, Christie BR, Phillips AG, Wang YT. 2007. Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. *Proc Natl Acad Sci U S A* **104**: 11471-11476.

Wood ER, Mumby DG, Pinel JP, Phillips AG. 1993. Impaired object recognition memory in rats following ischemia-induced damage to the hippocampus. *Behav Neurosci* **107**: 51-62.

Wood SJ, Proffitt T, Mahony K, Smith DJ, Buchanan JA, Brewer W, Stuart GW, Velakoulis D, McGorry PD, Pantelis C. 2002. Visuospatial memory and learning in first-episode schizophreniform psychosis and established schizophrenia: a functional correlate of hippocampal pathology? *Psychol Med* **32**: 429-438.

Xiang JZ and Brown MW. 1998. Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology* **37**: 657-676.

Yang J, Hou C, Ma N, Liu J, Zhang Y, Zhou J, Xu L, Li L. 2007. Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress. *Neurobiol Learn Mem* **87**: 257-263.

Yonelinas AP. 1997. Recognition memory ROCs for item and associative information: the contribution of recollection and familiarity. *Mem Cognit* **25**: 747-763.

Yonelinas AP. 2001. Components of episodic memory: the contribution of recollection and familiarity. *Philos Trans R Soc Lond B Biol Sci* **356**: 1363-1374.

Yonelinas AP, Aly M, Wang WC, Koen JD. 2010. Recollection and familiarity: examining controversial assumptions and new directions. *Hippocampus* **20**: 1178-1194.

Yonelinas AP, Kroll NE, Quamme JR, Lazzara MM, Sauve MJ, Widaman KF, Knight RT. 2002. Effects of extensive temporal lobe damage or mild hypoxia on recollection and familiarity. *Nat Neurosci* **5**: 1236-1241.

Yuste R and Bonhoeffer T. 2001. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu Rev Neurosci* **24**: 1071-1089.

Zalutsky RA and Nicoll RA. 1990. Comparison of two forms of long-term potentiation in single hippocampus neurons. *Science* **248**: 1619-1624.

Zhang Y, Cazakoff BN, Thai CA, Howland JG. 2011. Prenatal exposure to a viral mimetic alters behavioural flexibility in male, but not female, rats. *Neuropharmacology*. Epub ahead of print. **PMID**: 21376064

Zhu XO and Brown MW. 1995. Changes in neuronal activity related to the repetition and relative familiarity of visual stimuli in rhinal and adjacent cortex of the anaesthetised rat. *Brain Res* **689**: 101-110.

Zhu XO, Brown MW, McCabe BJ, Aggleton JP. 1995. Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. *Neuroscience* **69**: 821-829.

Ziakopoulos Z, Tillett CW, Brown MW, Bashir ZI. 1999. Input-and layer-dependent synaptic plasticity in the rat perirhinal cortex in vitro. *Neuroscience* **92**: 459-472.

Zola SM, Squire LR, Teng E, Stefanacci L, Buffalo EA, Clark RE. 2000. Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J Neurosci* **20**: 451-463.

Zola-Morgan S and Squire LR. 1985. Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav Neurosci* **99**: 22-34.

Zola-Morgan S and Squire LR. 1986. Memory impairment in monkeys following lesions limited to the hippocampus. *Behav Neurosci* **100**: 155-160.

Zuckerman L, Rimmerman N, Weiner I. 2003. Latent inhibition in 35-day-old rats is not an "adult" latent inhibition: implications for neurodevelopmental models of schizophrenia. *Psychopharmacology (Berl)* **169**: 298-307.

Zuckerman L and Weiner I. 2003. Post-pubertal emergence of disrupted latent inhibition following prenatal immune activation. *Psychopharmacology (Berl)* **169**: 308-313.

Zuckerman L and Weiner I. 2005. Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. *J Psychiatr Res* **39**: 311-323.