THE ROLE OF DOPAMINE D2 AND NEUREGULIN-1 RECEPTORS IN SCHIZOPHRENIA RELEVANT PHENOTYPES OF COGNITION, ATTENTION AND MEMORY

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ABSTRACT

Aberrant neurotransmitter function promotes cognitive deficits in schizophrenia. These abnormalities in functioning are seen as disruptions in attentional and information processing, as well as disruptions in the consolidation and retrieval of information. Tasks of attentional salience and memory that are used to model these disruptions include the latent inhibition (LI) task of attentional salience, prepulse inhibition (PPI) task of sensorimotor gating and an Episodic memory (EM) task, which is an index of memory for episodes at a particular point in time. Aberrant functioning of candidate genes that are associated with risk for schizophrenia may be seen as behavioural alterations in these tasks of schizophrenia relevant phenotypes. dopaminergic hyperactivity and hypofunction have been implicated in mediating disruptions on these cognitive tasks. Increased transmission in the dopamine system in the striatal region promotes schizophrenia symptoms, and indirect dopamine (DA) agonist Amphetamine worsens these symptoms in patients, and disrupts schizophrenia relevant behaviours on these cognitive tasks.

We investigated the effects of deletion of two genes relevant to schizophrenia on cognitive tasks known to be disrupted in the disorder. The effect of deletion of the dopamine D2 receptor (D2R) and trans membrane (TM) domain Neuregulin-1 (Nrg-1) receptor were investigated in mediating disruptions in cognitive processes in an animal model of schizophrenia. The role of the D2R in an attentional model of sensorimotor gating was assessed. PPI was attenuated in D2R knock out (KO), in a one day sensorimotor gating task. In a one day PPI test protocol, amphetamine

disruptions on PPI were spared in D2R WT and KO mice. Following on from previous reports of disrupted LI by a single low dose amphetamine injection, separated by 24h interval, we established a single vs. two low dose PPI protocol in order to facilitate a direct comparison of amphetamine induced disruption in LI with PPI. A one injection (prior to test only) vs. two injection (prior to habituation and prior to test) task was established. In the two day protocol, a single low dose of amphetamine disrupted PPI in D2R KO mice and reduced startle reactivity to the 120 dB pulse alone trials. Two low dose injections of amphetamine however, do not disrupt PPI in D2R KO or their WT littermates, and do not mimic low dose amphetamine disruptions in the LI task. These findings demonstrate that prior conclusions about the requirement of the D2R for amphetamine effects in PPI does not generalise to all dose regimens.

Episodic memory was also investigated as a measure of cognitive impairment in schizophrenia. D2R KO mice show sex specific dissociations on an EM task. Male D2R WT and KO animals show equal exploration of old vs. recent objects on the what-when component of the EM task, and female KO animals show enhanced memory for old vs. recent objects. Both D2R WT and KO mice show intact memory for displaced objects.

These deficits were also investigated in the TM domain Nrg-1 model. Nrg-1 has been implicated as a candidate gene for schizophrenia, and behavioural phenotypes assessing its role in cognitive impairment in schizophrenia were established. Intact LI is seen in both Nrg-1 WT and Het animals. Nrg-1 TM domain Het mutants also show deficits on the schizophrenia relevant PPI task. Nrg-1 Het mutants show attenuated % PPI compared to their WT littermates, which reflects interrupted sensorimotor gating in schizophrenia. Lastly, we found some evidence that reduced

function of TM-domain of the Nrg-1 gene disrupted episodic-like memory (what-where-when recognition) in males and improved it in females.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF ABBREVIATIONS

PANSS   Positive and negative Syndrome Scale

WCST   Wisconsin card sorting test

PFC   Prefrontal cortex

D1R   Dopamine 1 receptor

D2R   Dopamine 2 receptor

NMDA   N-Methyl-D-Aspartic

AMPA   α-amino-3-hydroxy-5-methyl-4-
                                            isoxazolepropionic acid

                                            receptor

mGLUR   Metabotropic glutamate receptor

PCP   Phencyclidine

PPI   Prepulse inhibition

KO   Knock out

GABA   Gamma -amino butyric acid

DAT   Dopamine transporter

mRNA	Micro ribonucleic acid
DISC -1	Disrupted in schizophrenia 1
LI	Latent inhibition
UCS	Unconditioned stimulus
CS	Conditioned stimulus
AMPH	D-Amphetamine
PE	Preexposure
APD	Antipsychotic drugs
NAC	Nucleus accumbens
ASR	Acoustic startle reflex
DLPFC	Dorsolateral prefrontal cortex
OBFC	Orbito-frontal cortex
Nrg-1	Neuregulin-1
COMT	Catechol-O-methyl transferase

DISC-1	Disrupted in schizophrenia 1
EGF	Epidermal growth factor like
Ig	Immunoglobulin like
TM	Trans membrane domain like
MK-801	Dizocilpine

Chapter 1: GENERAL INTRODUCTION

1.1 WHAT IS SCHIZOPHRENIA?

1.1.1 Definition

Schizophrenia is a mental illness that is best defined as a psychiatric disorder of perceptual, social and motivational deficits, as well as thinking and cognition. It was originally known as 'dementia praecox' and defined as a progressive syndrome (Kraeplin,1919), comprising a group of symptoms with unknown specific pathophyisiology, anatomical or molecular basis, that resulted in disease only when specific entities contributing to the disease could be identified (Kandel,1991). This idea further was refined by (Bleuler,1911) who proposed that schizophrenia entailed a splitting of the cognitive aspect of personality both from the emotional and affective states. However, signs and symptoms of illness are diverse and can be broken down in terms of: symptoms that precede a psychotic fit i.e. prodromal signs, symptoms that persist in the psychotic state and lastly, symptoms that occur in the non psychotic period (Kandel,1991). These symptoms have now been clearly assigned into three categories: positive symptoms (psychotic phase), negative symptoms (prodromal/non psychotic phase) and cognitive deficits, all of which are abnormalities that are characteristic of the schizophrenic mind.

1.1.2 Symptomatology underlying schizophrenia

The concordance rates for schizophrenia are about 41-65% in monozygotic twins on average and 0-28% in dizygotes (Cardno and Gottesman,2000), and monozygotic

twins have a higher genetic predisposition for schizophrenia than dizygotes (Kallmann, 1946). Moreover, genetic predisposition for developing schizophrenia is more prevalent in families with a psychotic member (see Fig.1a) where even prodromal symptoms have been observed in unaffected relatives of schizophrenic patients (Gottesman and Erlenmeyer-Kimling,2001). The positive symptoms of schizophrenia consist of hallucinations, delusional thoughts, feelings and actions, as well as disordered thoughts, incoherence and disorganized speech. The negative symptoms consist of social withdrawal and isolation, flattened affect, alogia, a lack of motivation, affective blunting of emotional expression as well as anhedonia in experiencing pleasure (Kandel,1991; Andreasen,1995). These symptoms are also accompanied by cognitive deficits in working memory, attention, semantic and episodic memory (Elvevag and Goldberg,2000).Evidence for cognitive impairment such as deficits in executive functioning, cued recall and recognition (Sullivan,1994; Joyce, Collinson et al.,1996; Hutton, Puri et al..,1998), may arise from dysfunction in specific anatomical areas or pathology (Lennox, Park et al.,2000; Perlstein, Carter et al., 2001). This will be discussed later.

1.1.3 Structural and functional changes governing schizophrenic symptoms

The aetiology of schizophrenia consists of dysfunction in structural brain regions and functional changes in the brain. Studies mapping neuroanatomical substrates in schizophrenia have shown reductions in temporal grey matter particularly in the temporal gyrus and cerebellum, in patients who experienced hallucinations (Neckelmann,2006). Furthermore reductions in grey matter have also been reported in the insula, medial prefrontal, medial temporal and striatal regions, as well as the dorso-medial frontal cortex, and lateral and orbital frontal areas (Fornito, Yucel et

al.,2009). Consequently, patients with schizophrenia with ventricular enlargement show the negative symptoms of illness, whilst patients with smaller ventricles show typical positive symptoms (Andreasen, Olsen et al.,1982). Moreover, reductions in white matter in the temporo-occipital cortex have been associated with the severity of negative symptoms in schizophrenic patients (Foong, Symms et al.,2001), thereby suggesting that cortical region changes are closely linked to the negative symptoms of schizophrenia. Furthermore, patients with schizophrenia also show a reduction in cerebral blood flow in the dorsolateral prefrontal cortex (Weinberger, Berman et al., 1986) whilst performing tasks related to working memory (Carter, 1998; Ho, Wassink et al.,2005) . When tested on the Wisconsin Card Sorting test (WCST) and a simple number matching test, drug naive patients with chronic schizophrenia had significantly reduced cerebral blood flow in the DLPFC which was correlated with the cognitive task; intact DLPFC functioning was accompanied by better performance on the card sort task. This indicates that dysfunction in neural circuitry may underlie the symptoms of schizophrenia pathophysiology.

Heritable Inheritance in Schizophrenia
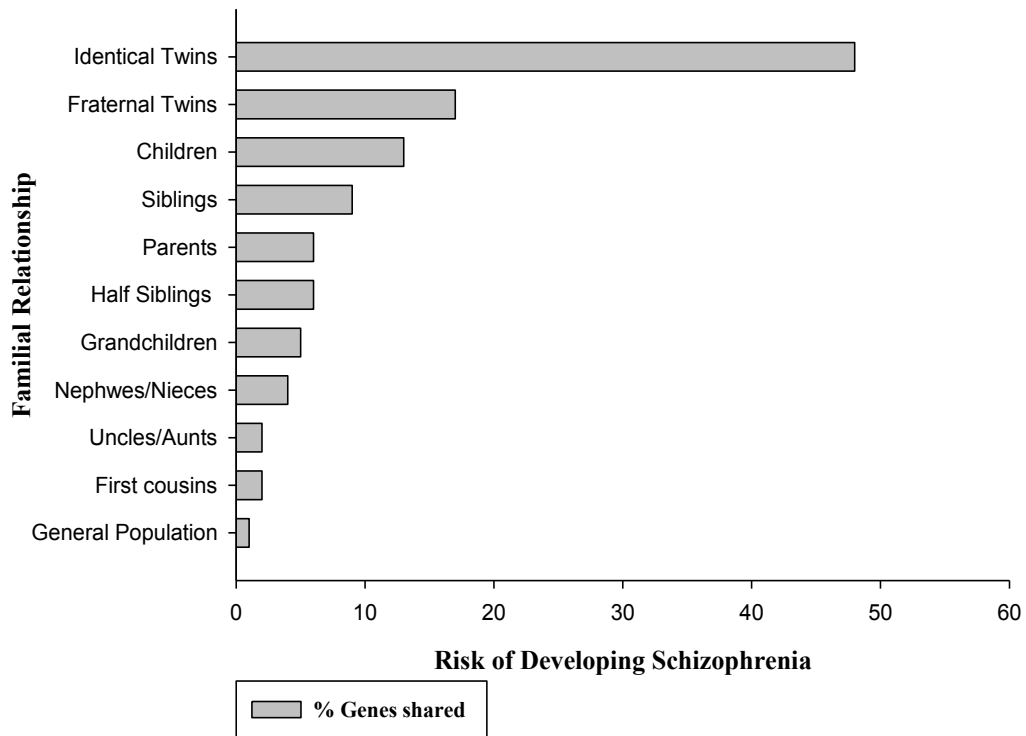
Figure 1a Figure depicting heritability in Schizophrenia. Adapted from Gottesman, 1991.

1.1.3.1 Structural changes and cognitive deficits in schizophrenia

Patients with schizophrenia have deficits in the processing of information as well as problems with involuntary attention (Braff, 1993). They have difficulty in selecting, categorizing and classifying masses of information, as well as deficits in the allocation of attention to multiple sources in a coherent manner. These deficits are linked to symptomatology of the illness. For instance a study reports that patients with negative symptoms showed impaired performance on a delayed match to sample task (Pantelis, Stuart et al.,2001). This task requires the participant to match the target stimulus to a different configuration of simultaneously presented stimuli of an identical pattern. Impairments were also seen on other tasks of fronto striatal

function and working memory such as the Cambridge Neuropsychological Automated Test Battery, and its component the Tower of London task; this is a planning task that incorporates aspects of initial and subsequent thought in task performance. Subjects were presented a set of stimuli (balls) at the bottom of a computer screen, which they were required to arrange so that the positions of the balls matched the configuration of another set of balls presented at the top of the screen. The initial position of the balls was changed so that the minimum number of solutions consisted of a minimum number of two, three, four or five moves. Planning and execution as indexed by initial thinking and subsequent thinking latencies were assessed. Patients with schizophrenia and those with frontal lobe lesions both showed disruptions on these tasks. Schizophrenic patients were slower at performing this task compared to controls and those with Parkinson's, as indexed by longer latencies in their subsequent thinking times as compared to their initial thinking times (Pantelis, Barnes et al.,1997).

Deficits in attention also form part of the cognitive impairments implicated in schizophrenia. Consequently, studies have looked at deficits in selective attention, i.e. the ability to filter out irrelevant information and process what is necessary. Schizophrenia patients presented with auditory stimuli both binaurally and dichotically showed decreased activation of the auditory cortices in the right super temporal gyrus compared to controls in processing these auditory stimuli, suggesting a temporal lobe deficit (O'Leary, Andreasen et al.,1996). Reductions in the volumes of brain tissue in the frontal, temporal, parietal and occipital lobes are also associated with cognitive abnormalities in schizophrenia (Andreasen, Flashman et al., 1994).Thus, implying that cognitive deficits are accompanied by changes in

anatomical regions relevant to cognitive processing of these tasks, accompanied by the symptoms of schizophrenia.

1.1.3.2 The striatal and frontal cortex as anatomically relevant regions for schizophrenia

The striatum and frontal cortex have been implicated in neurobiological dysfunction underlying schizophrenia symptoms. Studies have employed tests of working memory to investigate whether changes in activity in the prefrontal cortex in schizophrenia are associated with these cognitive deficits. More specifically, activations on the WCST in the mid dorsolateral and mid ventrolateral pre frontal cortex (PFC) are seen during the setting of rules to the task but not while implementing an action according to these rules. The mid dorsolateral PFC is activated in these tasks, and the cortical basal ganglia loop is also activated through the mid-ventrolateral prefrontal cortex, caudate nucleus, and mediodorsal thalamus, all of which govern the shifting of a mental state to initiate a new response set (Monchi, Petrides et al.,2001). Consequently, in a study employing the continuous performance task (a test of sustained attention) in ultra high risk patients, those with early onset and chronic onset of schizophrenia reported diminished activation of the frontal regions in the anterior cingulate gyrus, inferior frontal gyrus; and middle frontal gyrus (Morey, Inan et al.,2005). Both pre frontal and striatal activations were greater in patients with early onset rather than chronic schizophrenia (Morey, Inan et al., 2005; Thaden, Rhinewine et al.,2006). Furthermore, these fronto-striatal circuits play a role in the shifting of attention and behaviour, particularly in the processing of temporal information (Meck and Benson,2002). The authors suggest that PFC is involved in mediating deficits in attentional shifting and interval timing behaviour, in

mediating tasks of working memory and executive function in schizophrenia, and is also a source of altered dopamine neurotransmission in the schizophrenic brain.

Dopamine 1 receptors (D1R) have also been implicated in mediating working memory tasks in the PFC (Sawaguchi and Goldman-Rakic, 1991). A greater occupancy of dopamine 2 (D2R) receptors in the striatum is also seen in patients undergoing a psychotic episode (Kegeles, Abi-Dargham et al.,2010). Mice over-expressing D2R in the striatum showed impairments on performance in tasks of locomotor activity and sensorimotor gating of information processing. However, these mice exhibit altered dopamine transmission in the PFC and showed no deficits on the T maze or Morris water maze, indicating intact spatial memory (Kellendonk, Simpson et al.,2006).Thus, disruptions in working memory alone may involve the D2R (Luciana, Depue et al.,1992). Therefore, the aforementioned studies suggest that anatomical changes that are associated with cognitive deficits in schizophrenia may be attributed to the neurotransmitter circuit that innervates that anatomical region.

1.1.4 The role of neurotransmitters in illness

The neurobiology underlying schizophrenia is governed by a complex construct that is related to alterations in dopamine ,glutamate neurotransmission as well as GABA and 5 HT (Flames, Long et al.,2004, Li Woo,2007; Coyle,2004). We will focus on the two main hypotheses of schizophrenia in light of dopamine hyperfunction in predisposing to positive symptoms of illness, and glutamate hypofunction in mediating negative symptoms.

1.1.4.1 The dopamine hypothesis of schizophrenia

The dopamine hypothesis of schizophrenia, in its simplest form, attributes the formation of positive symptom like pathophyisiology of psychosis to an overstimulation of dopamine in the brain (Dargham, 2004; Meltzer and Stahl 1976). Aberrant salience through the hyper-function of this neurotransmitter promotes psychotic like symptoms (Howes and Kapur,2009); Antipsychotics (D2R antagonists) are known to selectively block dopamine receptors and are effective in the treatment of illness by decreasing dopaminergic activity (Carlsson and Lindqvist,1963;). Dopamine mimetic drugs such as L-DOPA and amphetamine induce the positive symptoms of psychosis (Seeman,1987). Dopamine receptors comprise of a large family of receptor subtypes that can be classified under the D1 and D2 receptor families as D1-like or D2-like receptors. The former includes the D1 and dopamine 5 (D5) receptor, whereas the latter includes the D2, D3 and dopamine 4 (D4) receptor subfamilies. These two subfamilies differ in their pharmacological and structural properties, specifically in their coupling to proteins, where the D1R stimulates adenylyl cyclase and the D2R inhibits its activity to produce intracellular responses (Jackson and Westlindanielsson 1994; Sibley 1999).

The key dopamine pathways in the brain comprise of the nigrostriatal tract, the mesolimbic tract, the mesocortical tract and the tuberoinfundibular tract. The dopaminergic cell bodies in these pathways innervate different brain regions, and control a variety of behaviours and functions. For example, the cell bodies of the nigrostriatal pathway are located in the substantia nigra and are responsible for coordinating motor control via the nervous system, whereas the cell bodies of the mesolimbic system are implicated in learning and memory processes. Conversely,

the mesocortical system is innervated by cortical dopaminergic and noradrenergic fibres and is linked to functions governed by the limbic system (Meltzer and Stahl, 1976).

Amphetamine, an indirect dopamine agonist, is commonly used as a pharmacological construct to investigate these neural substrates and behaviours relevant to psychosis. Amphetamine leads to a dopaminergic efflux in the striatal tracts, although the effects of amphetamine are dose dependent. In this dose dependent manner it mediates different neural substrates underlying diverse behaviours (Seiden, Sabol et al.,1993). Acute administration of amphetamine worsens symptoms in schizophrenia patients (Angrist, Rotrosen et al.,1980; Laruelle, Abi-Dargham et al.,1996). It also disrupts performance in tasks used to mimic cognitive and behaviour deficits in schizophrenia in rodent models of inattention i.e. latent inhibition (Weiner, Lubow et al.,1988) and spatial learning on the Morris water maze (Russig, Durrer et al.,2003) and sensorimotor gating (Swerdlow, Mansbach et al.,1990), where amphetamine induced disruptions on these tasks in rodents directly correlate with deficits in humans on these tasks (Gray, Pickering et al.,1992; Braff, David et al.,2001). Furthermore, administration of amphetamine in schizophrenic patients and rodents produces psychotic symptoms that are reversible by D2R antagonist drugs and antipsychotics, and has been used as a pharmacological tool to model tasks involved in investigating cognitive impairment in schizophrenia (Weiner, Lubow et al.,1988; Weiner, Shadach et al.,1996; Kapur,2003).

1.1.4.2 The glutamate hypothesis of schizophrenia

Glutamate is the major excitatory neurotransmitter in the central nervous system. The glutamate hypothesis of schizophrenia states that a hypofunction in glutamate

neurotransmission, via the aberrant functioning of the glutamate receptor N-methyl –
D-aspartate (NMDA) predisposes to schizophrenia-like pathophysiology (Goff and
Coyle 2001; Hashimoto K. 2004). Glutamate receptors consist of the ionotropic
receptors and the G-protein coupled metabotropic receptors. The ionotropic receptors
consist of three receptor subunits i.e. the NMDA (that consists of the NR1, NR2A-D
and NR3-A), AMPA (Glu receptors 1-4) and Kainate receptors (Glu receptors 5-7
and kainate receptors). Additionally, the metabotropic glutamate receptors are
divided into three groups: the group I mGLUR receptors (mGLUR 1, 2), group II
(mGLUR receptor 2,3) and Group III receptor (mGLUR 4,6,7,8) which are all
coupled to G proteins. Both the ionotropic and metabotropic glutamate receptors
have been implicated in memory impairments (Gao et al.,2000, Lisman et al.,1998,
Krystal et al.,2004). The hypofunctioning of these receptors contributes to the
behavioural symptoms in schizophrenia (Hirsch, Das et al.,1997).

 Phencyclidine and Ketamine, both non competitive glutamate antagonists, produce
schizophrenia like positive and negative symptoms, as well as cognitive dysfunction
in healthy humans (Snyder,1980;Javitt,1991), and worsen symptoms in
schizophrenic patients (Newcomer, et al.,1999). Acute administration of PCP and
Ketamine are associated with the blockade of glutamate receptors, and also with an
increase in cortical dopamine and glutamate (Moghaddam,1997). Administration of
these drugs leads to impairments on tests of cognitive functioning in the prefrontal
cortex and the striatal regions, by increasing dopamine transmission (Deutch, 1987)
and by NMDA receptor up regulation (Sircar,2003). mGLUR 1 and mGLUR 5
knockout mice with a blocked glutamatergic tone also show deficits in a task of
sensorimotor gating (Brody, Conquet et al.,2003). D2R antagonists reverse deficits in
PPI, but the antipsychotic raclopride has no effect in reversing disrupted PPI in

mGLUR deficient knockout mice(Henry, Lehmann-Masten et al.,2002), suggesting that metabotropic glutamate receptors mediate deficits in the sensorimotor gating in schizophrenia, that are not reversible by dopamine D2R antagonists/antipsychotic drugs. Thus, both the DA and glutamate systems have a complex relationship that mediates disruptions on cognitive tasks used to model Schizophrenia pathophysiology. Disturbances in one system may affect the functioning of other systems i.e. dopamine is known to inhibit glutamate release at the NMDA receptors (Jentsch and Roth,1999). These systems interactions in turn are further complicated by the inhibitory GABAergic and glutamate interactions and excitatory effects of glutamate in the prefrontal cortex (dopamine circuit), the functioning of which jointly contribute to schizophrenia pathophyisiology (Coyle,2004). Thus, schizophrenia pathophysiology is mediated by complex interactions between the dopamine and glutamate systems.

1.1.5 Methods of modelling schizophrenia

Schizophrenia is a complex disorder that has been investigated by using a variety of methods; in vivo, ex vivo and imaging studies in animals and humans**.** Schizophrenia is an illness that is not caused by the aberrant functioning of a single gene, but rather is the product of multiple genetic and environmental factors. The use of an endophenotype approach i.e. intermediate phenotypes that form the links between genetic predisposition and their overt expression of behaviour (Gottesman and Gould,2003) , provides clues to the neurobiology of Schizophrenia. Understanding the molecular mechanisms implicated in its pathology, including the neurotransmitter systems implicated in Schizophrenia and how they predispose to its symptoms, helps

establish a framework for this complex disorder (Cannon and Keller,2006), see table 1.1.

Animal models have been extensively used to model the symptoms of illness and its neurobiology by using approaches such as post mortem techniques and imaging to elucidate the role played by specific neurotransmitter systems in mediating these symptoms. Similarly behavioural indices mapping onto attentional and cognitive deficits as well as negative symptoms have been outlined. These can be measured using neuropsychological tasks that are behavioural correlates of the impairments inherent in Schizophrenia. For example, specific alleles may predispose to dysregulation in neurotransmitter systems i.e. altered glutamate neurotransmission in the hippocampus that manifests as disruptions in its behavioural correlate i.e. memory (Moghaddam,2003). NMDA receptors are involved in the encoding of long term memories that require the hippocampus, and in long term plasticity in the dentate gyrus-hippocampal circuit (Steele and Morris,1999). These models effectively measure the pathways influenced by aberrant gene function by challenging specific glutamate receptor ligands with glutamate agonist/antagonist drugs, which in turn promote or impede neurotransmission, and mimic or reverse abnormal gene function implicated in Schizophrenia.

The knock out approach has been extensively used to model human central nervous system (CNS) disorders. For example, transgenic mice overexpressing the human Tau isoform show age dependent onset and progression of Alzheimer's disease (Ishirhara, Hong et al.,1999). Other transgenic models of Alzheimer's disease include mice with mutations in the human amyloid precursor protein, that show oxidative brain damage indicative of disease, as seen by impairment on spatial learning and memory tasks; phenotypes used to model of memory loss as seen in

Alzheimer's (Butterfield, Galvan et al.,2010). Mouse models of other Parkinson's disease show that mutations of the LRRK2 gene causes the late onset of disease, and transgenic mice bearing these mutations show neuronal degeneration of the nigrostriatal dopamine pathway (Ramonet, Daher et al.,2011). This indicates that the transgenic approach is useful in elucidating the neurobiology underlying these disorders, and modelling disease relevant phenotypes.

These genetically modified mouse models are of importance as they help in identifying both susceptibility and causative genes implicated in these CNS disorders, and also help evaluate novel therapeutic drug targets (Morrisette, Parachikova et al.,2009). The common idea underlying the disease-common allele approach is that disease related mutations in the human genome consist of a single nucleotide polymorphism (SNP). However, in light of complex disorders such as schizophrenia, it is asserted that the illness is not a consequence of a single mutation but of a variation of large segments of DNA involving a number of genes (Kellendonk, Simpson et al.,2009).

 The two transgenic models we have focused on in this thesis include the dopamine D2R knockout model and the Neuregulin-1 partial knockdown model. A large number of SNP's within the Nrg-1 gene have been associated with schizophrenia, and schizophrenia relevant phenotypes such as measures of sensorimotor gating and hypofrontality (Stefansson et al.,2002, Hall et al.,2006). Knockdown of Nrg-1 ErbB receptors, as well as of the transmembrane-like domain, and immunoglobulin-like domain have been generated. Hetrozygous Nrg-1 mice, with deletions of the gene are hyperactive (O' Tuathaigh et al.,2007), but mice with mutations in the Nrg-1 gene that is either ErBB2 receptor coupled or ErbB3 receptor coupled do not show this phenotype (Golub et al.,2004).

Mouse models of the D2R model are centered around the dopamine hypothesis of schizophrenia i.e. dopamine 2 receptor transmission promotes schizophrenia-like symptoms, where an increased number and density of dopamine 2 receptors are also seen in the striatum (Dargham,Rodenheiser et al.,2000). Mice over expressing dopamine 2 receptor, show disruptions in prefrontally mediated cognitive endophenotypes (discussed later) of working memory and associative learning, that are also impaired in patients with schizophrenia, as well as to incentive motivation to food reward. Only the latter motivational deficit is reversed on switching off this over expression in the D2R mutant (Kellendonk, Simpson et al.,2009). This model is useful in understanding the underlying neurobiology of schizophrenia as it indicates that the mesostriatal pathways may be involved in mediating cognitive impairment in illness, and that blockade of D2 receptors may be beneficial in reversing this impairment.

We investigated the three schizophrenia relevant phenotypes of Latent Inhibition (LI), a test of conditioned attention; sensorimotor gating to startle i.e. Prepulse Inhibition (PPI) and an Episodic Memory (EM) task. Prepulse Inhibition has face, predictive and construct validity (discussed in detail, Refer to Section 1.4.1) and is associated with schizophrenia, and can be modelled in mice and rats. Dopamine agonists disrupt prepulse inhibition and antipsychotic drugs improve prepulse inhibition (discussed in detail, refer to section 1.4.1). With regard to LI, mice have been selectively bred (e.g. mice over expressing dopamine 2 receptors, knock out mice lacking the dopamine 1 receptor) to respond to antipsychotic drugs that do so by blocking dopamine transmission.

Episodic memory (memory for memory at a particular point in time) disruptions have been seen in schizophrenia patients (Park, Püschel et al.,1999; Park, Püschel et

al., 2003; Bonner-Jackson, Haut et al., 2005). These memory processes are regulated by dopamine; dopamine 2 receptors have been proposed to modulate verbal recall of memories (Chen, Kuang Yang et al.,2005).The dopamine 2 receptor is also involved in the memory for coding spatial information (Tran, Tamura et al.,2003), indicating that a transgenic model of memory would be a good schizophrenia relevant cognitive endophenotype. Thus, these models are useful for investigating latent inhibition and prepulse inhibition, and episodic memory as they would help elucidate whether aberrant gene function disrupts these cognitive endophenotypes, and also provide clues about the neurobiology underlying illness (Hitzemann,2000).

There are a number of advantages of using the transgenic approach, apart from the use to investigate cognitive endophenotypes for illness. This includes the investigation of a mutated gene in a population with a homogeneous genetic background, as well as the ability to study the onset of illness in a controlled environment. Additionally, transgenic approaches also make it possible to study the role of specific receptors with much higher specificity than pharmacological approaches .A disease could also be modelled prior to the onset of its overt symptoms, and its functional consequences can be studied (Piccioto and Wickman, 1998).

As mentioned previously, complex behaviours are attributed to a culmination of genetic effects, and although this is a useful rationale to employ the transgenic approach, it also acts as a limitation, as the allelic variation and expression levels of other genes can influence the phenotypes. If a phenotype is influenced by a number of genes, than knocking out a particular gene would lead to compensatory effects on the phenotype as a consequence of other genes. Additionally, the knockout approach is confounded by the background strain that the mutant is bred on. Some strains such as the C 57 show intact spatial memory, whilst others such as the 129/Sv show

disruptions on tasks used to model these memories. Some inbred mouse strains also show congenital deafness, or sight impairments (Crawley et al.,1997). These issues can be overcome by backcrossing with a mouse that has already been behaviourally phenotyped, but this might lead to a lethal mutation on the inbred background. It is suggested that since inbred strains show disruptions on complex behavioural task, utmost care needs to be taken in the strain selected for backcrossing (Piccioto and Wickman, 1998).

Structural changes corresponding to circuits regulated by different neurotransmitters are also used to measure these endophenotypes. Other models have investigated the effect of aberrant neurotransmitter function using lesion studies i.e. the disruption of dopaminergic-glutamatergic actions in mediating abnormal responses to activation of mesocortical dopaminergic areas takes place post puberty, following early hippocampal insult (Tseng, Lewis et al.,2007). These approaches have been outlined in the subsequent sections.

Schizophrenia symptoms	Tests used to model these symptoms	Neurotransmitter systems that can be investigated
I. Positive symptoms		
Psychomotor activation	Hyperlocomotion in response to novelty/stress Locomotor activity Locomotor activity to novelty	Dopamine, using dopamine agonists amphetamine to model enhancements in locomotor activity (Segal and Mandell,1974) Glutamate receptor agonists and antagonists
Enhanced sensitivity to psychotomimetic drugs	Augmented locomotor responses to NMDA antagonists PCP, Ketamine (Mohn, Gainetdinov et al.,1999) Locomotor response to amphetamine Increased sensitivity to tests of other drugs Dizocilpine, PCP on PPI	Glutamate
II. Negative symptoms		

Social withdrawal **Avolition** **Anhedonia**	Decreased interaction with juvenile conspecific Decreased preference for social novelty Decreased reinforcing properties of reward and drugs of abuse Altered aggression behaviour on resident intruder assay For example; mice with reduced NMDA receptor function show deficits in social interaction and motor activity (Mohn, Gainetdinov et al.,1999)	Glutamate
III. Cognitive symptoms		
Decreased working memory **Deficits in executive dysfunction** **Memory deficits**	Impaired alterations in the t maze, radial arm maze, attentional set shifting Delayed non match to sample task	Dopamine and glutamate agonist and antagonists
Deficits in attention/sensorimotor gating/	Decreased PPI Decreased LI	Dopamine and glutamate agonist and antagonists For example, to investigate predictive validity of antipsychotics (D2R antagonists) in the reversal of schizophrenia symptoms

		(Weiner and Feldon,1987) or PPI disruption (Yee,2004)
General cognitive deficits	Decreased spatial learning in the Morris water maze Radial arm maze	Same as the above; for instance by using PCP to affect glutamate neurotransmission and disrupt behaviour (Javitt and Zukin,1991)

Table 1.1 Mouse behaviours relevant to modelling symptomatology implicated in schizophrenia. Adapted from (Powell and Miyakawa 2006).

1.1.5.1 Cognitive endophenotypes for illness

Psychiatric disorders are a product of gene by gene interactions, gene by environment interactions, and epigenetic factors. These genetic interactions exist at multiple levels, and can be simplified by the use of endophenotypes, as they can quantify and delineate single gene functions, in a single activation circuit thus providing a more informative basis to the investigation of psychiatric illness (see Fig. 1b).

A number of alleles have been implicated as candidate genes for schizophrenia. These include Dopamine (DA), Neuregulin-1 (Nrg-1), Dysbindin and Disrupted- in - Schizophrenia (DISC-1) (Harrison and Weinberger,2005) where aberrant functioning of these genes is thought to lead to abnormalities that underlie schizophrenia pathophyisiology (Gottesman and Gould,2003). Abnormal functioning in each of these components can be indexed in behavioural paradigms investigating the symptoms of illness. Schizophrenia endophenotypes link genetic associations to behaviours, rather than to behaviours themselves (Gould and Gottesman,2006). Thus, the allocation of specific gene function that contributes to schizophrenia like can be indexed by behavioural measures. In order to study this, 'knockout' or 'knock-down' transgenic mouse models are created, that consist of a mouse strain lacking both functional copies of the allele or a certain percentage of the allele through a targeted mutation. For example, mice lacking dopamine transporter (DAT) have been used as a model of hyperdopaminergia in psychosis, as they show elevated synaptic levels of dopamine (Barr, Lehmann-Masten et al.,2004). This excess DA transmission promotes disruptions in attentional processing and can be modelled in the LI task of

attentional salience, as a schizophrenia relevant phenotype. Mice with dampened DAT function show deficits in sensorimotor gating and hyperactivity to a novel environment, which are cognitive endophenotypes of schizophrenia. These deficits are reversible upon the administration of a selective serotonin antagonist and D2R antagonists that are antipsychotic drugs (Zhuang, Oosting et al.,2001), thus demonstrating predictive validity in an animal model of schizophrenia. Thus, mice with aberrant gene function relevant to schizophrenia can be generated, and abnormalities implicated in cognitive behavioural tasks of relevance to schizophrenia, are a good measure, to determine genetic contribution to illness.
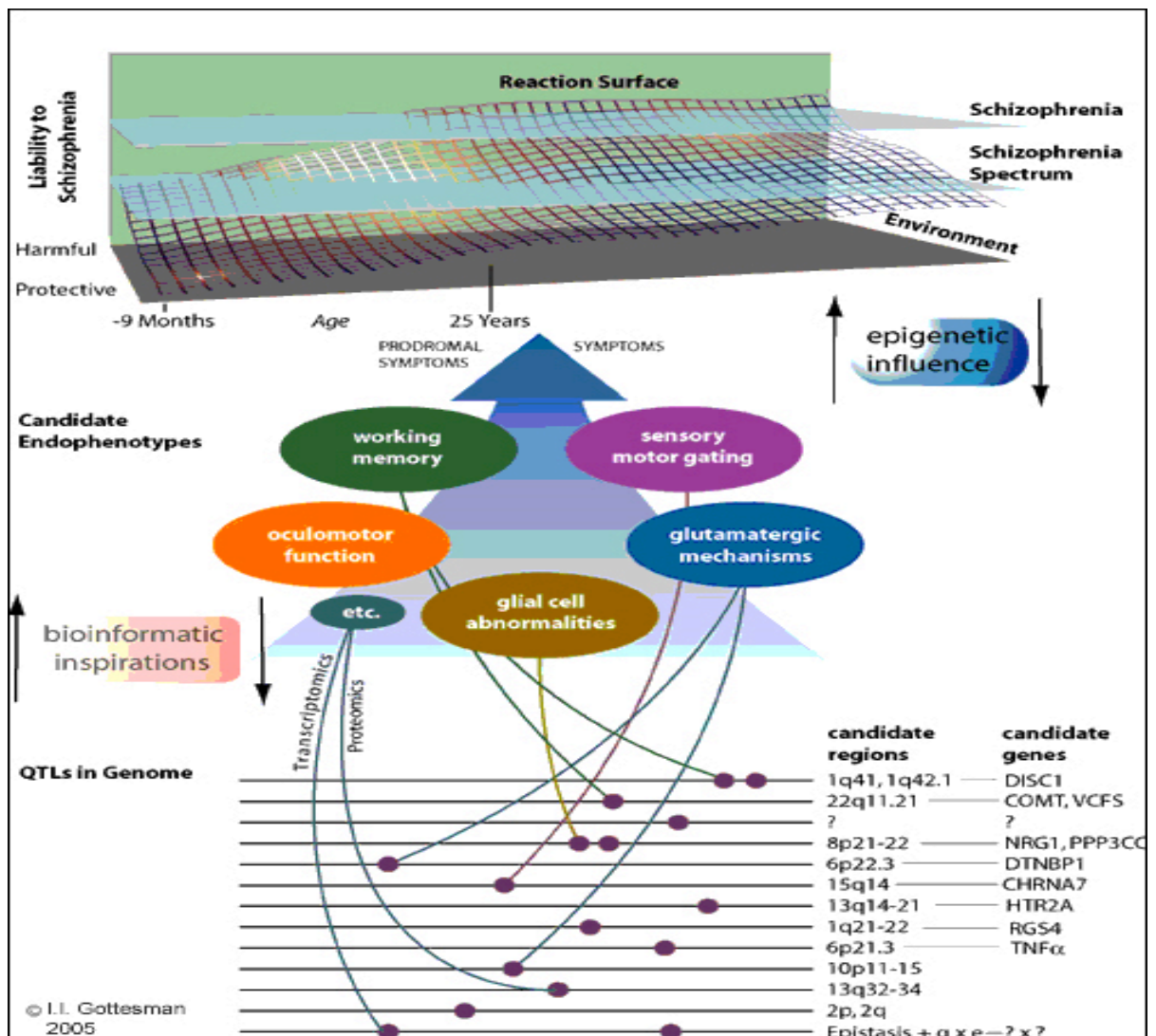
Fig. 1b Endophenotypes are indexed by single genetic/neurobiological antecedents that can be linked to specific neuronal circuitry and delineated in terms of specific functioning in their associations with psychosis. Adapted from Gould and Gottesman,2006.

1.1.5.2 Neural circuits, genes and behaviour

Histological measures are also used to investigate the effects of discrete genetic changes that contribute to deficits measurable on behavioural paradigms can be investigated in terms of their underlying neurobiology. Altered mRNA levels for proteins encoded by genes implicated in schizophrenia have been found (Farh,2005 and Perkins,2007). The DISC-1 is a candidate gene for schizophrenia that is associated with cognitive endophenotypes for schizophrenia (Li, Zhou et al.,2007). Animals with disruptions in the DISC-1 gene show deficits in spatial learning as well as locomotion and prepulse inhibition. Deletion of the DISC 1 gene that is specific to the 129S6/SvEv strain, indicates gross brain morphology is intact in mice homozygous for the gene, but abolition in the production of this protein structure were seen upon transference of the DISC-1 allele to the C57Bl/6J strain (Koike, Arguello et al., 2006).

Another gene that has been implicated in schizophrenia pathophyisiology includes the Dysbindin gene. A reduction of dysbindin-1 mRNA has been implicated in the formation of hippocampal synaptic plasticity in the CA3 region in the brains of schizophrenic patients (Wicker,2008) and may contribute to schizophrenia pathophysiology. Histological measures are also used for exploring protein changes is through lesion studies. Consequently, the hippocampus plays a role in remembering; in the consolidation and retrieval of memories (Costner, Goldman-Rakic et al.,2004), where hippocampal lesions disrupt the formation or retrieval of associations (For wood, Winters et al.,2005). More specifically, hippocampal damage is thought to affect exploratory preferences in rats and has been implicated in

studies of learning and memory. Altered inhibitory glutamate and GABAergic mechanisms also mediate neural plasticity in the hippocampus and promote these disruptions in behaviour (Impey, Mark et al.,1996; Abel, Nguyen et al.,1997; Malenka and Bear 2004; Forwood, Winters et al.,2005) which may be attributed to protein changes in the brain. This demonstrates a schizophrenia relevant endophenotype, as disruptions of schizophrenia relevant behaviours may be linked to changes in glutamate neurotransmission and aberrant gene function.

1.1.5.3 Neurodevelopmental approaches

Another approach to investigate aberrant gene function in Schizophrenia is by investigating gene*environment and gene*gene interactions, where, in the former a combination of genes and the environment may predispose to behavioural abnormalities as indexed by phenotypic measures. These may depend on experience at various stages of life, which interacts with a combination of genetic effects in predisposing to illness (Waddington, Corvin et al.,2007; Kirby, Waddington et al., 2009).

The neurodevelopmental hypothesis of schizophrenia posits that environmental insults that promote illness, usually take place in the prenatal or perinatal stages of development, where aberrant gene effects at these stages may manifest only at a later stage of development(Olney and Farber,1995). There is evidence for schizophrenia onset at adolescence(Thomsen,1996; Oise and Rund,1999) as indicated by volumetric gray matter changes in the dorsolateral pre frontal cortex during adolescence (Thompson, Vidal et al.,2001). These factors are mediated both by the environment and time of developmental insult onset at a later stage. The genes

implicated in schizophrenia, that have been investigated this neurodevelopmental approach include the DISC-1 and Neuregulin-1 genes. A study that has investigated the role of the DISC-1 in schizophrenia pathophysiology used DISC-1 knockdown mice, which show abnormalities in postnatal mesocortical dopaminergic maturation, as a consequence of disturbance to pyramidal neurons that mediate neuronal plasticity. This disturbance in brain maturation disrupts a sensorimotor gating in post pubertal rats (Niwa, Kamiya et al.,2010).Increasing evidence is emerging for mutations in the ErbB4 gene, encoded by Nrg-1, in disrupting developmental processes in schizophrenia (Walsh, McClellan et al.,2008). Nrg-1 regulates both inhibitory and excitatory neurotransmission in the brain, and alterations in the Nrg-1 ErbB4 pathway may influence plasticity by altering glutamate and GABAergic transmission to contribute to schizophrenia pathophysiology (Mei and Xiong,2008). Consequently, infant mice that were administered the Nrg-1β1 (which binds to the ErbB4 receptors) in the postnatal period, show impairments on prepulse inhibition, latent inhibition and social interactions tasks when tested at adulthood (Kato, Abe et al.,2010). The neurodevelopmental hypothesis has also been investigated using lesions, to investigate the effects of these lesions on schizophrenia relevant brain areas and their effects at maturation. Rats with ibotenic acid lesions in the ventral hippocampus on the seventh day of birth, show increased hyperactivity to a novel environment in early adulthood following amphetamine administration. Additionally, rats lesioned as neonates also show hyperactivity in the forced swim test, which has been attributed to increased mesolimbic dopamine responsivity to these lesions (Lipska, Jaskiw et al., 1993).

Other factors that contribute to schizophrenia pathology early on in development includes poor maternal nutrition, maternal infection, birthing season, urban births as

well as minor physical anomalies (Lewis and Levitt,2002). Consequently, mice administered protein regulator, immune challenging agent Polyl:C on gestation day 9 show deficits in latent inhibition, prepulse inhibition, as well as an enhancement in reversal learning and augmented locomotor activity in response to amphetamine, at a later stage of development (Meyer, Feldon et al., 2005). Therefore, these data suggest that maternal infection at mid gestation can promote deficits post puberty and promote schizophrenic pathophyisiology. Thus, neurodevelopmental approaches are useful measures of tracking changes at different stages of development, as they help delineate the role of environmental insult and their interaction with gene function in the course of development from birth to adulthood, where symptoms relevant to schizophrenia may only be evident at later stages of maturation.

1.2 Schizophrenia relevant phenotypes that investigate the dopamine hypothesis

The psychotic symptoms of schizophrenia have been attributed to a hyperdopaminergic state in the limbic regions; administration of a dopamine agonist amphetamine causes psychotic like symptoms that are reversible by neuroleptic drugs. Furthermore, the dopamine hypothesis suggests dopamine hypofunction in the cortex (Jentsch, Redmond et al.,1997). The imbalance of dopamine as a consequence of the deficit of dopamine in the cortical region and an excess in the sub cortical regions (Abi-Dargham 2004).Consequently, negative symptoms such as avolition to the reinforcing properties of rewarding behaviours and both of these models of hyper and hypodopaminergia together promote the formation of the positive, negative and cognitive symptoms of illness (Davis, Kahn et al.,1991). Dopamine is involved in motivational salience of environmental stimuli, where reward associated stimuli are

the focus of attention for an animal construct of attention in schizophrenia, and goal directed behaviour (Bridge and Robinson, 1998, Howes and Kapur, 2009).

1.2.1 The role of dopamine D2 receptors in schizophrenia

The D2R plays a role in mediating schizophrenia symptoms (Glut, Forgone et al., 2003; Debarred, Goya et al.,2004; Lawford, Young et al.,2005). There are an increased number of D2R present in the brain compared to other D2-like dopamine receptor subtypes (Holmes, Lachowicz et al.,2004); dopamine occupies a higher proportion of D2 receptors in the striatal brain regions of schizophrenics as compared to controls (Kegeles,Abi-Dargham et al,.;2001 Abi-Dargham, Rodenhiser et al.,2000). Overstimulation of dopamine produces DA hyperactivity and increased DA transmission in the striatal regions, and hypoactivation, or dampened DA activity in the cortical regions. Mice over-expressing D2 receptors in the striatum show deficits on a test of working memory as indexed by the delayed non match to sample task and attentional set shifting (Kellendonk, Simpson et al.,2006).

Dysregulation of sub cortical dopamine in the pre frontal cortex by D1 receptors has been implicated in the literature (Weinberger, Berman et al.,1992; Weinberger, 1993), and mediates the cognitive deficits in schizophrenia. The hypo activation of dopamine in the prefrontal region (Weinberger 1988) increases D2 turn over in the sub cortical areas, and DA receptor increases are dampened by D2 receptor antagonists/antipsychotic drugs (Deutch 1992). The D1 and D2 receptors modulate glutamate neurotransmission via projections to these regions. Reduced PFC activity owing to the secondary mechanisms of aberrant NMDA functioning, promotes decreased mesocortical DA activity. D2 receptor stimulation inhibits NMDA receptor mediated flow of information from the cortex to the striatum, (Laruelle,

Kegeles et al.,2003) and D1 receptor stimulation facilitates glutamate neurotransmission in the striatum(Centonze, Picconi et al.,2001). Thus, these studies suggest that D2 receptors are directly and indirectly (via D1R modulation) involved in the regulation of symptoms of illness. Indirect DA agonist Amphetamine induced disruption of schizophrenia relavant phenotypes is a model of hyperdopaminergia, by altering DA transmission; administration of amphetamine in rodents, induces schizophrenia like symptoms, and produces disruptions in schizophrenia relevant phenotypes (Centonze, Picconi et al.,2002). This is discussed in the next section.

1.2.2 Amphetamine induced disruptions in schizophrenia phenotypes

Schizophrenia symptoms are worsened in patients with schizophrenia that use amphetamine (Lieberman, Kinon et al.,1990), and in healthy humans administration of amphetamine even at low doses produces behaviour that resembles the positive symptoms of psychosis (Angrist and Gershon,1970). Amphetamine (AMPH) exerts its effect on multiple brain regions including the dopaminergic system (Seiden, Sabol et al.,1993; Seeman, Schwarz et al.,2006); PET and single photon emission computed tomography (SPECT) studies show increases in, synaptic dopamine levels in the striatal region in humans (Breier, Su et al.,1997) and rats (Wang, Pei et al., 2010) following amphetamine administration.

Amphetamine increases extracellular dopamine by vesicular release and reverse transport (Jones et al.,1998; Budygin et al.,2004). Amphetamine enters the dopamine vesicles and causes displacement of dopamine from the vesicles to the cytoplasm. Dopamine is released into the extracellular space by outward transport by the dopamine transporter. Via the plasma membrane transporter, the transport of dopamine takes place from one side of the plasma membrane to another, increasing

the rate of reverse transport, and thereby increasing the amount of extracellular dopamine across the plasma membrane (Jones et al.,1998, Floor and Meng,1996).

Amphetamine induced behaviours in rodents have been used to model the dopaminergic hyperactivity associated with schizophrenia (Alexander, Wright et al., 1996).For instance, amphetamine induced disruptions in D2 receptor wild type and knock out models have been used to investigate the role of these receptors in PPI (Swerdlow, Mansbach et al.,1990), LI (Solomon and et al.,1981) and locomotor activity(Braun and Chase 1986) that are attenuated in schizophrenia.

 Psychostimulants produce hyperactive and stereotypic (sniffing, licking, biting, head movements) behaviours in rodents (Braun and Chase 1986) in a dose dependent manner. More specifically, low doses of amphetamine (1mg/kg) disrupt latent inhibition and produce locomotor stimulation via the nucleus accumbens or ventral striatum (Weiner et al.,1988,Warburton et al. ,1993.Bay-Richter et al. 2008; Gray et al.,1991). High doses (5 mg/kg) on the other hand, lead to stereotypy via the dorsal striatum and nucleus accumbens (Weiner et al.,1988, Joseph et al.,2000, Gray et al,. 2005). Previous studies using the task of learned inattention latent inhibition (LI) have shown LI disruption by low, but not high doses of amphetamine; that is attributed to differential involvement of these doses with the mesolimbic and mesotriatal systems. At high doses, amphetamine is thought to predispose to anxiety related behaviours in mice (Bia, Grazyna et al.,2007) as well as stereotypy and preservative behaviours that create cognitive and affective disturbances (Groves and Rebec 1976; Braun and Chase, 1986). These cognitive tasks of relevance to schizophrenia have been discussed in more detail in the following sections.

1.3 Models of cognitive dysfunction implicated in schizophrenia

1.3.1 Latent inhibition and schizophrenia

LI is a retardation in learning to a stimulus, as a consequence of that stimulus being previously pre exposed, without any reinforced consequences (Lubow and Moore 1959; Lubow,1989), and has been observed in a variety of species including mice, rabbits, goats and humans (Lubow 1989), see Figure 1c. Psychosis is postulated to be a dopamine mediated state of aberrant salience (Kapur, Mizrahi et al.,2005), it is suggested that environmental and genetic factors predispose to dysregulated dopaminergic firing that promotes an assignment of salience to irrelevant stimuli. In order to justify this context-inappropriate salience attribution, schizophrenic patients experience hallucinations (distorted perceptions) and hallucinations (false beliefs); which thereby acts as a 'cognitive scheme' to guide and process further thoughts and action (Kapur, Mizrahi et al.,2005). D2 antagonists block dopamine to dampen the irrelevant salience of these symptoms, and serve to improve impaired cognitive functioning (Kapur and Mamo,2003; Mishara and Goldberg,2004).

Thus, LI is used as a model of 'learned inattention' i.e. ability to ignore irrelevant information, and maintain attention to the relevant stimulus and schizophrenia patients show disruptions on LI(Rascle, Mazas et al.,2001). Consequently, this task is an index of learning, of the rate at which an organism learns as association between the unconditioned stimulus (UCS) and the conditioned stimulus (CS), wherein, the 'to-be-CS' that occurs without consequences over a number of trials is compared with a stimulus that has not had the chance to become associated with anything

(Oades, R et al.,1997). Conditioning studies that used LI in humans use a variety of tasks; eye blink conditioning, electrodermal conditioning and taste aversion conditioning (Arwas, Rolnick et al.,1989) to demonstrate the LI effect.

LI can be indexed as a measure of conditioned emotional suppression of behaviours that comprises of three stages; pre exposure (PE), in which a stimulus that is to be conditioned (tone) to is presented repeatedly, conditioning where the pre exposed stimulus is paired with a reinforcement (foot shock) and a test phase which is indexed by the animal's learning to suppress licking.
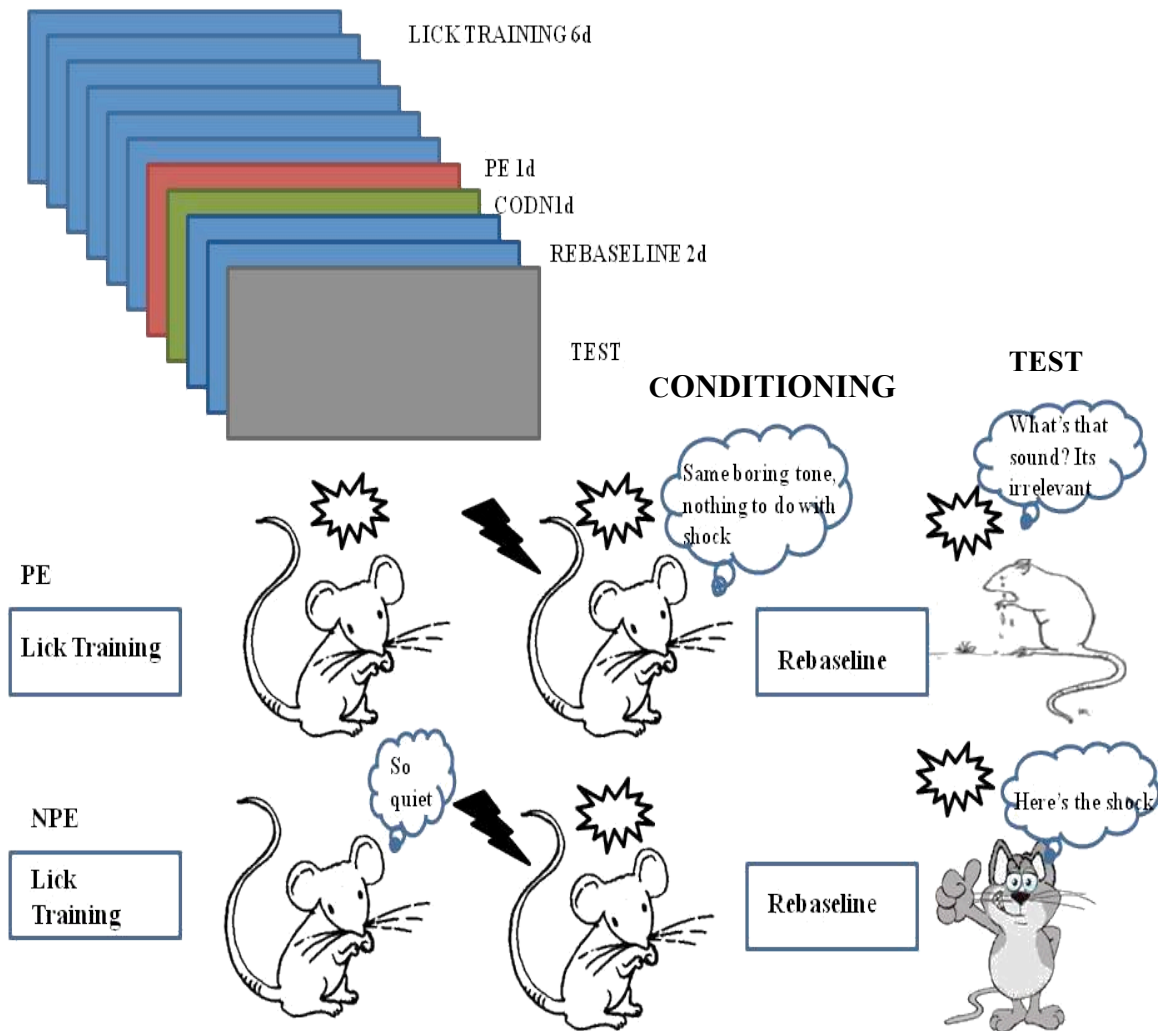
Fig 1c. Diagrammatic description of the LI protocol, depicting the PE and NPE stages. In the PE stage, the animal is conditioned to a tone, whilst NPE stage animals receive no tone. Upon conditioning, this tone is paired with a shock. In the test phase, the PE group exhibit retarded learning of the tone as a predictor of the shock, whilst NPE animals show that this association has been sufficiently learnt.

1.3.2 Mechanism of action of amphetamine and amphetamine mediated LI disruption in schizophrenia

As previously mentioned, Amphetamine increases extracellular dopamine by vesicular release and reverse transport (Jones et al.,1998; Budygin et al.,2004). Amphetamine stimulates the transport of dopamine, which permits the movement from one side of the plasma membrane to another, increasing the rate of reverse transport, and thereby increasing the amount of extracellular dopamine across the plasma membrane (Jones et al.,1998; Floor and Meng,1996). By blocking access to the transporter, the probability of reverse transport is lowered, thereby increasing extracellular dopamine (Carboni et al., 2001).

Amphetamine induced disruptions on behavioural models relevant to Schizophrenia are differentially mediated depending on the dose administered; low doses of amphetamine produce hyperactivity and high doses produce stereotypy in rodents (Feldon, et al.,1990). Previous studies using the LI task have shown LI disruption by low, but not high doses of amphetamine(Weiner,1987; Weiner, Lubow et al.,1988), which is attributed to differential involvement of these doses with the mesolimbic and mesotriatal systems. The mesolimbic system consists of the nucleus accumbens, a major site of termination of this system, along with its afferents and efferent's to other areas such as the amygdala, the hippocampus and the medial pre frontal cortex, and the mesostriatal system consists of the substantia nigra and the striatum. Both of these circuits are efferent fiber projections of the ventral tegmental area and jointly consist of the dopaminergic pathway. Low doses of amphetamine (1mg/kg) disrupt latent inhibition and produce locomotor stimulation via the nucleus accumbens or

ventral striatum (Weiner, Lubow et al.,1988; Gray,1991; Clea, Warburton, Joseph et al.,1994). High doses (5 mg/kg) on the other hand, lead to stereotypy via the dorsal striatum and nucleus accumbens (Weiner, Lubow et al., 1988; Gray, Joseph et al., 1995; Joseph, Peters et al.,2000) .However, administration of D1/D2 mixed agonist apomorphine, and indirect dopamine agonist, at low and high doses (0.3 and 1.5 mg/kg) did not produce LI disruptions (Feldon,1991). Consequently, failure of LI disruption at a high dose has also been seen (6mg/kg) (Weiner et al.,1987), where high doses of apomorphine and amphetamine DA agonists do not alter LI (Feldon, Barkai et al.,1990) This suggests that the amphetamine disruption of LI in schizophrenia is mediated by a low the dose which is sufficient to produce schizophrenia like cognitive deficits.

1.3.2.1 Disruptive effects of amphetamine are mediated by the injection schedule in LI

Different effects of amphetamine administration are seen depending on whether they occurred as part of a single PE (PE) session followed by a conditioning (COND) session, or as two sessions split by a 24 hour time interval. In a session where each of the stages (PE, COND, Test) were separated by 24 hours, acute administration of a high dose of amphetamine (6 mg/kg), prior to pre exposure and conditioning did not induce LI disruptions in rats (Weiner, Izraeli-Telerant et al.,1987). In the same study, chronic exposure to this high dose of amphetamine prior to both stages did not attenuate LI. Alternatively, a low dose of amphetamine (1.5 mg/kg) disrupts LI in rats when administered in both the PE and COND stages only, indicating that neither high doses not chronic exposure to amphetamine is needed to produce this disruption(Weiner, Lubow et al., 1988). In the session where PE and COND were not separated by a 24h interval, the administration of a single low dose of amphetamine

15 minutes prior to PE, and 15 minutes prior to COND, with a thirty minute interval between the PE and COND sessions did not disrupt LI. However, the same injection schedule did disrupt LI when the PE and COND sessions were 24 hours apart (Weiner, Lubow et al., 1988). This is corroborated by other studies that suggest that a single low dose of amphetamine (0.32 mg/kg, subcutaneously) administered 30 mins prior to the PE and COND phases attenuates LI (McAllister,1997). A later study showed that amphetamine administered 45-90 mins prior to conditioning, but not 15 mins prior to conditioning, also abolishes LI(Young, Moran et al.,2005). These studies indicate that amphetamine mediated disruption of LI are 1) sufficiently induced by low doses of amphetamine and 2) dependent on the injection schedule adopted.

1.3.2.2 Mechanisms for amphetamine disruption of LI: the 'switching' model of LI

The 'switching' model asserts that there are two models of LI i.e. one of LI disruption and one of abnormally persistent LI. LI is an acquisition deficit, which is characterized by an inability in acquiring the CS (tone) – reinforcement (shock) association as a consequence of non-reinforced procedures, thereby, decreasing the associability of the CS (tone) in predicting the reinforcer. However, LI disruption is not only dependent on the PE phase. Rather, according to the switching model, LI is a selection problem that involves learning conflicting contingencies i.e. a pre exposed phase accompanied by no reinforcement, and the conditioned phase accompanied by a reinforcer. The acquisition of information takes place in the PE and its expression in the COND. The cognitive switching phenomenon involves learning both the ability to ignore, and an inability to ignore, where certain

combinations in the number of PE's, number of conditioning sessions or context changes causes control rats to switch attentional mechanisms according to the appropriate stimulus-reinforcer contingency (Weiner 2003).

This model has been used to investigate the amphetamine induced disruption of LI and the neuroleptic reversal of amphetamine induced disruption of LI, in addition to LI potentiation by antipsychotic drugs (see Table 1.2). Furthermore, APD's block D2 receptors in the cortical regions, and mesolimbic DA system is critical in attribution of salience to DA (Kapur and Mamo 2003). APD's reverse amphetamine induced disruption of LI, under a low number of stimulus PE's, which does not lead to LI in control animals, as opposed to sufficient numbers of PE's which yield robust LI in control animals. This is dependent on the PE and COND parameters employed (Weiner 2003). APD's haloperidol (0.1 mg/kg), clozapine (5mg/kg) and Ritanserin (.6 mg/kg) administered in PE and/or COND, do not facilitate low level LI (40 PE). Using 5 conditioning trials and 40 PE's however, haloperidol and clozapine only facilitated the emergence of LI. Under conditions that did produce LI in controls, 40 PE and 2 conditioning trials, clozapine and ritaserine abolished LI(Shadach, Gaisler et al., 2000). This is consistent with studies in dopamine knockout mouse models, at low PE's (40) enhanced LI is seen in D1 female KO animals. Alternatively, D2R KO animals also show enhanced LI under these low PE's, with no LI in WT controls (Bay-Richter, O'Tuathaigh et al., 2009). The blockade of the dopamine neurotransmission promotes development of LI, and the enhancement of DA neurotransmission disrupts LI (Feldon, Barkai et al., 1990). DA agonist amphetamine and D2R receptor antagonist Haloperidol differentially mediate the expression of information in this switching context; AMPH mediates rapid switching in LI and haloperidol retards switching (Weiner,2003). Thus, amphetamine disruption of LI is

not only governed by the dose and schedule of amphetamine administration prior to

PE and COND, but also the number of pre exposures in the LI task.

LI effect	Compound	Dose Range tested	Dose effective	
Disruption of LI	d-Amphetamine	1 and 4 mg/kg, s.c.; 45 min prior to training	4 mg/kg	(Solomon and et al.,1981)
		2 injections X 1 mg/kg, 24 h and 15 min prior to PE, Cond and Test	Abolished LI in a single session	(Young 1992)
		1 mg, i.p. different CS stimuli used	1 mg/kg, but only with flashing house light	(Ruob,Elsner et al.,1997)
		1.5 mg/kg i.p; different PE durations of 3, 30 and 150 mins	1.5 mg/kg only at 30 PE duration	(De la Casa, Ruiz et al.,1993)
		.5,1.5, 3 i.p. mg/kg .5 mg/kg i.p.	Non significant LI attenuation	(Killcross, Dickinson et al.,1994)

	PCP	1 and 5 mg/kg prior to PE, Cond, or both	No effect	(Weiner and Feldon 1992)
		2.5 mg/kg i.p. (15 min pre treatment) or 8.6 mg/kg i.p (20 h pre treatment)	No effect at 2.5 mg/kg, abolished at 8.6 mg/kg	(Turgeon, Auerbach et al.,1998)
Reversal of amph induced disruption of LI	Haloperidol	0.2 and 0.5 mg/kg Against 1 mg/kg i.p. Amphetamine	0.2 and 0.5 mg/kg	(Warburton, 1996)
	Clozapine	1-10 mg/kg Against1 mg/kg amphetamine	2 and 5 mg/kg	(Moran, Fischer et al., 1996)

Table 1.2 Table showing LI disruption by amphetamine, and its reversal by neuroleptics, and neuroleptic induced potentiation of LI. Adapted from (Moser, Hitchcock et al., 2000)

1.4 PPI and schizophrenia

LI is a task of learned irrelevance that is used to model irrelevant attentional information that mimics attentional deficits in schizophrenia. Prepulse Inhibition is another attentional cognitive phenotype of relevance to schizophrenia. Rather than the ability to allocate attentional processes to ignore irrelevant stimuli, it is used as an index to 'gate' or filter information in schizophrenia. PPI is a measure of sensorimotor gating where the Acoustic Startle Reflex of an animal (ASR) is reduced, when the startling stimulus is preceded by a low intensity prepulse. Sensorimotor gating is a means to 'gate' the flow of information received from the environment that is processed by the brain, which schizophrenic patients are unable to do. More specifically, PPI is a measure of gating attentional and cognitive deficits that are present in schizophrenia. Disruption of this process in schizophrenia leads to an inability to process the "flood" of information and disorganized thoughts that are characteristic of the illness (Venables,1960). This measure of assessing startle gating possesses continuity across species, and has been observed in mice (Kokkinidis, 1986; Csomor, Vollenweider et al.,2008; Powell, Zhou et al.,2009), pigs (Lind, Arnfred et al.,2004) and humans. Deficits in PPI have been seen in drug naïve first episode schizophrenics (Ludewig,2003), stable outpatients and inpatients (Parwani, Duncan et al.,2000). These deficits have seen as impairments in the inhibitory mechanism in the acoustic startle response.

PPI measures salient sensitivity to a stimulus, and can be used to model sensorimotor gating deficits as seen in schizophrenia, on a schizophrenia relevant phenotype, disruptions in PPI can be induced by administration of DA agonists, and the ability of APD's to reverse of these disruptions can be modelled (Swerdlow,2008). PPI has

face, construct and predictive validity for illness (Swerdlow,1994). Face validity indicates that changes in PPI impairment in Schizophrenia in humans' parallels changes in PPI in rodent models; dopamine agonist induced disruptions in PPI, mimic PPI deficits in schizophrenia patients. This task also has predictive validity, where indirect dopamine agonist induced disruptions on PPI are reduced by treatment by typical or atypical antipsychotics, but not by psychoactive drugs that lack antipsychotic activity (Hoffman and Donovan 1994).Thus, PPI disruption holds predictive validity as it predicts the efficacy of antipsychotics (D2R-like antagonists) in reversing dopamine agonist mediated disruptions. Lastly, this measure has construct validity because disruptions on PPI are produced by dopamine agonists, serotonin 5HT-2A agonists and NMDA receptor antagonists which reproduce abnormalities implicated in schizophrenia (Geyer,Mark et al.,2001; Swerdlow, 2008).

The indirect DA agonist amphetamine facilitates DA release and causes disruptions of these gating processes in schizophrenia, and animal models of schizophrenia used to index PPI, mimic these amphetamine induced disruptions in PPI (Mansbach, Geyer et al.,1988; Ralph, Varty et al.,1999; Sills 1999; Ralph, Paulus et al., 2001; Tenn, Kapur et al.,2005). Furthermore, antipsychotic drugs reverse PPI deficits in schizophrenia patients (Meltzer, Park et al.,1999; Salimi, Jarskog et al., 2009). Typical antipsychotic drugs such as haloperidol and risperidone, atypical antipsychotics such as clozapine, and drugs that function as D2R-like antagonists are able to reverse PPI deficits in patients with schizophrenia (Hoffman and Donovan 1994; Geyer,Mark et al.,,2001; Varty, Walters et al.,2001). Thus, PPI is used as a cognitive phenotype for illness.

1.4.1 Dopaminergic modulation of amphetamine induced disruption of PPI

The direct nonspecific dopamine agonist apomorphine and indirect DA agonist amphetamine have been shown to disrupt PPI in rats and mice (Mansbach; 1988, Davis et al.,1990, Varty et al.,2001). Neither apomorphine (5 mg/kg) nor the more selective D1 selective receptor agonist SKF82958 (0.3 mg/kg) altered PPI in D1R KO mice, although both compounds disrupt PPI in D2R WT and KO mice, suggesting that the D1R alone might modulate PPI in mice. However, the NMDA antagonist Dizocilpine (0.3 mg/kg) induces similar PPI deficits in D1R and D2R KO mutant mice, confirming that the influences of the NMDA receptor on PPI are dependent on both D1Rs and D2Rs in rodents. Thus, both D1Rs and D2Rs modulate PPI deficits differently (Williams et al.,2002), suggesting that different drug-receptor interactions affect PPI differently in this mouse model. Robust PPI and acoustic startle have been seen in D2R, D3R and D4R KO mice (Ralph, Varty et al.,1999). However, administration of amphetamine (10 mg/kg), only disrupts PPI in D3 and D4 KO mice, but not in D2R KO mice., but amphetamine induced disruption of PPI in D2R (+/-) Heterozygous mice is seen(Ralph, Varty et al.,1999). Subsequent studies have reported amphetamine induced disruptions in PPI in D1R WT and KO mice and in D2R WT, but not D2R KO mice, suggesting that the D2R may not play a role in modulating PPI (Ralph-Williams et al.,2002). As reported previously, amphetamine (10 mg/kg) failed to disrupt PPI in D2R knock-out mice (Ralph et al., 1999), supporting a unique role of the D2R in the amphetamine disruption of PPI.

1.4.2 Other considerations: The influence of sex and strain on PPI

Sex differences have been seen in studies of PPI and the acoustic startle response (ASR). PPI is expressed as a percentage inhibition of the ASR magnitude. In humans, the administration of d-amphetamine attenuates the inhibition of the prepulse responses in both men and women (Hutchison and Swift 1999). Studies' report that women show less prepulse inhibition than men (Kumari, Gray et al., 2003). Females showed less PPI on a single prepulse paradigm as compared to men, but exhibited prepulse facilitation, when inter stimulus interval lasts over 50 msecs) on when two sets of prepulses were administered in succession. This effect may be mediated by protocol, and may also be subject to oestrogen and menstrual cycle changes (Chavez, Gogos et al.,2009). Menstrual cyclic changes affect mesostriatal dopamine activity; an increase in oestrogen levels is accompanied by a reduction in prepulse inhibition (Meziane,2007).

Other studies attribute time of day (circadian rhythms) rather than menstrual cyclic changes, in disrupting PPI in a sex specific manner (Adams, Hudson et al.,2008; Gogos,2009). In line with this, hormonal fluctuation in the four stages of oestrous cycle-proestrous, oestrous, metestrous and diestrous of the menstrual cycle only altered PPI magnitude in the BALB/By strain. More specifically, the behaviour of the C57 females was consistently stable despite oestrous cycle modulations in PPI and other behavioural tests when compared to the BALB/cByJ females (Meziane et al.,2007). This suggests sex specific differences in PPI may be strain specific (Paylor and Crawley, 1997;Taylor ,Markham,et al.,2011;Kilpatrick et al., 2010).

1.4.3 Low and high dose injections as well as aministration schedules affect amphetamine induced disruption of PPI

Previous studies show LI disruption by low, but not high doses of amphetamine at a 24h interval. This is attributed to dose dependent involvement of amphetamine with the mesolimbic and mesotriatal systems. The mesolimbic system has been implicated in low dose amphetamine mediated disruption of PPI. At low doses, amphetamine is coupled to impulse flow, and promotes augmented release of DA in the C57 mouse strain (Ventura et al.,2004). At high doses, amphetamine becomes uncoupled to impulse flow; and promotes preservative/restricted behaviours in this strain (Ralph et al.,2001).Consequently, dopamine depletion by 6-hydroxydopamine lesions in the nucleus accumbens, olfactory tubercles and anterior striatum reverse amphetamine induced disruption on PPI, but do not disrupt AMPH potentiation of ASR. As the ventral striatum is associated with these limbic structures, it is suggested that increased mesolimbic DA activity governs AMPH-induced disruption of PPI(Swerdlow, Mansbach et al.,1990) .

Disruptions in PPI are also affected by the time window between administration of injections and test sessions. Amphetamine affects the release of dopamine in the mesolimbic region between a 1-3 hour time window(Gold, Swerdlow et al.,1988). It is seen that amphetamine (0, 0.3, 1, or 3 mg/kg) attenuated PPI when tested 10 mins after amphetamine administration, and when the prepulse stimuli were 5 dB above 65dB of background noise. It is suggested that background noise influences amphetamine disruption of PPI; amphetamine dose dependently disrupts PPI when it is administered immediately after an amphetamine injection or ten minutes prior to

test, when the prepulses need to be at least 5dB above 65 dB background noise (Sills 1999).Consequently, a 3 mg/kg dose was found to effectively disrupt PPI when prepulses were 10db above background noise only. These disruptions were seen at the 10-40 min time window between amphetamine administration and test; at 40-70 delay between administration and test only a high dose of amphetamine disrupted PPI. At a 60-90 min time window between amphetamine administration and test, none of the doses of amphetamine disrupted PPI(Sills, Onalaja et al.,1998).Thus, longer delays between injection and test prevent amphetamine induced disruptions. It is suggested that PPI should be tested within a relatively narrow time window between injections and test, as a 40-60 min delay leads to a complete loss in the PPI attenuating effect as mediated by amphetamine (Sills,1999).

Sensitization studies indicate that PPI is also disrupted as a consequence of different numbers of injections of amphetamine over a number of days. Following an escalated dose regime (3 injections per day X 6 days), where amphetamine administration was increased from 1-10 mg/kg, disruptions in PPI were seen (Peleg-Raibstein, Sydekum et al., 2006). However, escalating doses from 1-3 mg/kg with 3 injections per day also disrupted PPI (Tenn, Kapur et al.,2005). These sensitization studies corroborate previous studies and suggest that low and high doses of amphetamine both modulate PPI differently.

1.5 Memory impairments in schizophrenia

Both LI and PPI are measures of cognitive function that are disrupted in schizophrenia and these disruptions may be induced by the aberrant functioning of the DA system. Deficits in memory are another subset of these cognitive impairments that are modulated by aberrant neurotransmitter function in

schizophrenia. Dopamine is involved in the cortical modulation of working memory in the PFC as well as in spatial memory, where DA agonists produce deficits in spatial memory and amphetamine mediated deficits on recognition memory are seen in rodents (Stefani and Moghaddam, 2002; Bisagno, Ferguson et al.,2003; Castner, Goldman-Rakic et al.,2004). Schizophrenic patients show deficits in multiple facets of memory i.e., working memory and memory for cued recall and recognition (Sullivan EV 1994; Joyce, Collinson et al.,1996; Hutton, Puri et al., 1998).They also show impairments on verbal and non verbal measures of long term memory, logical memory(Toulopoulou, Rabe-Hesketh et al.,2003), and episodic memory (Tendolkar, Ruhrmann et al., 2002; Bonner-Jackson, Haut et al., 2005).

Tests for retrospective memory are species specific and have been developed for birds, dolphins and rodents. The formation, storage, consolidation and retrieval of memories involves multiple substrates(Leavitt and Goldberg 2009), where complex interactions between the glutamate and dopaminergic systems mediate the formation and storage of these memories (Rushe, Woodruff et al., 1999; Arco and Mora,2009). Memories for remembering 'what, where, and when' (memory for episodes) have involved the caching and recovery of food in scrub jays (Clayton and Dickinson 1998,Dere et al.,2006) as well as food preference tasks in non human primates(Hampton, Hampstead et al.,2005). These models of declarative and reference memories place emphasis on hippocampal connections underlying the formation of memories(Castner, Goldman-Rakic et al.,2004).

1.5.1 The role of dopamine and glutamate neurotransmission in mediating memory in schizophrenia

1.5.1.1 Neuroanatomy of episodic memory

The pre frontal cortex is involved in the encoding and retrieval of memories(Nyberg et al. 2003, Israel 2010, Wheeler, Stuss et al. 1997) and contributes to the formation of these memories. For instance, the dorsolateral prefrontal cortex (DLPFC) integrates information provided by sensory and motor systems in mediating working memory and executive functioning, as well as long term memories and encoding. Prefrontal dysfunction in an episodic memory task has also been seen in schizophrenics (Ragland, Gur et al., 2001). Alternatively, the dorsomedial PFC may play a distinct in episodic memories by processing information that is relevant to the self and self encoded personality, and initiating a concept of memory based on one's experience(Brand 2008). Other studies support PFC involvement in mediating recognition memory for objects, PET studies have shown decreased cerebral blood flow in the PFC during the recognition of new objects and greater increases in cerebral blood flow in the left PFC in the recognition of objects that they had previously experienced (Heckers, Curran et al., 2000).

Both prefrontal and hippocampal activation has been seen in a test of episodic retrieval in schizophrenia; increased regional cerebral blood flow in the DLPFC and reductions in hippocampal activation of the conscious recollection memories has been seen in a schizophrenic cohort (Heckers 1998). This suggests, there is a dissociation in the encoding and retrieval of memories by the hippocampus and PFC in episodic memory (Dietrich 1998;).

1.5.1.2 Pharmacology of Episodic Memory: Rodent Studies

Evidence for hippocampal mediation of memories and novelty detection has also been reported (Ennaceur, Neave et al.,1997; Mumby,2002,). The hippocampus is involved in memories for actions and places that make up discrete events and also in the sequential organization of these memories (Eichenbaum,2004). This encompasses both semantic and episodic memory, but not the disposition of priming, learning of a skill set or conditioning to a test of learning and memory (Squire 1992). The hippocampus also incorporates temporal information from the frontal lobes, and provides a basis for the episodic memory function. This is demonstrated on a one trial paired association task where rats were required to distinguish for food flavour (what) and place (where), to determine the correct location of the food, when paired with its associate flavour. Hippocampal blockade of glutamatergic NMDA receptors affects the memory for paired associates (recall) (flavour-place) but not retrieval. However, upon an 8 week training schedule in rats with glutamate AMPA receptor blockade, recall was not affected (Day, Langston et al.,2003). Encoding for what-where glutamate mediated memories in paired associates is found to be impaired following hippocampal lesions (Burgess, Maguire et al.,2002). These studies suggest glutamate dissociates in mediating memory disruption depending on the components of cued recall and memory that are tested.

Complex interactions between the DA and glutamate systems underlie episodic memory deficits in schizophrenia in the hippocampus and cortical regions. The hippocampus receives DA enervations from the mesolimbic DA region, and regions such as the CA1, are abundant in D1R-like an D2R-like receptors (Csernansky, Kerr et al.,1988). The hippocampal and DA relationship is marked by interactions in reward mediated behaviour; DA acts as predictor for rewards, both in the unexpected

occurrence as well as the omission of a reinforcer in signalling these rewards(Schultz,2004) . Thus, in an object recognition /episodic memory task, novelty may act as intrinsic reward and motivate exploration (Schott,2008). Glutamatergic- dopamine interactions in the mesolimbic regions do not modulate these memories alone, but dissociation in the retrieval and encoding of these memories is seen in the frontal and striato-limbic regions, owing to altered DA transmission. This suggests that disruptions in hippocampus mediated recognition memories may be indirectly modulated by dopamine receptor function in the cortical regions.

1.5.2 Episodic memory in DA mutant models

Episodic memory is measured in terms of object discrimination. The typical task of object recognition memory consists of one trial object recognition, where rats are familiarized with a familiar old object (sample phase) and then tested with a copy of this familiar object, along with the addition of a novel object (test phase). Animals spend a longer period of time exploring the new object as compared to an old object (Ennaceur and Delicious, 1988).Episodic memory in mice however, has been measured using a modified version of the object recognition task , that incorporates a temporal component This is different from the object recognition task as it encompasses when the object was presented at a particular location, at a certain point in time. This task of episodic memory measures the what, where and when components of memory.

Additionally, individual components of the memory for 'what', 'when' and 'where' have been assessed as separate tasks to measure memories for object recognition, place recognition and recency (Dere et al.,2005,2006;). For example, the object

recognition task has been used in rats to explore two equal objects in a two trial design (sample trial- test trial) in which animals are presented with a novel object. Rodents spend more time exploring the novel object, which thus indicates that a familiar object was recognized; this measures the 'what' component of memory. Another modification of the task measures the memory for a change in place of an object. Two familiar objects are presented in one location in the sample trial, and one copy of the object is displaced in the test trial. Here, animals spend more time exploring the object in the new location than the old location (Bussey, Muir et al., 1999).Another variation of this task is the memory for temporal order that measures the 'when' component for two objects that were presented in the past. This consists of a two phase sample trial, where two copies of a familiar object are presented (A) in the first sample trial, and two copies of a second new object are presented (B) in a second sample trial. In the test trial, two copies of each object A and object B are presented. Animals spend more time exploring the familiar old object (A) as opposed to the newer object (B), based on the regency of their occurrence (Hansson, Howland et al.,2004).

Both the D1 and D2 receptors have been implicated in memory disturbances in schizophrenia (Abi-Dargham, Malawi et al.,2002). The D1 receptor has been implicated in working memory and spatial memory deficits (Zahra, Taylor et al., 1997; Castner, Williams et al.,2000; Abi-Dargham, Malawi et al.,2002). It is also involved in both temporal order and place order memory. Administration of a high dose of D1-like agonist facilitates memory retrieval, after a 4h delay and the administration of both a low and high dose of D1-like agonist impairs memory after a short 1h delay in a temporal order recognition memory task (Hotter, Naudon et al., 2005).

A dissociation exists between dopamine receptor subtypes in mediating memory processes behaviours; the D2R stimulation spares memory on a delayed alteration WM tasks, whereas D1 receptor stimulation promotes disruptions on delayed alteration WM and spatial memory tasks in rats(Zahrt, Taylor et al.,1997). It is suggested that the D2R affects the consolidation and retrieval of memories by increasing D1 receptor turnover in the medial PFC, and thus also promotes behavioural inflexibility in attentionally mediated tasks of working memory (Kellendonk, Simpson et al.,2006). Switching off the D2 over expression in adult animals does not reverse cognitive impairment on working memory tasks, and this has been primarily attributed to D1 receptor imbalance in the PFC. This indicates that the D2R may indirectly mediate memory disruptions in the consolidation and retrieval of delay dependent memories schizophrenia.

Both the D1R and the D2R are implicated in reward mediated behaviours and spatial learning. Pre reward oriented excitatory responses are governed by the D1R and mediate reward oriented memory processes, as indicated by mice lacking the D1R that show spatial memory and incentive reward related deficits(Tran, Tamura et al., 2005). This indicates the D1R is required for reward mediated spatial memories. D2R KO mice show slower learning of place recognition and show partial alteration in their coding of spatial information to an open field to rewarding stimuli ,as well as slower acquisition of place reward associations (Tran, Tamura et al.,2003). Additionally, spatial memory deficits have been reported in rats administered a DAD2R antagonist raclopride (Wilkerson and Levin 1999) and delay interval dependent disruptions are seen in D2R KO mice(Glickstein, Hof et al.,2002).This indicates that D2R is required for spatial memory.

Neuregulin-1 and cognitive impairment in schizophrenia relevant phenotypes

1.6 Neuregulin-1 and schizophrenia

A number of genes that have been implicated in risk for schizophrenia i.e.Nrg-1, DISC-1, Catechol-o-methyl transferase (COMT), DA (Holmes, Hollon et al., 2001; Shifman 2002; Shen, Lang et al., 2008) and have been investigated in behavioural phenotypes relevant to the illness. More specifically, mutant mouse models lacking a single or entire functional copy of these gene exhibit behavioural abnormalities that are related the characteristics of schizophrenia. These include hyperactivity, isolation rearing and sociability, and PPI (Mohn,1999; Geyer, Mark et al.,2001; Clapcote, Lipina et al.,2007; O' Tuathaigh, et al.,2007). Studies investigating the role of these candidate genes associated with risk for schizophrenia do so using schizophrenia relevant phenotypes in animal models of schizophrenia where aberrant gene function leads to deficits in cognitive behavioural tasks. We investigated the involvement of one such candidate allele, Neuregulin-1 (Nrg-1) as an at risk gene for Schizophrenia(Stefansson H. 2002) in mediating schizophrenia like disruptions on schizophrenia relevant cognitive tasks.

1.6.1 What is the Neuregulin-1 gene?

Neuregulin-1 (Nrg-1) has been identified as a candidate gene for schizophrenia, originally shown by way of a genome wide scan in an Icelandic population, where it

was known to map onto the 8p12-p21 locus (Stefansson H. 2002; O'Tuathaigh C.M.P 2009).This association of Nrg-1 to a high risk for developing schizophrenia has been replicated in the Han Taiwanese, Portuguese, as well as the British/Irish population (Petryshen, Middleton et al.,; Williams, 2003; Hong, Huo et al., 2004; Hong, Wonodi et al., 2008). Nrg-1 is a protein encoded by the Nrg-1 gene which plays a vital role in neuronal plasticity and development in the adult brain (Inta, Monyer et al.,2009). It has multiple isoforms Nrg types I-VI, that are implicated in schizophrenia (Hashimoto, 2004). Nrg-1 type I,II and III are part of an epidermal growth factor like domain, which consists of the Immunoglobulin domain (Ig), the trans- membrane domain (TM) and proteins that signal through the its receptor; ErbB 2,3,4 receptor tyrosine kinases (Corfas, Roy et al., 2004). Nrg-1 signals primarily by being released as a soluble protein that binds to the ErbB receptor family, or as a transmembrane (TM) protein- like domain which activates the target cell receptors via cell to cell contact (Corfas 2004). Preferential binding to the ErbB4 receptor also modulates glutamatergic receptor activity where type I,II and III are the major constituents (Kwon 2005; Li, Woo et al., 2007).

1.6.1.1 Nrg-1 and its role in developmental processes

Nrg-1 plays a primary role in regulating key developmental processes which include synapse formation, cardiovascular functioning, neuronal migration, myelination, dendritic growth as well as long term potentiation, all of which are key processes in neuronal development (Harrison and Weinberger 2005; Lemmens, Doggen et al., 2007; Desbonnet, 2009). The contribution of Nrg-1 in these developmental processes indicates that the aberrant functioning of this gene prior to adulthood may manifest as impairments at different stages of maturation, and promote disruptions in tasks of

social learning, thus mimicking social withdrawal and avoidance behaviours characteristic of the negative symptoms of psychosis (Walsh, McClellan et al., 2008). Consequently, neonatal mice that were administered the Nrg-1β1 protein (which binds to the ErbB4 receptors) show elevations in dopamine and dopamine metabolism, as well as impairments' on PPI, LI and social interaction tasks when in adulthood (Kato, Abe et al.,2010). Amongst other behaviours, exploratory hyperactivity has also been seen in TM domain heterozygous (+/-) Nrg-1 mice. This exploratory activity was inhibited in animals housed in a deprived environment as opposed to animals housed in an environmentally rich housing environment (Karl, Duffy et al.,2007). Thus, suggesting the involvement of Nrg-1 in Schizophrenia relevant phenotypes.

1.6.1.2 Nrg-1 and phenotypes relevant to schizophrenia

Nrg-1 plays a role in behavioural phenotypes associated with schizophrenia. Nrg-1 TM domain mice hypomorphic for this gene show hyperactivity, deficits in novel object recognition and PPI (Mohn, Gainetdinov et al.,1999; Duffy, Cappas et al., 2010; Kato, Abe et al.,2010). Consequently, increases in both exploratory activity and social dominance related behaviour have also been seen in Nrg-1 TM domain animals (O'Tuathaigh, Babovic et al.,2007; Moy, Troy Ghashghaei et al.,2009; O'Tuathaigh, Harte et al., 2010). Thus, schizophrenia relevant phenotypes have been observed in mice hypomorphic for the Nrg-1 gene. Additionally, mutant mice with partial deletions of epidermal growth factor like (EGF) domains show disrupted mismatch negativity and reduced sociability, which is a similar behavioural profile to the TM domain mice (Duffy, Cappas et al.,2008). This indicates that Nrg-1 and its isoforms play a role in schizophrenia phenotypes.

Altered Type III Nrg-1 signalling leads to functional deficits that are related to schizophrenia. These include dendritic arborization, which promotes abnormal cortical plasticity thus leading to altered neural circuit connections (Pederique and Farazzi, 2010,Chen et al. 2010). Altered expression of type 1 and type IV isoforms of Nrg-1 are seen in schizophrenia, and in alleles implicated at a high risk for schizophrenia (Harrison and Law, 2005). Nrg-1/ErbB$ signalling is involved in the wiring of cortical inhibitory circuits, and modulates synapse formation in these circuits (Rico and Marin,2011). However, little is known about how aberrant TM function of Nrg-1 isoforms disrupts different neurotransmitter systems. It is suggested that abnormalities in Nrg-1 functioning may lead to altered excitatory or inhibitory signalling of glutamate, GABA or acetylcholine receptors, which affects neuronal plasticity, and resembles altered cortical connectivity in schizophrenia. For Neuregulin is also thought to play a critical role in GABAergic signalling (Russig, Murphy et al., 2002; Gajendran, 2009) that may impact cognitive processing and thus attribute to schizophrenia-like symptoms (Corfas,2004).

Enhanced Nrg-1 ErbB4 function blocks NMDA receptor activity in the human PFC and rodent cortex (Hahn, Wang et al., 2006), thus modelling NMDA hypofunction like phenotype as seen in schizophrenia. Heterozygous (Het) TM domain Nrg-1 mice habituate readily to a novel environment compared to their WT littermates, and on acute challenge with MK 801 show disruptions on PPI (Duffy, Cappas et al., 2008). Nrg-1 also reverses long term potentiation of hippocampal glutamatergic excitatory synapses via the activation of dopamine D4 receptors (Kwon, Paredes et al., 2008), and The Nrg-1 ErbB4 receptor is implicated in the maturation of glutamatergic synapses(Li B Woo,2007).Administration of glutamate antagonists Dizocilpine (MK-801) or Phencyclidine (PCP) induces reduced sociability and preference for social

novelty, in both TM domain Nrg-1 mutant and wild type animals. Alternatively, untreated animals showed increases in both exploratory activity and social dominance related behaviour (O'Tuathaigh, Babovic et al.,2007; Moy, Troy Ghashghaei et al., 2009; O'Tuathaigh, Harte et al.,2010). This indicates that glutamate may modulate Nrg-1 mediated disruptions on schizophrenia relevant phenotypes. However, these studies do not imply a causal relationship between Nrg-1 signalling and glutamate and GABA systems. Rather, they suggest a complex relationship may exist between Nrg-1 isoforms and inhibitory glutamate and GABA signalling, that may not generalise to non ErbB receptor coupled proteins.

1.7 Nrg-1 and LI

Nrg-1 mediated is involved in the processing of stimulus salience (O'Tuathaigh, 2003), but Nrg-1 is thought to be involved more in identifying novel and unfamiliar stimuli, rather than their discrimination on prior exposure to the stimuli (De Leonibus, Verheij et al., 2006).

Nrg-1 gene has also implicated in modulating cognitive tasks that act as behavioural phenotypes of schizophrenia, but disruptions on these tasks as a consequence of altered Nrg-1 functioning have not been investigated in attentionally mediated cognitive phenotypes of relevance to schizophrenia.

Nrg-1 Ig domain mice have shown deficits in LI. Ig domain hets show impaired LI as compared to their wild type (WT) littermates, but the lack of control groups in this study deems it inconclusive (Rimer, Barrett et al.,2005). To date, studies in LI in TM domain Nrg-1 mutants have not been carried out. The Nrg-1 is a relatively novel gene, with respect to its association with high risk for schizophrenia, and studies

have not looked at the role of all isoforms of the Nrg-1 gene as behavioural correlates of schizophrenia pathophysiology. Furthermore, an LI paradigm that uses suppression of behaviour as an index of learning has not been employed in these mutant mice, and there is no evidence of paradigm specific deficits in LI. This is of importance as transgenic lines of the Nrg-1 mouse differ in their phenotype and performance on behavioural tasks depending the Nrg-1 isoform mutations; ErbB4 receptor coupled, or TM domain knockout mice (Mei and Xiong, 2008).

1.8 NRG-1 and PPI

Reduced PPI has been observed in humans carrying the mutation of the single nucleotide polymorphism Rs3924999 of the Nrg-1 gene, where schizophrenic patients with abnormal PPI show an over expression of this mutation, as compared to controls(Hong, Wonodi et al., 2008).

A study investigating the PPI deficit in TM domain Nrg-1 mutants, found no effects of PCP or MK-801 or amphetamine on PPI or startle habituation or locomotor activity in these animals. Only treatment with a 5HT-1a receptor agonist 8-OH-DPAT disrupted PPI in Nrg-1 Hets (van den Buuse,2009), supporting the lack of a generalized impairment in Nrg-1 mutants of different isoforms. However, other studies show that MK-801 and PCP attenuate disruptions in PPI dose dependently (Yee 2004). Studies have shown PPI deficits in mice lacking glutamate receptors (Bubenikova, Vera et al.,2008, Duncan, Moy et al.,2004). However, interaction with the glutamate systems is limited to ErbB2,4 receptor Nrg-1 proteins as previously mentioned, and little is known about other isoforms. PPI disruptions have not been consistently replicated in Nrg-1 mutants.

Furthermore, PPI in TM domain mutants is sensitive to changes in protocol; no deficits are seen in PPI in Nrg-1 Het mutants compared to their WT littermates; however, reduced baseline startle is seen only at high levels of startle (110db, 115 db,120 db), in a site specific protocol(Karl 2011). The reliability of the Nrg-1 TM domain mutants in mediating PPI task is not clear, as a replication of the original study showing disrupted PPI in Nrg-1 mutants(Stefansson H. 2002) has not been produced to date.

1.9 NRG-1 and episodic memory

The role of Nrg-1 in mediating memory disruptions relevant to schizophrenia has been investigated in TM domain Nrg-1 mutants. Tests of spatial learning and working memory in report intact memory processes in Nrg-1 mutant mice, as assessed by the Barnes maze and Y mazes (O' Tuathaigh et al., 2007). Furthermore, spatial memory is found to be intact in these Het mutants mice with even better retention of spatial memory following brain trauma (Lok,2007). This suggests spatial working memory is intact in Het Nrg-1 mice. Other studies also support this notion of intact spatial learning and working memory in the Nrg-1 TM domain mutants (Duffy, Cappas et al.,2010).Deficits in Het mutants are only seen on tests of contextual fear conditioning, cued aversion and novel object recognition in these mice (Duffy, Cappas et al.,2010).

 Nrg-1 mutant mice with partial deletions of epidermal growth factor like (EGF) domains on all three forms (Type 1,2,3) of the gene show disrupted mismatch negativity and reduced sociability(Enrichment, Luminais et al.,2009). To date, TM domain Nrg-1 animals show deficits social novelty oriented exploratory behaviours(O'Tuathaigh et al.,2006) and novel object recognition (O' Tuathaigh et

al.,2008). This indicates Nrg-1 involvement in socially relevant behaviours and social novelty.

Thus, it follows that novelty oriented tasks may be good constructs to test deficits in recognition memory in Nrg-1 mutants. A complete test of episodic memory (what, where, when) using object recognition has not yet been conducted in TM domain Nrg-1 mutants. This is a coherent test of memory that measures the spatio-temporal aspects of memory and encompasses the consolidation and recall of memory for an object in a particular location and at a particular point in time. Thus, it measures the memory for particular episodes that are not governed by semantic rules or knowledge but rather the memory for what object was investigated, where it was seen and at what point in time it was seen. This would be a good construct of both partial, temporal and recognition memory in Nrg-1 mice, as it provides consistent delay intervals between phases and adequate training provide robust effects , and act as phenotypic indices for episodic memory impairment in schizophrenia.

1. 10 Other considerations

Sex specific differences in Schizophrenia phenotypes are seen in Nrg-1 TM domain mutants. Decreased PCP induced hyper locomotion in male mice Heterozygous for the Nrg-1 gene as compared to female Het animals is seen(O'Tuathaigh, Harte et al., 2010). Furthermore, reductions in preference for social novelty (time spent in chamber with stranger 1 or stranger 2) and sociability (preference in time spent in a chamber containing a mouse as opposed to an empty chamber) are seen, as well as in dyadic social behaviours (time spent in stranger 1 vs. stranger 2 chamber, switched; novel chamber- unfamiliar mouse; opposite empty chamber-familiar mouse). Overall levels of chamber entries are markedly increased in female Nrg-1 mutants than

males. Moreover, locomotion, rearing and exploratory behaviour are attenuated over long time intervals, in Nrg-1 mutant mice, as compared to the WTs (O'Tuathaigh, Harte et al., 2010). Thus, indicating deficits for schizophrenia relevant phenotypes in male mutants Heterozygous for the Nrg-1 gene. The authors propose that shifting of exploratory behaviour was increased in female mutants but reduced in male mutants; where a reduction in grooming behaviour was observed in the male mutants only. Hence, these studies suggest that the partial knockout of a functional copy of the Nrg-1 gene, dissociates for behavioural deficits in schizophrenia like phenotypes in male Nrg-1 mutants only, where Nrg-1 mediates sex specific dissociations in disrupted behaviours as a consequence of the genetic mutation itself.

1.11 Thesis Objectives

The studies undertaken in the thesis seek to investigate animal models of attention and cognitive deficits implicated in schizophrenia. The role of aberrant neurotransmitter function can be tested using transgenic animal models lacking functional copies of candidate genes associated with schizophrenia pathophysiology. The candidate genes used to investigate cognitive and behavioural impairments in schizophrenia consisted of the dopamine D2 receptor (D2R) (-/-) and the Neuregulin-1 (Nrg-1) (+/-) mutant mouse models. The D2 receptor plays a major role in schizophrenia, by affecting both striatal and mesolimbic DA neurotransmission. Increased transmission of the dopamine system in the striatal region promotes Schizophrenia symptoms, and as indirect DA agonist Amphetamine has been known to induce these psychotic like symptoms in healthy humans, and worsen these symptoms in schizophrenia patients(Seeman 1987). Moreover, disruptions in paradigms of attention and cognition as a consequence of altered DA neurotransmission have been seen in tasks of inattention and gating such as Latent Inhibition (LI) and Prepulse Inhibition (PPI), and have been used as behavioural correlates of schizophrenia. D2R -/- mice show a reduced DA tone and have proved to be useful models to understand the function of D2 receptors in behaviours and antipsychotic drug effects that are relevant to schizophrenia.

Disruptions on cognitive tasks have been induced by Amphetamine, which is an animal model of hyperdopaminergia. Amphetamine causes an elevation of DA and promotes schizophrenia like psychotic symptoms, as a consequence of increased DA neurotransmission. Low doses of amphetamine promote psychotic like effects in humans and rodents, whereas high doses promote preservative/stereotyped

behaviours. Amphetamine induced deficits in cognitive and behavioural tasks have been used as pharmacological models of schizophrenia, as these disruptions can be reversed by D2 antagonist drugs. High doses of amphetamine promote stereotypy and preservative behaviours, and low doses produce psychotomimetic effects in humans and rodents. Administration of amphetamine affects attention, sleep and sensorimotor gating(Gainetdinov 2001). Studies using dopamine D2 receptor null mice suggest that the D2R is necessary for amphetamine-induced disruption of LI and PPI (Weiner, Bernasconi et al.,1997; Ralph, Varty et al.,1999). These studies suggested that both the number of injections as well as high and low doses of amphetamine can have dissociable behavioural and neural effects in these tasks.

We investigated whether a single low dose of amphetamine disrupts PPI, and whether this is dependent on the amphetamine administration schedule adopted. To determine the relevance of this receptor subtype in mediating amphetamine induced disruptions in mice, we used 'knockout' mouse models to elucidate whether knocking out the D2 receptor abolished the disruptive effects of amphetamine on these tasks. As D2 -/- mice show deficits memory tasks i.e. in spatial memory (where) and novel object recognition (what), a test of episodic memory deficits that incorporated memory for 'what', 'where' and 'when' was also established for the first time in these mice, thus mimicking episodic memory deficits seen in schizophrenia patients.

We investigated all three schizophrenia relevant tasks in the Nrg-1 mouse model, as Nrg-1 has been associated as a candidate gene for Schizophrenia (Stefansson H. 2002; Tosato, Dazzan et al.,2005). This is a relatively novel mutation that has been associated with schizophrenia and schizophrenia like phenotypes(Gerlai, Pisacane et al.,2000), and these behavioural tasks have not been carried out in the TM-domain

Nrg-1 Het (+/-) mouse model. Homozygous or complete deletion of the Nrg-1 receptor is lethal in these mice(Sanchez-Soria and Camenisch 2010), and therefore we investigated whether partial 'knockdown' i.e. lacking one functional copy of the allele (Heterozygous mice), mimics cognitive deficits in schizophrenia. The tasks used are LI, PPI and Episodic memory, which have been repeatedly used in DA mutant mouse studies. Using these models, we seek to elucidate whether mutations in TM domain of the Nrg-1 gene produces cognitive and attentional deficits in behavioural phenotype relevant to schizophrenia.

CHAPTER 2

PREPULSE INHIBITION (PPI) IN D2R WT and KO

ANIMALS

2.1 Introduction

PPI is a measure of sensorimotor gating where the startle reflex of an animal is reduced when the startling stimulus is preceded by a low intensity prepulse. It is a good test for measuring the processing of information in schizophrenia, as patients with schizophrenia show deficits on tasks that require the gating of information (Braff 1993).PPI is a robust phenomenon that has been seen in different strains of mice and rats(Ralph and Caine,2005). Mice lacking the dopamine transporter show deficits in PPI(Ralph, Paulus et al.,2001).Furthermore, decreased startle in PPI in D2R KO mice has been seen (Geyer 1999).This indicates that PPI is DA receptor dependent. Consequently, the D1 receptor is required for DA agonist induced disruptions in PPI, but D2R involvement in these disruptions remains inconclusive (Doherty, Masten et al.,2008).However, disruption of PPI by dopamine agonists is species specific i.e. non-selective DA agonist Apomorphine disrupts PPI and leaves acoustic startle unaffected in rats, where D1R antagonists reverse apomorphine induced disruption of PPI. However, D2R antagonist raclopride leaves this unaltered (Williams,2002).

DA releasing drugs like amphetamine disrupt LI and dopamine D2R antagonists potentiate LI (Weiner, Feldon et al.,1987; Weiner, Lubow et al.,1988). At low PE's (40) enhanced LI is seen in D1R female KO animals. D2R KO animals also show enhanced LI under these low PE's, with no LI in WT controls (Bay-Richter,

O'Tuathaigh et al.,2009). Two low doses of amphetamine administered 24 hours apart have been shown to disrupt LI in both D2R WT and KO mice.

The indirect dopamine agonist amphetamine induces a range of behavioural effects that are reversed by antipsychotic drugs e.g. hyperactivity, attentional (LI), prepulse inhibition (Kelly, Rubinstein et al., 1998; Ralph, Paulus et al.,2001).Amphetamine induced disruption of PPI is prevented in mice lacking the D2R, but not in mice lacking the D3 and D4 receptors(Ralph, Varty et al.,1999). High doses of amphetamine (10 mg/kg) decrease PPI in both D1R WT and KO mice, and D2R WT but not D2R KO mice(Ralph-Williams, Lehmann-Masten et al.,2002). A very high dose of amphetamine disrupts PPI; although these effects are prevented in D2R KO mice (Ralph et al.,1999).At low doses it promotes disruptions in LI, which are not blocked in D2R KO mice (Bay-Richter, O'Tuathaigh et al.,2009).Prior studies in rats suggest that amphetamine induced disruption of LI depends on the drug administration schedule adopted. A single low dose of amphetamine (1.5 mg/kg) administered 15 mins prior to the pre exposure and conditioning stages at a 24h interval only, disrupts LI (Weiner, Lubow et al.,1988).The time delay is critical in the disruptive effects of amphetamine on LI as a single low dose injection but not two injections given 30 mins prior to PE and 30 min prior to COND disrupts LI. Other studies that suggest a single low dose of amphetamine (.32 mg/kg, sub cutaneously) 30 mins prior to PE and COND phases attenuates LI(McAllister,1997). Consequently, amphetamine administered 45 -90 mins prior to the conditioning phase only, but not 15 mins prior to it, also abolishes LI (Young, Moran et al., 2005). The following studies investigated whether a low dose of amphetamine disrupts PPI in D2R KO and WT animals, and whether like LI the schedule of amphetamine administration is important in mediating disruptions in the PPI task.

2.2. Materials and Methods

2.2.1 Animals

The original F2 hybrid strain (129/Sv x C57BL/6J) containing the mutated DRD2 receptor allele were generated as reported previously (Kelly et al.,1997). The targeted gene deletion was constructed in 129/Sv embryonic stem cells and male chimaeras mated with C57BL/6 females to produce Heterozygous mutants (DRD2 +/-). Congenic DRD1 and DRD2 lines were established by backcrossing heterozygous mutants to wild type C57BL/6 for 14 generations. Homozygous KO mice (DRD2 -/-) and wildtype (DRD2 +/+) littermates were bred by heterozygous intermatings of the congenic Heterozygote mutants imported from Royal College of Surgeons, Dublin, Ireland. Congenic strains have significant advantages over mixed background strains in reducing inter-animal variability and maximising the specificity of the phenotype to the targeted gene (Kelly et al; 1997). DRD1 -/- mice had low body weight and poor growth and were weaned later than DRD2 -/- mice to facilitate growth and survival. They were given a wet mash diet in the home cages after weaning; this has been reported previously in these mice (Doherty et al., 2007). Male and female KO (n=28) mice and wildtype (WT=29) littermates were between 16-24 weeks old prior to being used in these experiments. Animals were housed 1-4 per cage under a 12 h light: 12 h dark cycle. Mice were housed at constant temperature of 22 degrees and 45% humidity controlled environs, with food available freely. Mice were housed 1-4 per cage under a 12 h light: 12 h dark cycle (lights on 07:00 hrs), constant temperature (20+/-C) and humidity (40-60%) with food available *ad libitum*. Experiments were performed during the light period. Mice were subjected to daily water restriction periods of 23 h throughout the LI experiments with one hr free access to water in their home cages

after the experimental session. All experiments were carried out in accordance with local and national rules on animal experimentation, and with appropriate personal and project licence authority under the Animals (Scientific Procedures) Act, UK 1986. UK home Office Project licence No: 40/2883.

2.2.2. Behavioural Testing

2.2.2.1 Drugs

d-Amphetamine sulphate (Sigma –Aldrich, Gillingham, UK) dissolved in 0.9% saline and administered at a dose of 2.5 mg/kg. Injections of 2.5 mg/kg amphetamine or saline were administered intraperitonealy (i.p.) 30 mins prior to behavioural testing at a volume of 10 ml/kg. This low dose of amphetamine was based on previous LI studies conducted in the laboratory, that sufficiently produced disruptions in LI. Animals were administered one single low dose amphetamine injection thirty minutes prior to the PE stage and thirty minutes prior to the COND stage, that were separated by a 24h interval. Animals were drug naive during the test session.

2.2.2.2 Apparatus

Two startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used to measure startle reactivity. Each chamber consisted of a sound attenuated, lighted and ventilated cabinet holding a single Plexiglass chamber (5 cm inner diameter). All acoustic stimuli were produced by a high frequency loudspeaker mounted inside the chamber. In order to ensure consistent stabilimeter sensitivity, a calibration system was employed across both chambers. A high frequency loudspeaker produced continuous background noise of 65db, in addition to the acoustic stimuli. Vibrations of the plexiglass cylinder caused the whole startle response of the animal to be

recorded as analog signals, which were then stored by a computer. Eighty four readings were taken at stimulus onset, and the during the entire test session. Each session had a block of 6 120 db pulses interspersed with 68dB, 72dB, 80dB and 90 dB prepulses. The SR-LAB calibration unit was used to ensure consistent stabilimeter recordings between test chambers over time (Fig. 2.1). Sound levels in dB were measured as previously defined (Dulawa et al.,1997).

Figure 2.1 A PPI test chamber. The mouse is placed in a plexiglass chamber, and the startle response of the animal to a series of loud prepulses is recorded within the chamber.

2.2.2.3 Acoustic startle session/ PPI

The PPI test session consisted of a one day session of startle trials (PULSE ALONE) and prepulse trials (PREPULSE+PULSE), intermixed with no-stimulus trials (NOSTIM). The pulse alone trial consisted of 40 ms of 120-db pulses of noise. PPI

was based on acoustic prepulse intensities that consisted of noise prepulses 68db, 72db, 80db and 90 db presented for 20 ms (3, 7, 15, 25 dB above 65 db background noise). After the mice were placed in the startle chambers, a 65 dB background noise level was presented for 10 min acclimation period and continued throughout the test session. Each session began with blocks of 6 and ended with another block of 6 PULSE ALONE trials, which were used for measuring habituation andwere not included in the analysis of PPI or mean acoustic startle response. In between these two blocks, each trial type (pulse alone, prepulse+pulse for the four prepulse intensities) was presented 12 times in a pseudo-random order (see Fig. 2.2). The NO STIM trial consisted of background noise only. There was a delay of 7 sec between trials. Two startle chambers were used for testing, and each animal was always tested in the same startle chamber in the two day session.
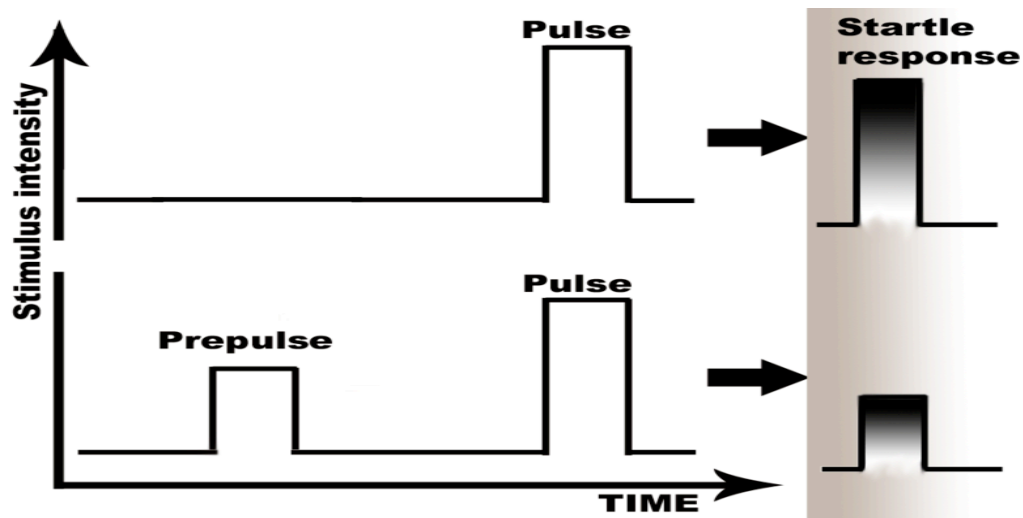

Figure 2.2 Diagrammatic depiction of the PPI task. The amount of startle to the pulse alone, interspersed with prepulses is measured. PPI is measured as percentage inhibition of the startle response to the prepulses.

2.2.2.4 Data Analysis

PPI was calculated as a mean percentage score for each prepulse+pulse trial, where % PPI= [(Mean of startle to the120 dB pulse- Mean % 68/72/80/90 dB pulse)/(mean 120 pulse) X100]. The ASR to the 120 dB pulse alone trials (excluding block of 6 at beginning and end) was also analyzed. Habituation to the 120dB tone was calculated as: (Mean initial 120dB startle-mean 120 dB startle)/ mean initial startle*100

This habituation was a measure of baseline startle and was based on the initial block of six 120 dB pulses at the beginning and block of six pulses at the end of the session. All scores were expressed as percentages.

Percentage PPI was analyzed using ANOVA to compare differences in startle to prepulses in WT and KO animals. The computations were carried out using SPSS statistical software. % inhibition to each prepulse intensity (68 db, 72 db, 80 db and 90 db) was the within subjects factor. Genotype (WT/KO) and treatment (Amphetamine / SALINE) were between groups factors. Prepulses were not administered in a particular sequence, but in a random order in the session. Two cohorts of animals were merged in this experiment to create a complete database with sufficient sex, genotype and drug matched controls. A repeated measures ANOVA indicated no significant effect of cohort on the data sets ($p > 0.05$)

2. 3 Results
Effects of experiment (dataset 1 vs. dataset 2) in D2R WT and KO animals

A repeated measures ANOVA indicated no significant effect of cohort on the data sets (p>0.05) A repeated measures ANOVA showed no significant effect of cohort on % PPI [f(1,49)= .011,p>0.05] nor was there a genotype by cohort interaction on % PPI [f(1,50)= .071,p>0.05].One animal (KO) was removed from the analysis because its level of startle responding was below the minimum 0 on the scale, see Appendix 2.

Effects of amphetamine administration on % PPI in D2R WT and KO animals

A repeated measures ANOVA showed that there was a significant effect of prepulse intensity on % PPI[f(3,156)=12.689,p<0.05]. Furthermore, a significant main effect of genotype on % PPI was seen [f(1,52)= 6.337,p=.015]. This indicates that %PPI was attenuated in D2R KO animals compared to their WT littermates. No significant effect of treatment on the % PPI was seen [f(1,52)= 1.126,p>0.05]. Furthermore, no genotype by treatment interaction on % PPI was seen [f(1,52=.899,p>0.05] (Fig. 2.3).
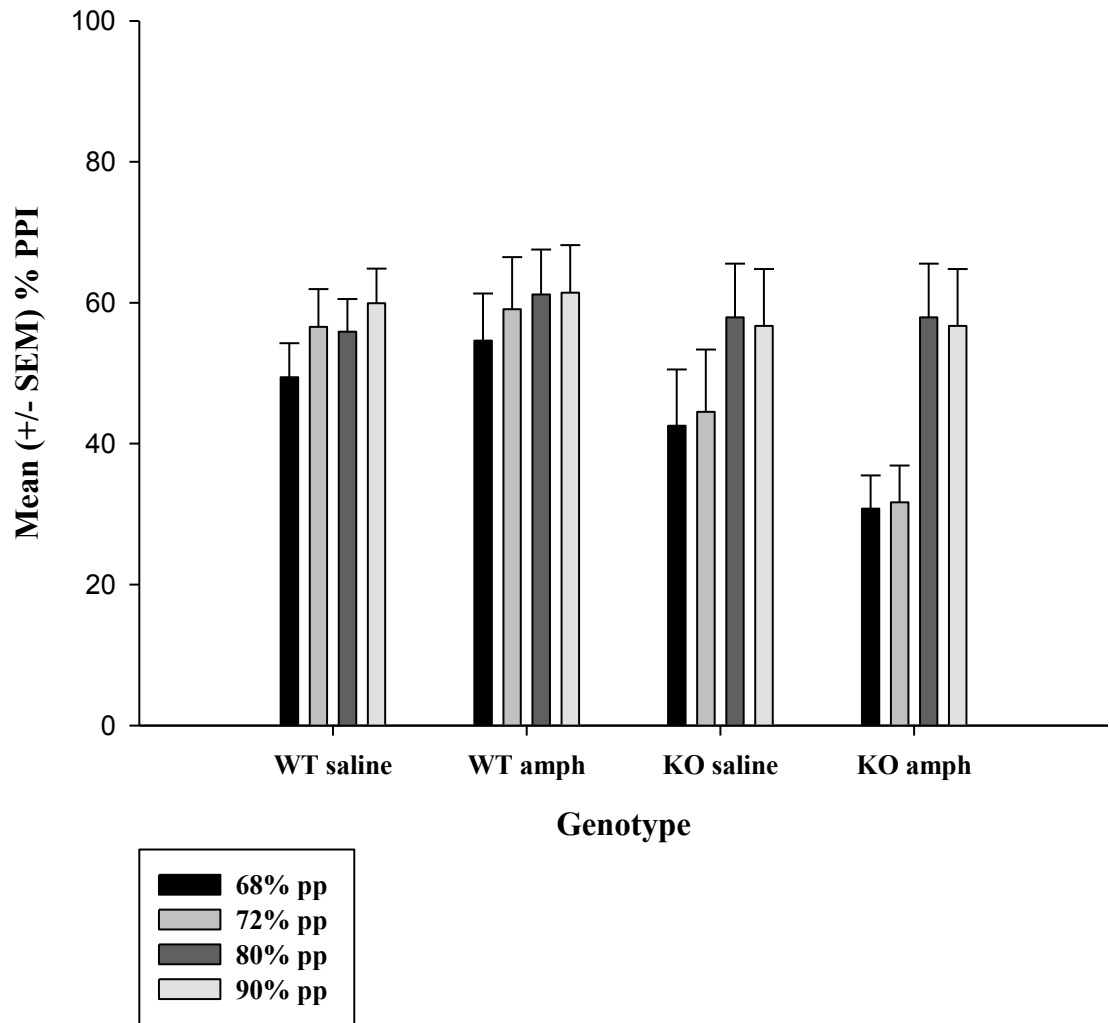
Figure 2.3 Results indicate that there was a significant main effect of genotype on % PPI. (68-90%pp= 68,72,80,90 dB % ppi).

Effect of treatment on startle to 120 dB pulses in D2R WT and KO animals

ANOVA showed that there was no significant effect of genotype on the 120 dB pulse alone trials [$f(1,29)= 2.655,p>0.05$].Thus, indicating no differences in startle to the 120 dB pulse between D2R KO and WT animals. No significant effect of treatment on the startle to 120 dB pulse was seen [$f(1,29)=4.031,p>0.05$].

Furthermore, no genotype by treatment interaction on the startle to 120 dB pulse was seen [$f(1,29)= 4.012,p>0.05$]. This indicates that neither treatment nor genotype influenced baseline startle to the 120 dB pulses.

Effect of treatment on ASR on prepulse+pulse trials in D2R WT and KO animals

A repeated measures ANOVA showed that there was a significant effect of prepulse intensities on ASR [$f(1,156)=9.368,p<0.05$].There was no significant effect of genotype on ASR [$f(1,52)= .766,p>0.05$].Thus, indicating no differences between D2R KO and WT animals on ASR. No significant effect of treatment on the ASR was seen [$f(1,52)= 3.043,p>0.05$].Furthermore, no genotype by treatment interaction on the ASR was seen [$f(1,52)=.121,p>0.05$] (Fig.2.4).
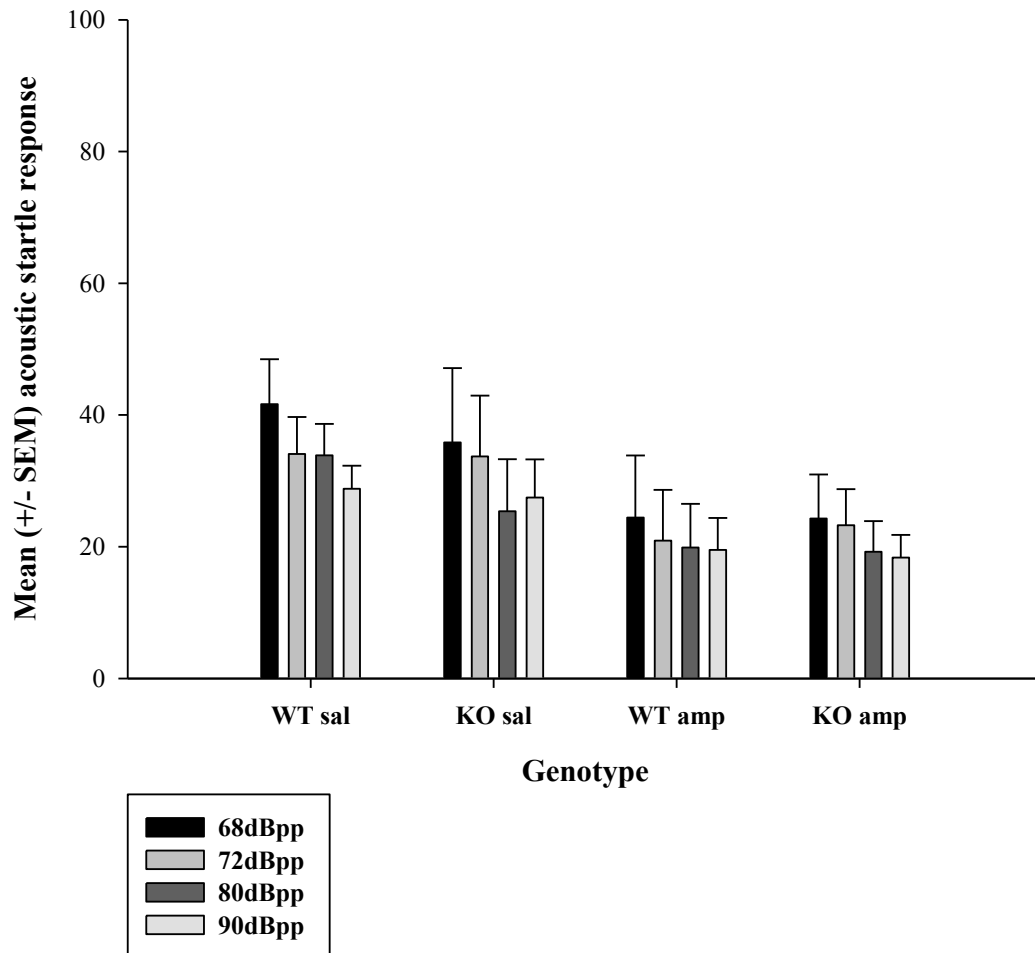
Figure 2.4 Data showing a significant ASR in D2 WT (+/+) and KO (-/-) animals. There were no differences in ASR between D2 WT or KO animals, both in drug naive or drug treated groups.

Effect on Habituation

A significant main effect of genotype on % habituation [$f(1,52)= 6.268, p=0.015$] was seen. Thus, indicating that D2R KO mice habituate differentially to D2R WT mice. No significant main effect of treatment on % habituation [$f(1,28)=.860, p>0.05$] was seen (fig 2.5). No genotype by treatment interaction on % habituation was seen [$f(1,52)=.328, p>0.05$]. Independent samples t tests indicated that D2R KO animals show attenuated %habituation compared to D2R WT ($t= 2.338, df=54, p=.023$), that precluded analysis with amphetamine treatment.
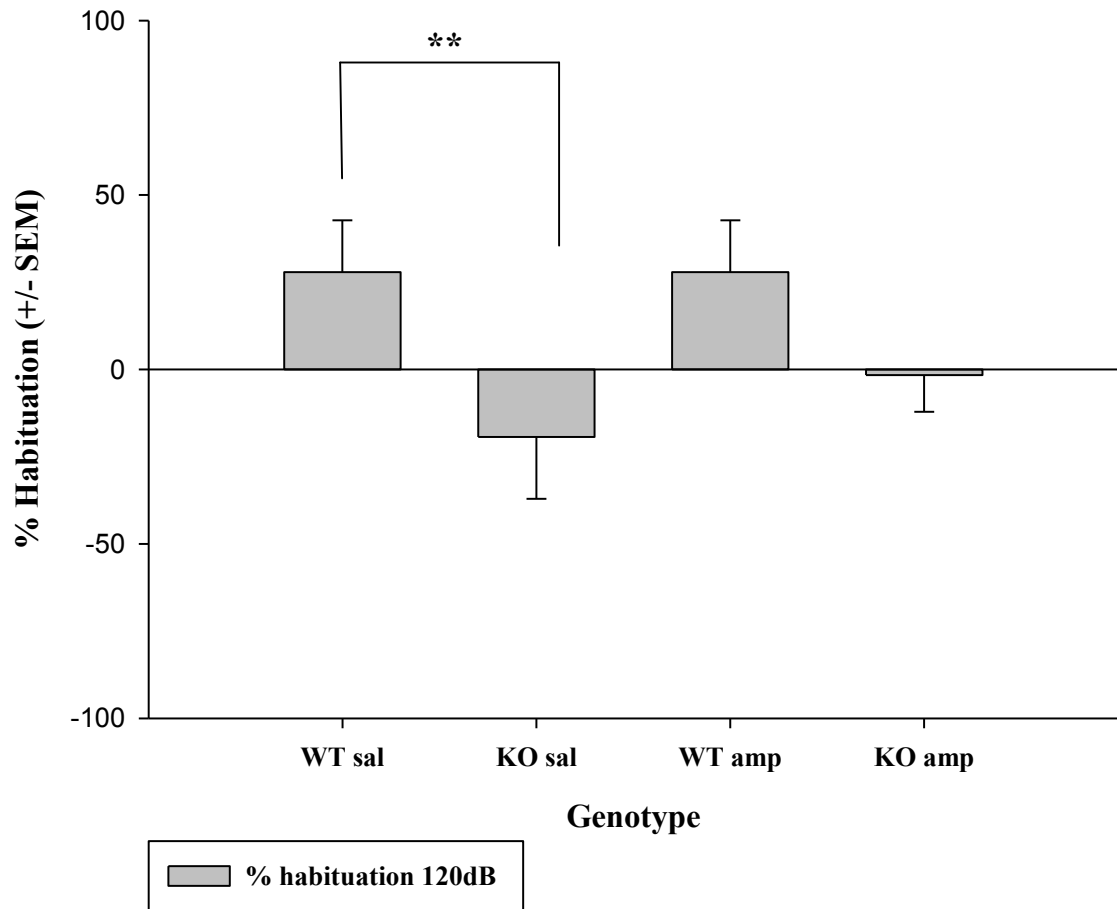
Fig 2.5 Data indicating a significant difference of the percentage of habituation to the 120 dB pulses in D2R WT and D2R KO animals (p<0.05). Treatment with amphetamine did not promote differences in % habituation in either D2R WT or KO littermates.

2. 4 Discussion

The results from this study indicated that D2R mutants showed lower levels of % PPI compared to their WT littermates. Furthermore, there is a trend for a single low dose of Amphetamine treatment to disrupt %PPI in D2R KO animals, but overall there was no significant effect of amphetamine treatment on % PPI in WT animals. Results do suggest however, that amphetamine can disrupt PPI in the absence of D2R when administered at a low dose. Conversely, Ralph et al's investigation (1999) showed that a high dose of amphetamine prevented PPI disruptions in the D2R KO. The present results suggest that this is not the case for a single low dose of amphetamine. These findings suggest amphetamine mediated disruption of PPI may be governed by D2R dependent mechanisms, but we are unable to make conclusions regarding the dependence of amphetamine disruptions in PPI on the D2R, as amphetamine did not produce any significant disruption in the D2R KO at a single low dose. Furthermore, stable baseline startle responding to the prepulses is seen, in both genotypes. However, disruptions in habituation in KO animals were seen compared to their WT littermates.

 D2R KO animals show deficits in habituation compared to their WT littermates, with a trend for amphetamine to improve these deficits in both WT and KO animals. Schizophrenia is marked by deficits in habituation, and schizophrenic patients show impairments in the habituation to startle (Geyer,1987). Previous reports have not indicated any habituation deficits in DAD2R KO mice. However, impaired habituation in DAT knockdown mice in a locomotor activity were seen to be inhibited by amphetamine administration (Zhuang, Oosting et al.,2001). Our results demonstrate a similar a trend toward inhibition of impaired habituation by

amphetamine. Alternately, it is suggested that impairments in habituation may account for hyperactivity. However, D2R KO mice are hypoactive, so differences in habituation cannot be attributed to hyperactivity in our cohort. It is suggested that differences in the habituation of startle may be mediated by strain, but animals on the C 57BL/10J background show high levels of ASR amplitude and low prepulse inhibition, but no impairment in either PPI or ASR (Paylor and Crawley,1997; Dulawa and Geyer, 2000). Our animals were bred on this background, and although they show low levels of prepulse inhibition, and they also show a habituation deficit to the 120 dB pulses. This may be independent of the level of prepulse inhibition; if animals did not habituate to the 120 dB stimulus, the inhibition to the prepulses would not be reduced (refer to definition of PPI in the Introduction). This is corroborated by our results that show no difference in baseline startle in the D2R KO compared to D2R WT animals, but attenuations in PPI are seen in D2R KO animals. Thus, suggesting that habituation may be an independent phenomenon to prepulse inhibition.

There is wider evidence that the behavioural effects of amphetamine depend on the dose schedule adopted. More specifically, a single exposure to amphetamine (5 mg/kg, i.p.) leads to sensitization of locomotor activity(Vanderschuren and Tilders 1999). Alternatively, multiple daily low amphetamine injections (2.5 mg/kg) augment locomotor activity as well as promote increased stereotypy. When tested again with the same dose of amphetamine 8 days after long term treatment was discontinued, the offset of stereotypy and hyperactivity resembled animals treated with short term amphetamine administration (Segal,1996). Other studies corroborate this administration schedule dependent role of amphetamine in disrupting behaviours; a low dose of amphetamine (1.5 mg/kg) disrupts LI in rats when

administered in both the preexposure (PE) and conditioning (COND) stages only, indicating that neither high doses not chronic exposure to amphetamine is needed to produce this disruption(Weiner, Lubow et al.,1988). In the session where PE and COND were not separated by a 24h interval, the administration of a single low dose of amphetamine 15 minutes prior to PE, and 15 minutes prior to COND, with a thirty minute interval between the PE and COND sessions did not disrupt LI. However, the same injection schedule did disrupt LI when the PE and COND sessions were 24 hours apart (Weiner, Lubow et al.,1988). This indicates that different injection schedules interact with the dose of amphetamine administered to modulate behaviour differentially.

We therefore compared one and two administrations of a single low dose of amphetamine on PPI, where the two dose task consisted of a single low dose of amphetamine administered once prior to habituation and prior to test, and was separated by a 24h time interval. Thus, we seek to investigate whether one and two injections of a low dose of amphetamine disrupt PPI differentially in D2R WT and KO animals.

2.5 Materials and Methods

2.5.1 Animals
Male and female D2R KO (n=22) mice and wild type (WT=17) littermates were between 16-24 weeks old prior to being used in these experiments. Refer to previous experiment for colony details (Refer to Section 2.2.1).

2.5.1.2 Behavioural Testing
The testing protocol was the same as the aforementioned experiment (Refer to page 176-178). However, prior to testing the animals in the PPI apparatus, animals were given a one day habituation session. On day 1, animals were removed from their

home cages and placed in the PPI apparatus for 25 minutes. The house light and white noise were turned on. They were left in the startle chamber for the entire duration without any pulses. On day 2, they were subjected to the startle session as described in the previous experiment i.e. blocs of 120 dB pulses, intermixed with prepulses of varying intensities.

2.5.1.3 Drugs

d-Amphetamine sulphate was obtained from (Sigma –Aldrich,Gillingham, UK) dissolved in 0.9% saline and administered at a dose of 2.5 mg/kg. Injections of 2.5 mg/kg amphetamine or saline were administered i.p. thereafter, 30 mins prior to behavioural testing at a volume of 10 ml/kg. Animals were injected thirty minutes prior to the test session. The dose was the same as in the previous study. On DAY1, animals were injected thirty minutes prior to being placed in the PPI boxes. After which, they were allowed to habituate to the boxes for a period of 25 minutes. On DAY2, animals were again injected thirty minutes prior to being placed in the boxes, for a test session of twenty five minutes in total that incorporated 5 minute habituation to the 120 dB pulse.

2.5.1.4 Apparatus

The apparatus is the same as the previous experiment.

2.5.1.5 Acoustic Startle Session/ Prepulse Inhibition session

The startle session is the same as the aforementioned.

2.5.1.6 Data Analysis

The data analysis is the same as the prior experiment.

2.6 Results

Effects of amphetamine administration on % PPI in D2R WT and KO animals

A repeated measures ANOVA showed that there was a significant effect of prepulse intensities on % PPI [$f(3,81)$=7.731, p=0.000]. No significant main effect of genotype [$f(1,27)$=.068,p>0.05] or sex [$f(1,27)$= 4.164,p=0.05] on %PPI was seen. A significant main effect of treatment type [$f(2,27)$=13.646,p<0.001] on % PPI was also seen. A significant interaction of %PPI intensities by treatment type was seen [$f(6,81)$= 2.367, p=.037]. Furthermore, a genotype by treatment type interaction on % PPI was also seen [$f(2,27)$=4.430,p= .023]. Moreover, significant genotype by sex by treatment type interaction on %PPI [$f(2,27)$=4.907,p=.015] was also seen. Additionally, a significant genotype by sex interaction on % PPI was seen [$f(1,27)$=18.639, p<0.001].

In order to explore these interactions further, data was split by genotype, and a separate ANOVA was conducted, with treatment and sex as a between groups factor. In WT animals, there was no significant effect of prepulse intensities on %PPI [$f(3,33)$=2.593,p>0.05]. A significant main effect of sex was seen [$f(1,11)$=14.008,p=.003] reflecting higher No significant main effect of treatment [$f(2,11)$=2.880,p>0.05] or any interaction between treatment and sex was seen [$f(6,33)$=.973,p>0.05]. In the D2R KO animals, a significant main effect of prepulse intensities on % PPI was seen [$f(3,48)$=6.342,p=.001]. No significant main effect of sex was seen [$f(1,16)$=3.66,p>0.05]. A significant main effect of treatment [$f(2,16)$=15.767,p<0.001] was seen. No interaction between PPI, treatment and sex was seen [$f(6,48)$=.200,p>0.05].

In D2R KO animals multiple comparisons (Fishers LSD) indicated a significant difference of the effect of a single low dose of amphetamine treatment as compared to saline treatment (p=.002). This indicates that a single dose of amphetamine disrupts %PPI in D2R KO animals. Post hoc tests indicated no differences % PPI in KO animals treated with two doses of amphetamine when compared to their saline treated controls (p>0.05) but significant differences on % PPI were seen in D2R KO animals treated with a single dose vs a double dose of amphetamine (,p<0.05). (See Fig 2.7-2.8).
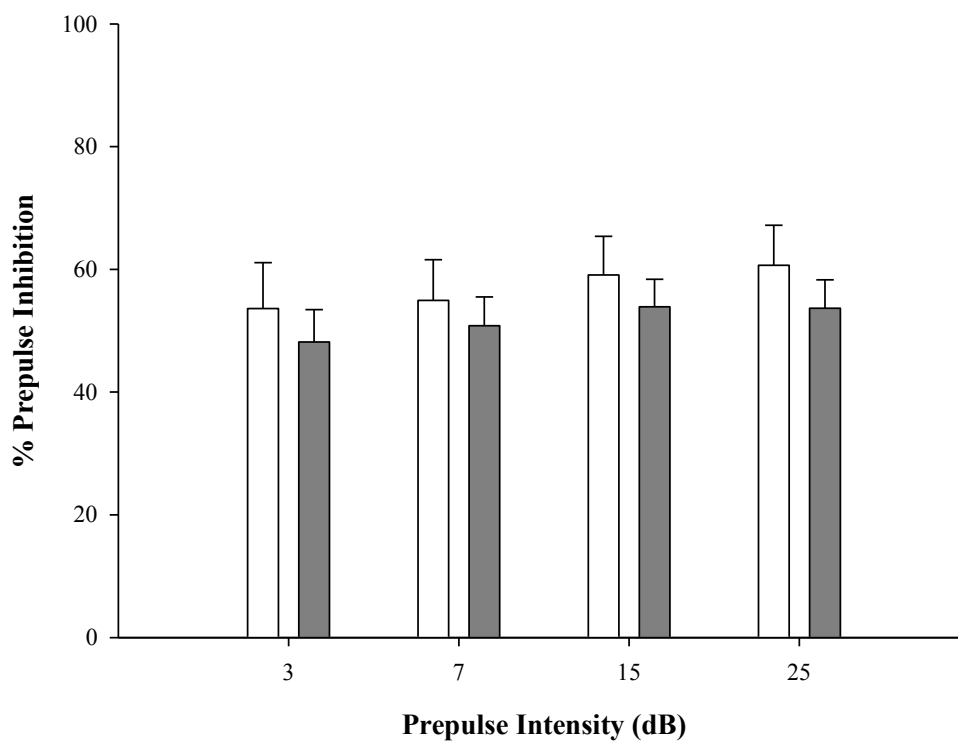
Figure 2.6.Baseline PPI for D2R wild –type controls (+/+) (0.9 % saline; white bars) and knock-out(-/-) (0.9 % saline; grey bars) mutant mice.
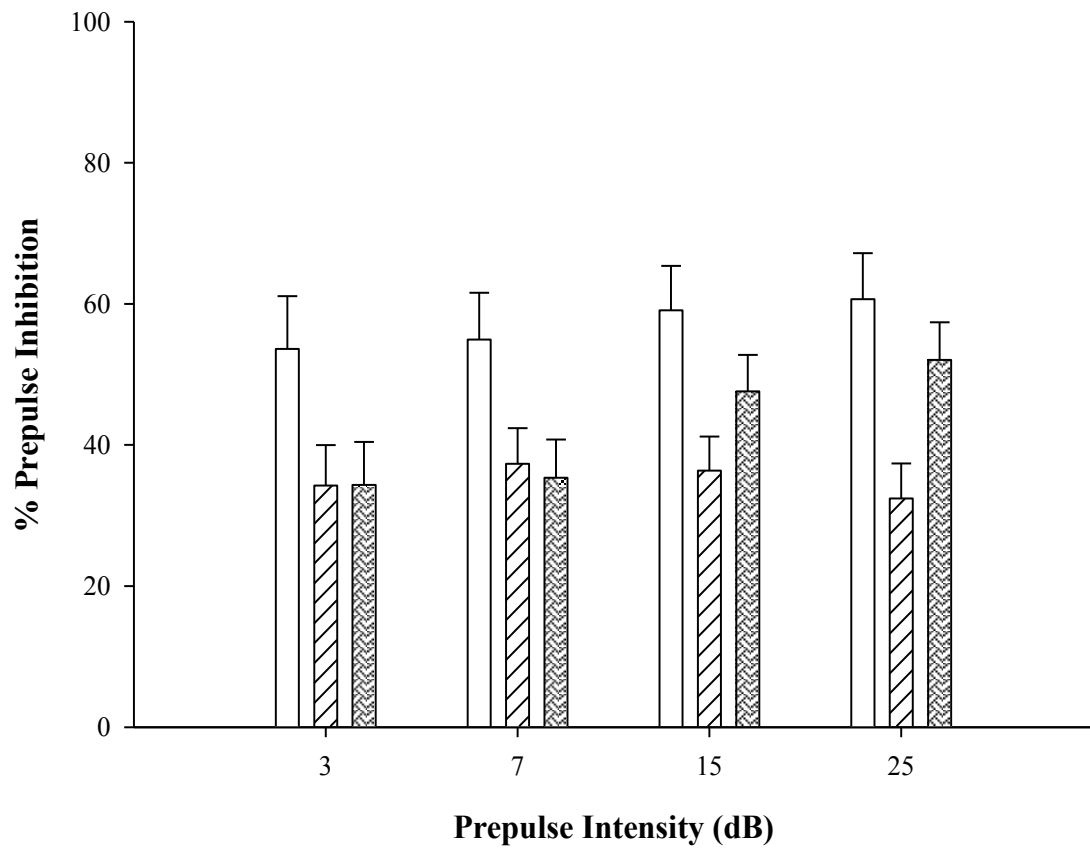
Fig 2.7 Baseline PPI for in D2R wild-type mice with vehicle control (0.9% saline; open bars), single low dose amphetamine (2.5 mg/kg; hatched bars) and two low doses amphetamine animals (2.5 mg/kg; patterned bars). Post hoc Analysis showed that a single low dose of amphetamine disrupts %PPI in D2R WT and animals (p<0.05), but two low doses spare these disruptions.
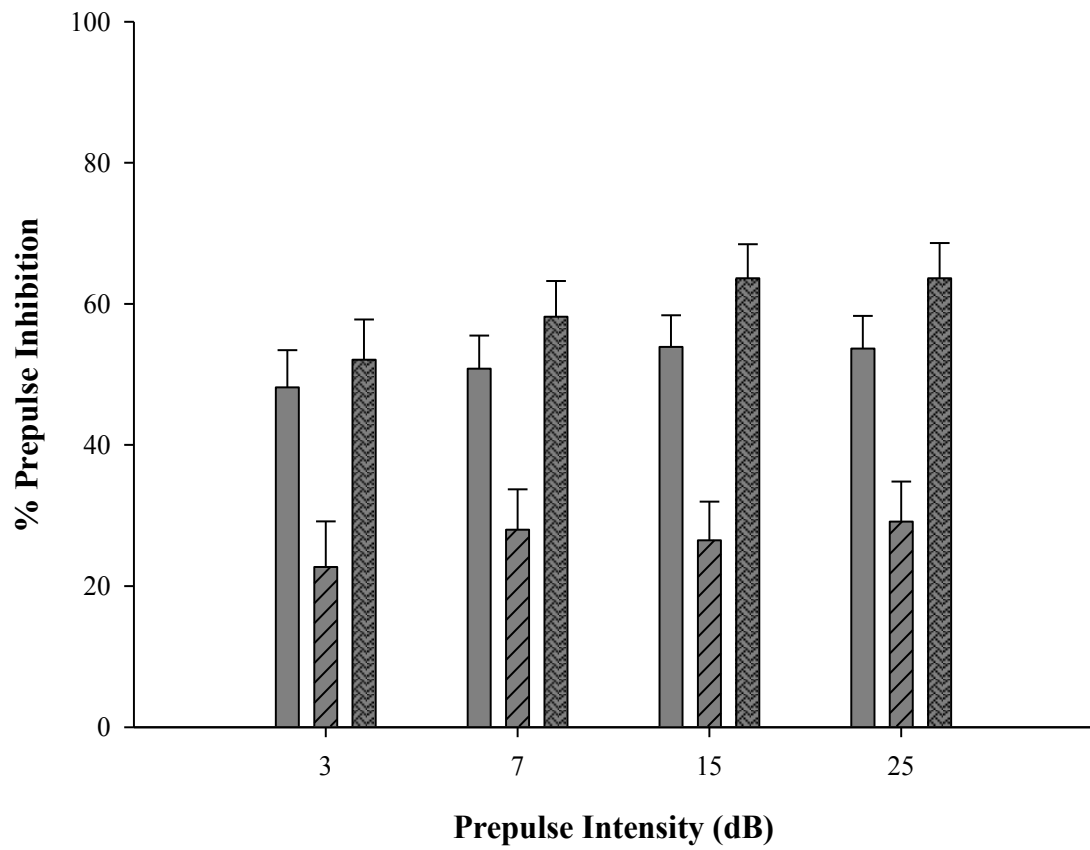
Figure 2.8 Baseline PPI for in D2R KO mutant mice with vehicle control (0.9% saline; open grey bars) (0.9% saline; open bars), single low dose amphetamine (2.5 mg/kg; hatched grey bars) and two low doses amphetamine animals (2.5 mg/kg; patterned grey bars. Post hoc Analysis showed that a single low dose of amphetamine disrupts %PPI in D2R KO animals (p<0.05), but two low doses spare these disruptions.

Effect of treatment on baseline startle to the 120 dB pulse trials

A univariate ANOVA indicated no significant main effect of genotype on startle magnitude to the 120 db pulse alone trials [$f(1,66)= 2.587, p>0.05$]. However, a significant main effect of treatment on startle magnitude to the 120 db pulse alone trials [$f(2,66)= 4.610, p=.013$] was seen. A significant main effect of sex on startle magnitude to the 120 db pulse alone trials [$f(1,66)= 4.265, p=.043$] was also seen. However, no genotype by treatment by sex interaction was seen [$f(2,66)= .379, p=.686$] (Fig. 2.10).

Effect of treatment on ASR on prepulse+pulse trials

A repeated measures ANOVA showed that there was a significant effect of prepulse intensities on ASR [$f(3,81)= 6.514, p=0.001$]. Furthermore, no significant effect of genotype [$f(1,27)= 1.433, p>0.05$] or sex [$f(1,27)= .316, p>0.05$] on ASR was seen. Thus, indicating no differences in baseline startle between D2R KO and WT animals. There was also no effect of treatment on ASR [$f(2,27)= 2.209, p>0.05$]. Furthermore, no genotype by treatment interaction on the ASR was seen [$f(2,27)=.912, p>0.05$] (Fig. 2.9).
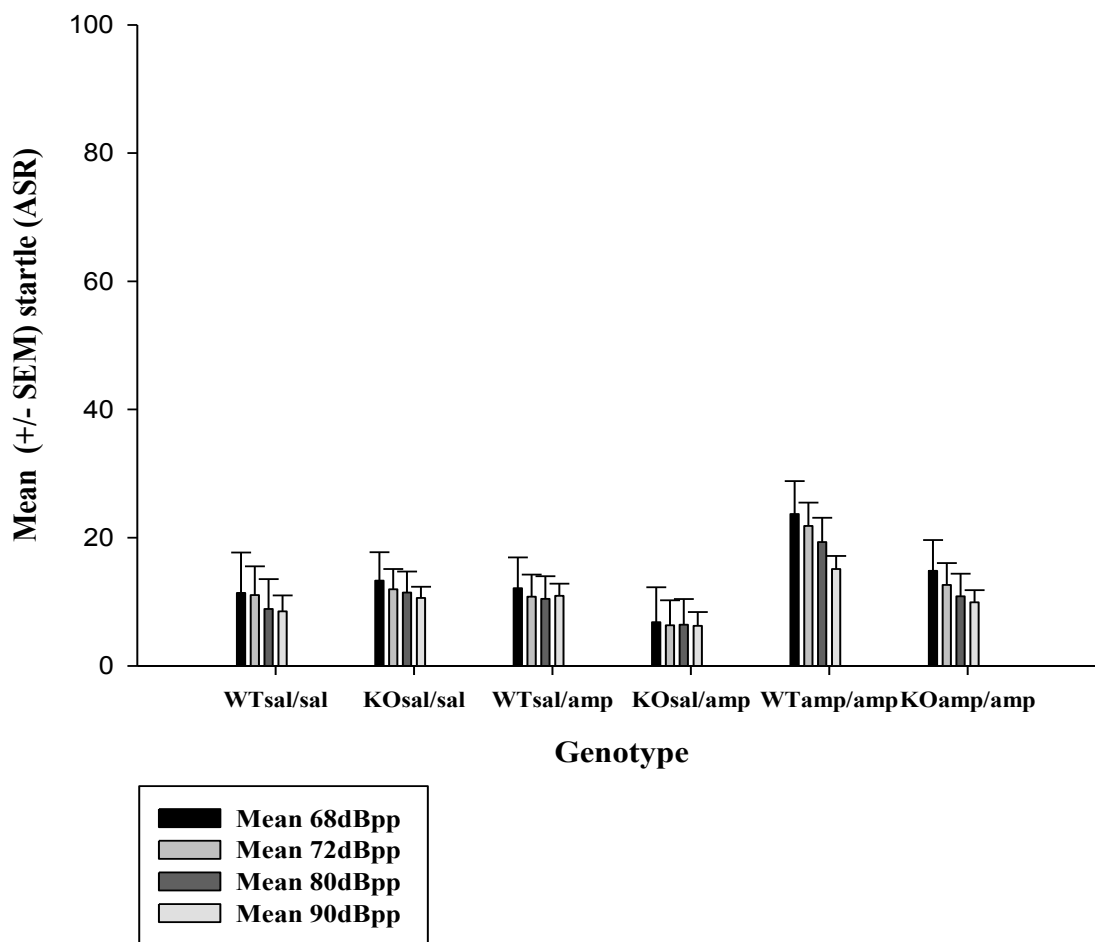
Figure 2.9 Data showing a significant ASR in D2 WT (+/+) and KO (-/-) animals. There were no differences in ASR between D2 WT or KO animals, both in drug naive or drug treated groups.
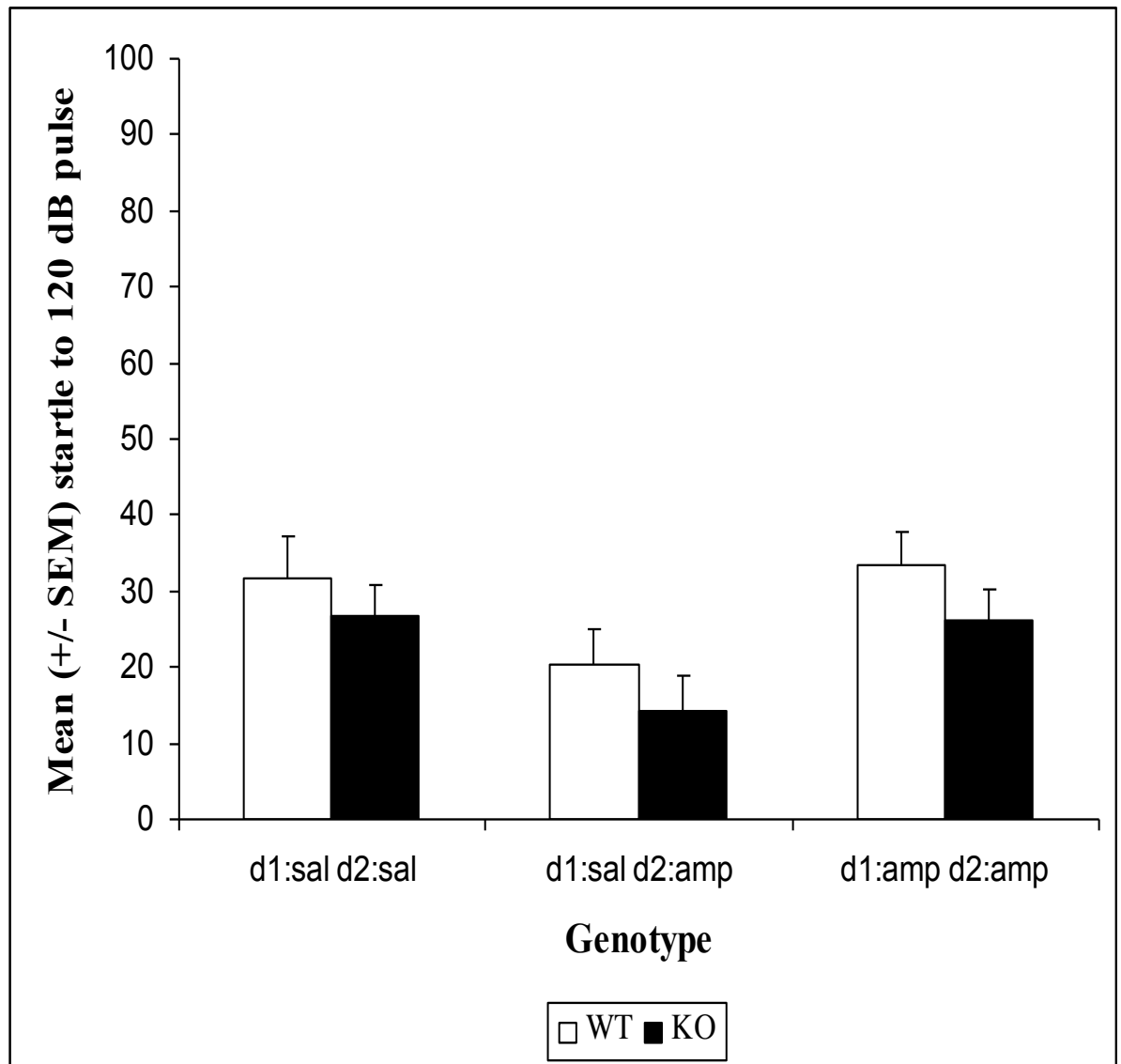
Figure 2.10 Data showing ASR to 120 dB pulses in D2 WT (+/+) and KO (-/-) animals. There were a significant main effect of treatment on ASR to the 120 dB pulse (p<0.05) in D2R WT or KO animals, but no interaction between genotype and treatment (p>0.05).

Effect on Habituation

There was no significant main effect of the to the 120 dB prepulses to % habituation $[f(11,27)=1.288, p>0.05]$. No significant main effect of genotype $[f(1,27)= 1.313, p>0.05]$ on % habituation was seen. No significant main effect of treatment $[f(2,27)=3.289, p>0.05]$ on % habituation was seen (fig 2.11).
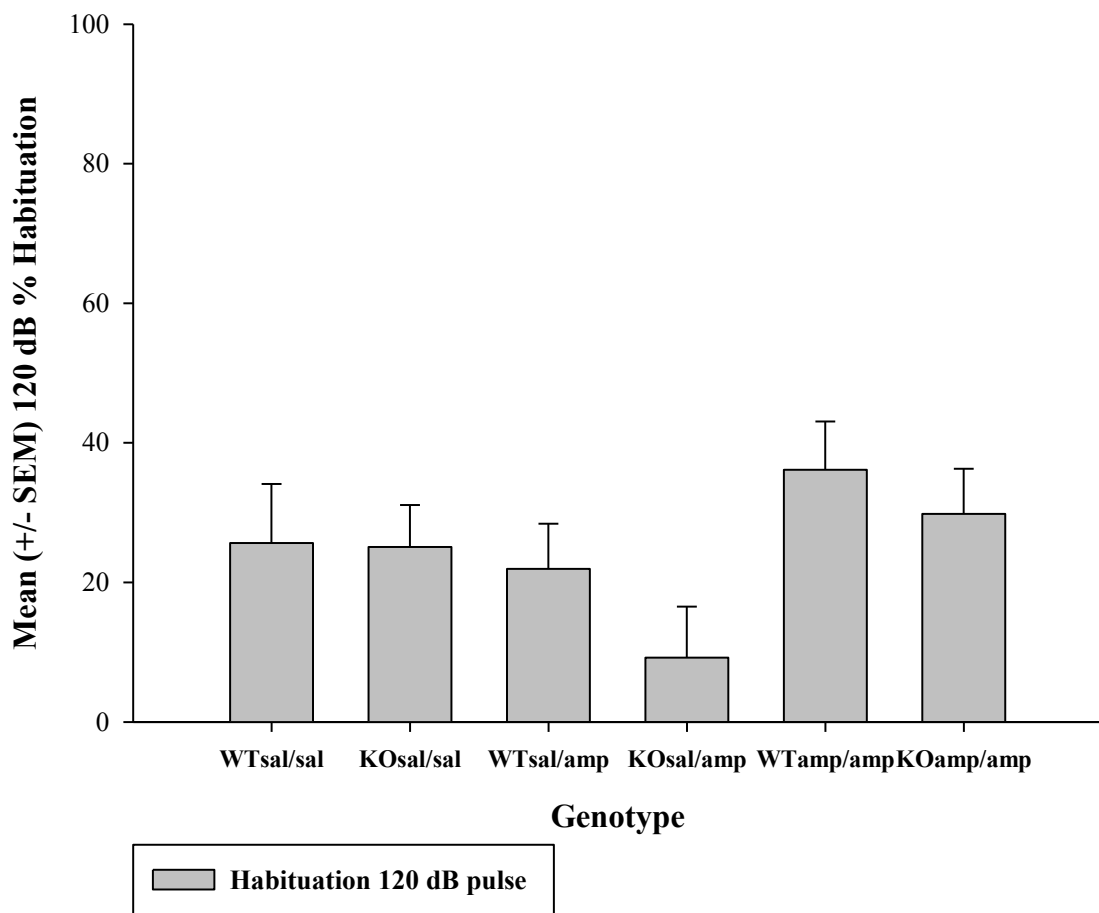
Fig 2.11 Data indicating no significant difference of the percentage of habituation to the 120 dB pulses in D2R WT or D2 KO animals ($p<0.05$). Treatment with amphetamine did not produce deficits in % habituation in either D2R WT or KO littermates.

2.7 Discussion

Our results indicated that a single administration of low dose amphetamine disrupted PPI in D2R KO while two administrations 24hrs apart prevented disruptions in WT and KO mice only. This suggests that the D2 receptor may not be required for amphetamine induced disruption of PPI by a single low dose of amphetamine. These findings demonstrate that previous findings suggesting that the D2R is required for amphetamine effects in PPI does not generalise to all doses, and further that the effect of one administration is not necessarily the same as the effect of two administrations.

These data contradict prior studies suggesting that amphetamine disruption of PPI is blunted in D2R KO mice (Ralph et al.,1999).As mentioned previously, low doses of amphetamine produce disruptions in LI via the mesostriatal areas and high doses produce behavioural stereotypy via the mesolimbic region. Sensitization to amphetamine disrupts PPI and LI (Tenn, Kapur et al.,2005). This suggests the same neural mechanisms may be involved in LI and PPI. The amphetamine induced disruption in both these processes might be mediated by a common circuit; the limbic system has been implicated in the regulation of PPI. Dose dependent disruptions may be mediated differentially by these regions in PPI (Swerdlow, Mansbach et al., 1990). Sensitization studies provide evidence for differential effects of amphetamine on PPI disruption and ASR following multiple in injection schedules at escalating doses, which that suggest that different dose schedules govern PPI differently (Russig, Murphy et al.,2002). Additionally, it is suggested that indirect agonists affect PPI by inhibiting GABA receptors (Swerdlow, Braff et al.,1990), and activate

VTA mediated neural circuits. Direct DAD2R agonist Pergolide reduces PPI at low doses, and eliminates it at high doses. It is suggested that with increased D2R stimulation, the information contained in the prepulse is less vulnerable to interference by stimuli in immediate temporal proximity, but more vulnerable to interference by stimuli at longer durations(Swerdlow,Geyer et al.,2001). This suggests that variations in PPI may be mediated by protocol differences such at the time of occurrence of prepulses and prepulse intensity (Karl,2011).

Our results from experiment 1 and experiment 2 support the importance of protocol specificity in mediating PPI. In experiment 1, PPI was seen to be attenuated in the D2R KO, but these attenuations in PPI were not seen in experiment 2. Experiment 1 consisted of a one day PPI test session, which consisted of five minutes of habituation to the chamber, which was incorporated into this session. Experiment 2 consisted of a two day session, where day 1 consisted of habituation to the chambers without any stimulus presentations and day 2 consisted of a PPI test session that was identical to experiment 1. This suggests that a longer habituation period to the test chambers may produce more stable PPI. Baseline acoustic startle responses to the prepulses however remain unaffected by the lack of habituation day in experiment 1. These findings thus indicate that PPI and ASR may be differently mediated by depending on the amount of habituation to the test session.

Furthermore, no changes in baseline startle and habituation to the 120 dB pulses by amphetamine, indicate that disruptive effects on PPI in the test session are not simply an artefact of disrupted baseline startle due to stress of injections administered prior to this session. Animals do not show the habituation deficits, seen in the previous experiment. Other studies report disruptions in the habituation of acoustic startle at moderate (5mg/kg) and high (10 mg/kg of d-amphetamine, but not at a low dose (2.5

mg/kg) in the NMRI nude mouse strain. This suggests that amphetamine disruption of startle habituation may also be dose dependently mediated. However, basal startle reactivity to the 120 dB pulse alone trials are affected by amphetamine treatment. More specifically, treatment with amphetamine attenuated baseline startle responses to the 120 dB pulses. However, no effect of amphetamine treatment was seen on the startle magnitude to the prepulse and pulse trials, and this lack of change in the startle reflex may be a floor effect (Swerdlow et al.,2000) of amphetamine treatment induced disruption in the pulse alone trials. Therefore, the disruption in %PPI may not reflect a true disruption of sensorimotor gating and needs to be interpreted with caution (see General Discussion).

3.8 Conclusions

Low dose amphetamine disruption of PPI in D2R animals is dependent on the dose schedule and protocol adopted. A baseline PPI phenotype is seen in D2R KO mice, in the one day and two day protocol. Alternately, % PPI is attenuated in the D2R KO compared to their WT littermates in the one day protocol only. Furthermore, dissociation between a single and double dose of amphetamine exists with regard to D2R involvement in PPI. The data from our first one day experiment suggested attenuated PPI in the D2R KO, and a trend toward amphetamine disruption by a single low dose in both D2R KO animals. Our second two day experiment found no attenuation in PPI in the D2R KO, however, it was seen that a single low dose of amphetamine disrupted % PPI in D2R KO mice, but two injections prevented amphetamine mediated impairment in this task in across both genotypes. Taken together, our results suggest indicating that a low dose of amphetamine disrupts PPI

in the D2R mutant model, in a protocol and drug schedule dependent manner where these effects do not generalize to all doses of amphetamine.

CHAPTER 3

EPISODIC MEMORY IN D2R WT and KO

ANIMALS

3.1 Introduction

In addition to cognitive deficits that span LI and sensori-motor gating, patients with schizophrenia also show disturbances in memory. Impaired episodic memory is seen in schizophrenia patients and DA agonist amphetamine produces schizophrenia like impairments in memory in non human primates (Castner and Goldman-Rakic 2003; Castner, Goldman-Rakic et al., 2004).

Impairments in multiple facets of memory i.e. working memory, spatial memory and episodic memory have been well documented in schizophrenia patients (Park, Püschel et al.,1999; Tendolkar, Ruhrmann et al.,2002; Park, Püschel et al.,2003; Bonner-Jackson, Haut et al.,2005). These memory processes are regulated by dopamine; D1 receptors modulate working memory in the PFC(Granon, Passetti et al., 2000) and D2 receptors have been proposed to modulate verbal recall of memory the striatum (Chen, Kuang Yang et al.,2005).The D2R is also involved in the memory for coding spatial information (Tran, Tamura et al.,2003). Spatial memory deficits have been reported in rats administered a dopamine D2R antagonist raclopride in the radial arm maze test (Wilkerson and Levin,1999), suggesting that D2R blockade induces deficits in spatial memory. D2 receptors in the striatum have been associated with episodic memory tasks(Cervenka, Bäckman et al.,2008), and

dopamine stimulation in this region may regulate activity in the hippocampus through the ventral tegmental area (VTA) -hippocampal circuits or via ventral striatal-thalamic-cortical circuits(Alexander,DeLong et al.,1986). The cortical and hippocampal regions are the functional substrates of these memory operations (Rajah and D'Esposito,2005).

D2R KO mice show blunted PFC activation on a spatial working memory task; where augmented impairments in spatial memories are seen with increasing delays in this task (Glickstein and Schmauss,2002). This suggests that memory disruptions in D2R KO's are sensitive to long delay intervals in a memory task. However, to date episodic memory or novel object recognition has not been investigated in dopamine deficient mice, and it is not known if D2R KO induces disruptions on this form of memory.

This chapter seeks to investigate episodic memory in D2R KO mice. We seek to investigate episodic memory in the D2R KO mouse model, in a task that establishes their memory for 'what', 'where' and 'when' based object discrimination. This task incorporates temporal memory for an object at a particular point in time, and memory for spatial displacement of objects (Refer to Section 1.5.2 in the Introduction). This task combines recency discrimination for a novel object as well as spatial memory for objects based on their occurrence at a point in time, as opposed to only novelty based object discrimination. It allows the simultaneous investigation of the discrimination for objects depending on the point of time at which they occurred, for what an object was, and where it was displaced to, with reference to the point in time it occurred at. This task allows the investigation of a novel object not only in terms of familiarity discrimination but also in terms of its temporal occurrence, and whether animals are able to detect a spatially displaced object based

on these recency and novelty driven properties. Thus, novelty and spatial displacement object properties individually form constituents of an episodic memory for 'what', 'when' and 'where', that are governed by the temporal point, at which they occurred. . Antipsychotic drugs disrupt spatial memory (memory for where only) (Skarsfeldt, 1996) and mice lacking the D2R receptor show impairments in spatial memory (where) (Glicksteinet al.,2002). Thus, indicating that the D2R is required for intact spatial memory. Furthermore, antipsychotic drugs that are D2R antagonists do not improve impairments in episodic memory in schizophrenic patients (Goldberg et al.,2007). Thus, we wanted to investigate whether deletion of the D2R in KO's promotes deficits in an episodic memory task to investigate whether this model mimics these APD effects in the episodic memory task and its spatio-temporal component that is governed by recency of object occurrence as well as its displacement (memory for what-where).

3.2. Materials and Methods

3.2.1 Animals

Dopamine receptor (D2R) wildtype and knockout mice were used in this experiment. Male and female KO (n=16) mice and wildtype (WT=16) littermates were between 16-24 weeks old prior to being used in these experiments. (Refer to Material and Methods in Chapter 2 for full details).

3.2.2. Behavioural Testing

3.2.2.1 Apparatus
Open Field

Object exploration was assessed in an open field (8 boxes; 30 cmX 30 cmX 40 cm), chamber that consisted of enclosed boxes made out of clear plastic. Two sides of each

chamber were covered with black Fablon adhesive plastic, so as to prevent animals in each chamber from seeing animals in neighbouring chambers. Multiple spatial cues were also provided i.e. the left wall to the chamber was blue; the wall behind the chambers was covered in a white sheet with a stripe pattern, and its adjacent wall had a large red triangle pasted onto it. A video camera, connected to a video recorder was mounted 70 cms above the field to record and store activity videos on computers for analysis. Diffuse white light provided illumination in the centre of the chamber. The chamber had a closed ceiling, where the activity boxes were kept, and the door was closed whilst testing. A fan created white noise and was located at the top right corner of the chamber and also acted as a cue. After each trial, the apparatus was thoroughly cleaned with a 75% ethanol solution to remove any odour cues.

Fig. 3.1 Mice were placed in open field chambers during the habituation phase, to acquaint them to novel objects.

Objects

Two different objects (four copies of each), with varying texture and made of different materials were used i.e. a glass conical bottle and a golf ball. The objects were cleaned thoroughly with 70 % ethanol solution so that they could not be distinguished by odour cues. In the habituation session to a novel object, objects unrelated to those presented in the test phase were used (Lego blocks), so that there was no prior exposure to a similar object. Pilot studies ensured that the mice could discriminate between both objects, and to ascertain that there was no preference for one of these objects.

HANDLING: Day 1-7

The mice were habituated to the handling procedure prior to the 'what', 'where' and 'when' episodic memory task for seven consecutive days. The animals were picked up and released in the open field for an exposure of 1 minute, after which they were placed back into their home cage.

HABITUATION: Day 8-10

After handling, mice were familiarized to the test apparatus that was devoid of any objects for three consecutive days. They received 5 minutes of open field exposure per day.

HABITUATION WITH AND WITHOUT OBJECTS: Day 11-12

On the following two days, in order to habituate the animals, the mice received 3 more daily sessions with exposure for 10 minutes. Two objects were placed in the corners of the open field (lego blocks), and each exposure was separated by a 20 minute inter trial

interval (fig. 3.1). These objects were not used in the 'what', 'when', and 'where' object exploration task.

TEST OF MEMORY FOR 'WHAT', 'WHERE' AND 'WHEN' PARADIGM IN MICE BY COMBINING DIFFERENT VERSIONS OF THE NOVELTY PREFERENCE PARADIGM: Day 13

Each mouse received two sample trials and a test trial. On the first sample trial, mice were placed in the centre of the open field that contained four copies of a novel object in a triangle shaped configuration; where one object was placed in the centre of the northern wall (NC), one at south-west corner of the box (SW), one at the south -centre and the last object at the south -east corner of the box (as shown in Fig.3.2). Animals were allowed to explore these objects for a period of 10 minutes. After a delay of 50 minutes, animals received a second sample trial that was identical to the first, except the four novel objects were arranged in a different spatial orientation in the open field. Objects were arranged in a quadratic formation, where one object was arranged in the top left north-west corner (NW), north–east (NE), south–west (SW) and south-east (SE). The objects determined for each mouse were counterbalanced across both sample trials. After a second delay of 50 minutes, mice received a test trial identical to the second sample trial, where two copies of the old familiar object were places in the SW and NE corners, in their stationary (as-before) and displaced (not as-before) positions respectively and two copies of the recent object were placed in the NW and SE corners. The what-when task shows a preference for the old objects vs the recent objects, and the what-where component of the old objects over their subsequent locations (stationary and displaced).

Old Objects　　　　　　**Recent Objects**　　　　　　**Discrimination Test**
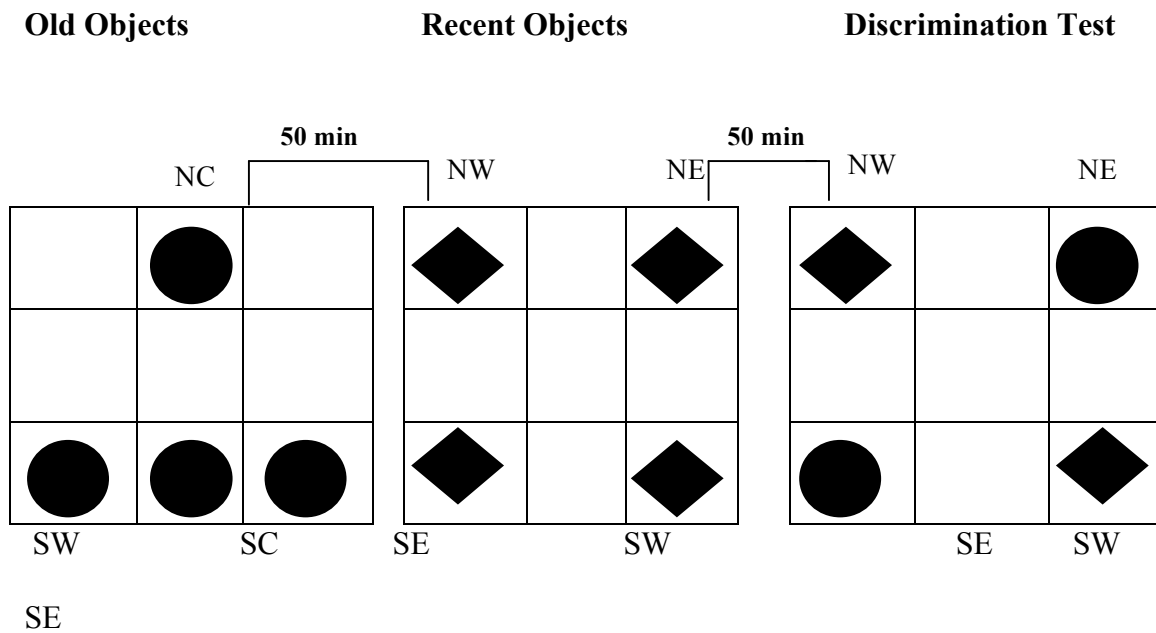
Fig. 3.2 Schematic drawing of the episodic memory task encompassing the 'what', 'where' and 'when' components. Object locations: NC= north corner, SW= south-west, SC= south-centre, SE= south-east, NW= north-west, NE= north-east. Adapted from Dere et al. 2005

3.2.2.2 Data Analysis

Data was analysed using mean exploration times in exploring the old and new objects and exploration times in exploring the stationary and displaced objects. Two repeated measures ANOVAs with object type (old vs recent and stationary vs displaced) and genotype and sex as within groups factors were used. Paired t tests were also conducted as a post hoc measure to ascertain differences in exploration between groups. The Bonferroni correction (α= .0125) was also applied.

Additionally, discrimination ratios were also calculated, to obtain a measure of object discrimination, which takes into account individual differences, as it measures exploration of a preferred object as a proportion of the total amount of exploration time, as opposed to total exploration times of all objects (Dix and Aggleton,1998). Discrimination ratios (DI) that expressed the preference/discrimination of one object as a proportion of the total amount of time spent exploring both objects was calculated as follows:

DI (old vs recent ratio) = Time spent at old object (in seconds)/ Time spent at old object+ time spent at recent object (in seconds)

DI (stationary vs displaced ratio) = Time spent at stationary old object (in seconds)/ Time spent at stationary old object+ time spent at displaced old object (in seconds).

A one way univariate ANOVA was used with DI as the DV and genotype and sex as between groups' factors. One sample t tests against a chance level of .5 were also conducted on these DIs. The Bonferroni correction was applied (α= .0125) to all t tests. Three animals were removed from the analysis based on the lack of exploration in each of the sample stages (< 20 sec). See Appendix 3.

3.3 Results
Recency discrimination: What-when analysis using mean exploration times

A repeated measures ANOVA showed that there was a significant main effect of the familiarity of old and recent objects on the mean amount of exploration of old familiar objects and the recent objects [$f_{(1,55)}$=5.123, p<0.05]. No significant main effect of genotype [$f_{(1,55)}$= 3.424, p>0.05] or sex [$f_{(1,55)}$= 1.168, p>0.05] on the mean exploration times of the old familiar objects and recent objects was seen.

However, a genotype by sex interaction on the exploration of old and recent objects [f(1,55)= 7.485, p=.008] was also seen.

To explore this interaction further, data was split by genotype. In D2R WT animals, no significant main effect of the familiarity of old vs recent objects on the exploration times of old and recent objects [f(1,27)=.226, p>0.05] was seen. A significant main effect of sex was seen [f(1,27)=8.113, p=.008], but no interaction on object familiarity and sex was seen [f(1,28)=3.064,p>0.05]. In D2R KO animals a significant main effect of the familiarity of old vs recent objects on the exploration times of old and recent objects [f(1,28)= 11.505, p= .002] was seen. No significant main effect of sex was seen. However, genotype by sex interaction on discrimination was seen [f(1,28)= 4.778, p<0.05].

Paired T tests indicated that male WT animals [t= -.658, df=13, p>.0125, two tailed] did not differ significantly in their exploration of the old and recent objects, similarly to male KO animals [t=.992, df=15, p>.0125, two tailed] that did not exhibit these preferences. Female WT animals [t=.027, df=14, p>.0125, two tailed] did not differ significantly in their exploration of the old and recent objects either. However, female KO [t= 5.185, df=13, p<.0125, two tailed] showed a significant difference in exploration of the old objects as compared to the recent objects, indicated by an increased preference for the old object. This suggests an enhancement in the exploration of old vs new objects in females D2R KO animals. However, independent samples t tests indicated no significant differences in exploration of the old object between female D2R WT and KO animals [t= -904, df= 13,p>.0125].See Fig 3.3
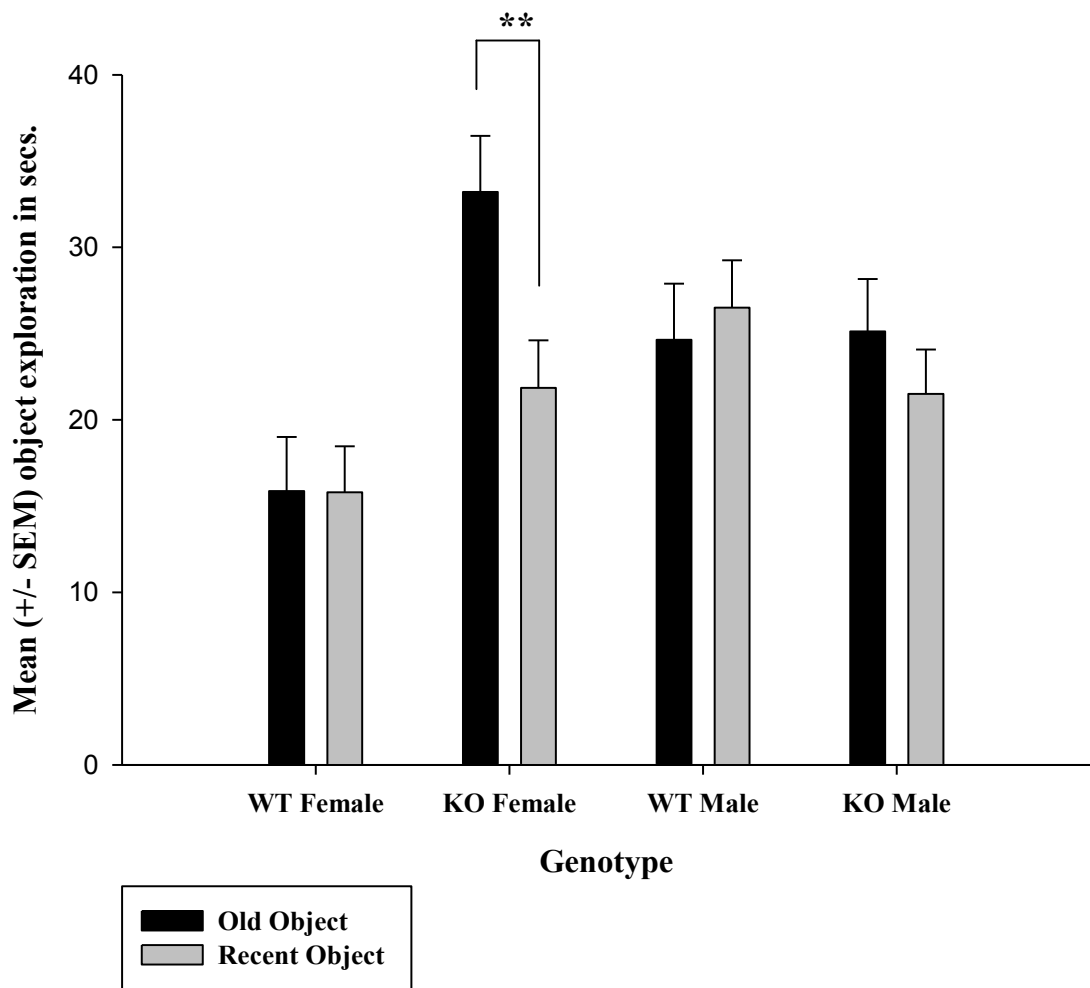
Figure 3.3 Graph showing exploration of old vs recent objects in D2R WT and KO animals. Male and female WT animals do not exhibit any differences in their preference for old rather than recent objects. However, female animals KO animals show a difference in exploration of the old objects vs recent objects (p<0.05) and this increased preference for the old object is enhanced in the female KO.

Recency discrimination: What When analysis using Discrimination ratios

Using the DR measure one way between groups ANOVA showed no significant main effect of genotype [f(1,55)= 5.479, p>0.05] on the preference (discrimination ratios) for the old vs new object. No significant main effect of sex [f(1,55)= 3.426, p>0.05] on the preference for the old vs new object, and no significant genotype by sex interaction [f(1,55)= .019, p>0.05] was seen. See Fig 3.4
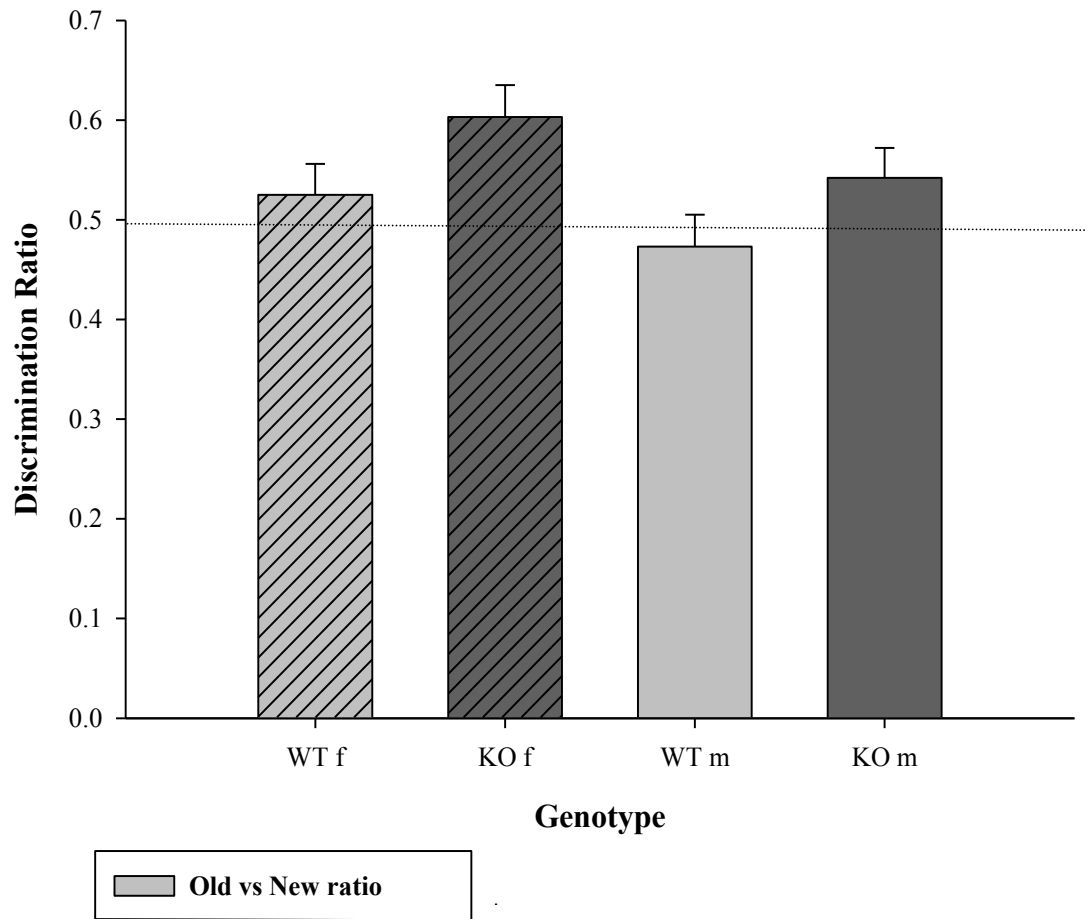
Figure 3.4 Graph showing discrimination ratios for old objects vs recent objects in male and female D2R WT and KO animals. Only KO female animals show a significant exploratory preference for the old objects vs recent objects (p<0.05).

What-where analysis using mean exploration times

A repeated measures ANOVA showed that there was a significant main effect of the familiarity of the old displaced and stationary objects on the on the exploration times of old displaced vs old stationary objects [$f(1,55)= 29.406$, $p< .001$]. A significant main effect of genotype [$f(1,55)= 7.642$, $p=.008$] on the mean exploration times of the old displaced vs stationary objects was seen. Furthermore, a genotype by sex interaction [$f(1,55)= 6.823$, $p=.012$] was also seen, but no interaction involving displacement was significant. This indicates there were no differences in memory between sex or genotype (Fig 3.5).
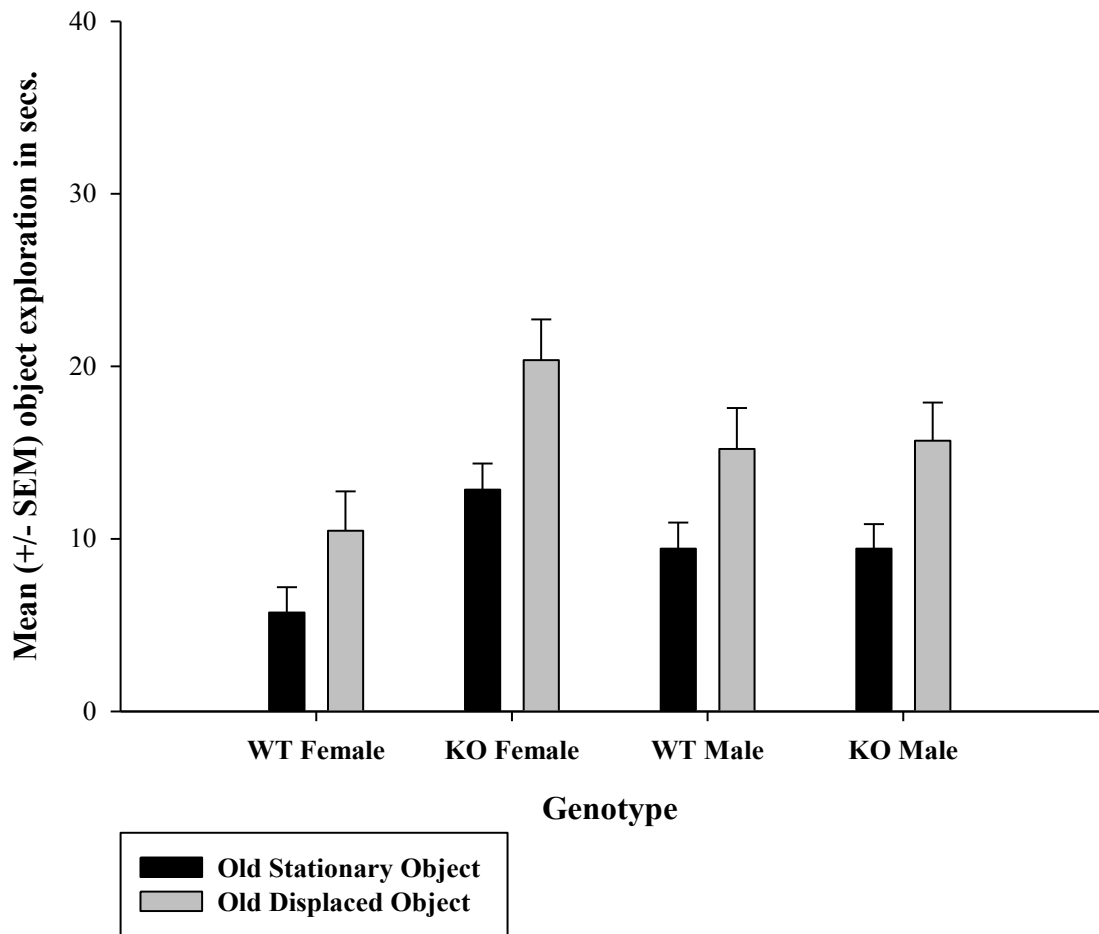
Figure 3.5 Graph showing exploration of spatially displaced old vs stationary old objects in D2R WT and KO animals. Female animals KO animals show a difference in exploration of the old displaced objects vs old stationary objects (p<0.05) and this effect is enhanced in the female KO compared to their WT littermates.

Recency discrimination: What where using discrimination ratios

A one way between groups ANOVA showed no significant main effect of genotype [f(1,55)= .005, p>0.05] or sex[f(1,55)= 1.254, p>0.05] on the preference for stationary vs displaced objects. No genotype by sex interaction on the preference for stationary vs displaced objects [f(1,55)= 1.046, p>0.05] was seen. One sample t tests indicated that WT female animals show a preference in exploration for the stationary vs displaced objects [t= -3.470,df=14, p<.0125] as do female KO animals [t= -4.226,df=13, p<.0125]. Male WT animals do not show a preference in exploration of the stationary vs displaced object [t= 1.853,df=13, p>.0125]; but male KO animals [t= 2.470,df=15, p<.0125] did show this preference in exploration. See fig 3.6
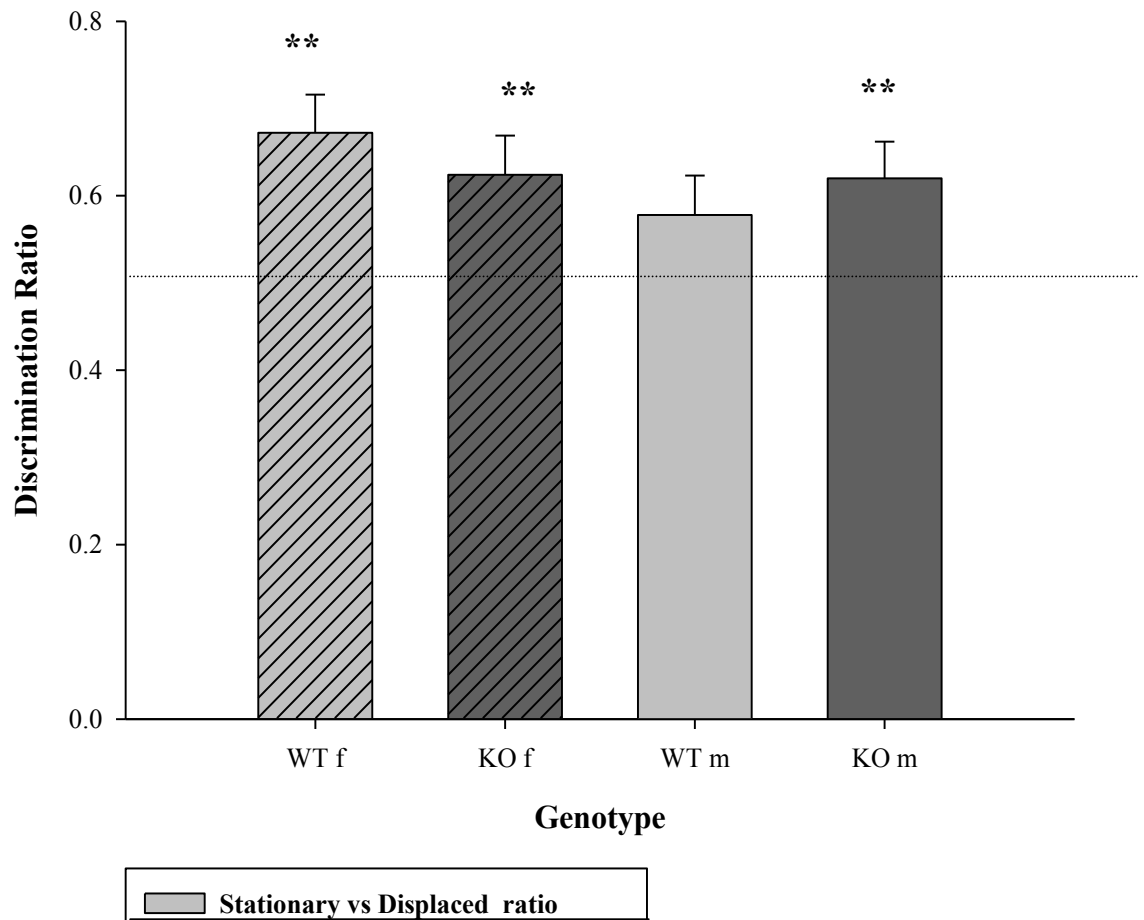
Figure 3.6 Graph showing discrimination ratios for spatially displaced old vs stationary objects in male and female D2R WT and KO animals. Only female WT and KO animals and male KO animals show a significant discrimination of the displaced objects compared to the stationary objects (p<0.05).

3.4 Discussion

Our results suggest intact spatial memory in D2R WT and KO mice on the what-where component of the episodic memory, but no object discrimination on the what - when recency mediated aspect of object recognition memory. D2R WT and KO animals show no differences in the exploration of the recency- oriented memory. An enhancement towards preference for the old familiar object over the recent object is seen in D2R female KO animals, but we are unable to make a conclusion regarding this due to a lack of discrimination in controls. Thus, we are unable to make conclusions regarding recency discrimination on the episodic memory task.

Our results further indicate that D2R deficient female mice show sex specific preferential exploration of the what where aspect of the episodic memory task, reflecting intact processing of this component of episodic memory. Furthermore, both male D2R WT and KO animals show preferential exploration for the old displaced object over the old stationary object, reflecting intact memory for displacement. Only female D2R WT and KO mice show intact spatial memory in their preferences for their memory for a displaced object that was not in its original position to when they had previously encountered it, where female KO animals showed an enhancement in their exploration of the displaced object as compared to their WT littermates. Thus, reflecting enhanced spatial memory for what and where in female D2R KO mice only.

In a recency based task of episodic memory (Dere, Huston et al.,2005) purport that rats and mice spend a greater proportion of time exploring the old objects as compared to the recent objects, and also spend a greater amount of time exploring the spatially displaced object compared to the stationary object. According to this, the

more recent object is explored less than the less recent (old familiar) object, as a representation of the less recent object still exists in memory. However, tests of novel object recognition involve greater preference of the novel object rather than the older, familiar one. Here, more exploration of an old familiar object indicates weak memory for this object not improved memory. Additionally, it is suggested that novelty preference does not wane over multiple exposures or over time (Ennaceur and Delacour 1988; Ennaceur 2010) rather, the memory for a familiar stimulus decays over time, thus making the older stimulus look novel. This encourages the exploration for the old, slightly familiar object as well as the never before seen novel object, which is manifest as equal amounts of exploration for each object or a short term increase in exploration of the old object. In line with this, D2R WT female mice have intact memory for the what and when recency component of the task.

We conducted a secondary analysis that investigated preferential exploration of the old objects vs recent stationary objects, to determine whether exploration was greater for novel/ more recent stationary objects as compared to older, familiar stationary objects, and that this exploration was not a consequence of the displacement of the older objects (See Appendix 4) This analysis shows that animals spend more time exploring the recent objects as compared to the old objects, which is in line with theories of novel object recognition, but not recency based discrimination. Thus, our results mimic the pattern proposed by Ennaceur et al., (2010) a lack of exploration of the old objects by D2R WT animals may be due to the increased exploration of the novel object, which is in line with previous literature.

It is suggested that the recency aspect in episodic memory encompasses the memory for 'what' a specific occasion was in terms of its elapsed time, as opposed to a single point in time i.e. 'when' (Eacott and Easton,2010), and is not a true measure of

episodic memory. It is also suggested that equal amounts of exploration for both the old and recent/new objects is not a failure in the ability to discriminate between objects (Ennaceur 2010). Rather, animals recognize both objects as familiar or are both explored as novel (as previously described), and therefore it is not possible to model the 'when' aspect. Therefore, the failure to discriminate between objects in male animals may have been interpreted differently, where equal or no differences in object exploration may not be a true indicator of learning.

On the measure of spatial memory i.e. the where component, D2R KO female mice did not show deficits in their preference for displaced vs stationary objects. Spatial memory has been previously assessed more as a measure of working memory by using tests such as the T maze, radial arm maze and the delayed matching non matching procedure. In our test, the stationary and displaced objects are presented in the same locations; in Sample 1, the old familiar objects are present in this location and in Sample 2 the recent objects are present in the same location. Thus, the context-place association does not change, only the objects do, thus making it difficult to ascertain if animals discriminate between object presentations at particular points in time. It is also suggested that the spatial displacement element involves recall of memories rather than a simple judgement based on familiarity as rats manipulate the location of the object compared to what had been previously experienced rather than learnt, which cannot be delineated as the temporal element – spatial element (context-place) does not change, thus, preference for the displaced object may be an artefact of place preference, rather than a true discrimination for the displaced object.

Paradigmatic differences aside, female D2R KO's are able to identify the displaced object based on their recall of the old objects (enhanced exploration for old objects), thus displaying intact episodic memory.

3.5 Conclusions

D2R WT nor KO's showed any preferences in exploration in the what- when aspect of the episodic memory task. However, intact spatial memory is seen in D2R WT and KO mice.

CHAPTER 4

LATENT INHIBITION IN C 57/BL6J MICE

4.1 Introduction

The LI paradigm has been extensively used as a model of inattention in schizophrenia. LI is a model of attentional salience, which requires an organism to ignore irrelevant stimuli. Subsequent non - reinforced pre exposures to a conditioned stimulus retard learning to that stimulus, as opposed to sufficient learning in a non pre exposed group. This difference in learning between the pre exposed group that shows poorer learning to the stimulus, and the non pre exposed group, that shows better learning reflects LI.

We used a conditioned emotional response paradigm that uses the suppression of behaviour to measure LI. The emotional suppression of eating or drinking behaviours as a consequence of the tone- shock (CS-US) pairing is used as an index of learning behaviour. An aversive shock is paired to a stimulus, to form a CS-US association, in a pre exposed (PE) and non-pre -exposed (NPE) group of animals. The PE phase involves pre exposure to a stimulus (tone) in the chamber, and the NPE group receives exposure to the chamber only. The conditioned stimulus consists of the tone and the unconditioned stimulus of a foot-shock. The test phase consists of the suppression of drinking behaviour, which is used as an index for measuring LI, where animals that learn the CS-US association (NPE group), and suppress licking behaviours as they learn to make the tone shock association and refrain from water consumption (Lubow, 2010;Weiner and Shadach,1996; Weiner and Feldon,

1992,Lubow, 1959) . However, animals that have been pre exposed to the CS, do not exhibit this learning, as they deem the tone as being irrelevant to the shock, and do not suppress licking behaviours. This paradigm has been repeatedly used in the majority of the literature; both rats and mice show LI (Nakajima, Ka et al., 1999; Chang, Meyer et al., 2007; Lipina, Weiss et al., 2007; Lubow 2010) .

However, in models of conditioned suppression, it is proposed that the aversive nature of the stimuli (loudness of tone) and the context (that it is presented in), may affect the conditioned emotional behavioural response (Davis 1972). The degree of aversiveness of a stimulus promotes a decline in the rate of stimulus responses, as a consequence of the stimulus being paired to a strong predictor (Azrin and Hake, 1969). More specifically, an aversive event (loudness of tone) or novelty (not having been pre exposed to the tone) may predispose to an anxiety like emotional state in the context the stimulus is presented in (Estes and Skinner,1941) and promotes behavioural suppression. Thus, it promotes a lack of responding behaviours in the NPE group, not previously exposed to the tone. Exposure to a tone in the test phase may deem the tone or context as aversive /novel stimuli to the NPE group, and lead to a suppression of behaviour. This mimics the suppression of behaviour as expected from NPE group animals in response to the tone shock association in LI. Additionally, animals may not form an association between two stimuli as they are suppressing behaviour to the stimulus itself, and not as a consequence of the associative CS-US properties, thereby promoting an artefactual LI effect, or unconditioned suppression.

In this chapter, the aim of the experiment is to verify that the LI effect occurs due to the PE-NPE association to the paired stimuli i.e. LI and is not attributed to the aversive/novel properties of the CS (tone) alone. Our paradigm consists of a non

contingent tone-shock pairing in the unconditioned suppression non contingent (NC) group and paired tone-shock in the NPE group. Animals in the NC group are hypothesised to not differ from animals in the PE group, as they do not learn to suppress behaviours, owing to a lack of association (non contingent pairing) between the tone and shock. Animals in the PE group would be unaffected due to prior pre exposure to the tone, and they do not deem it as aversive/ novel and show licking behaviours as predicted in LI. However, animals in the NPE LI group would show a suppression of behaviour and differ from the PE group as they have learned to make the association in of the tone being able to predict the shock. Thus, indicating that LI is not an artefact of the nature of the aversive stimulus or context but a robust index of learning an association between contingently presented stimuli, in our hands, and as seen in previous studies. This is also a pilot study that differentiates between the LI effect and unconditioned suppression, as a pilot LI study in the C57 strain of mouse.

4.2. Materials and Methods

4.2.1 Animals

18 naive male C57BL/6 mice obtained from Biomedical Services Unit, University of Nottingham Medical School, UK) were used in the study. At the beginning of the experiment, animals were 12-28 weeks old and were an average weight of 25-30 gms. They were housed three per cage, under a 12:12 hour light-dark cycle, maintained at constant temperatures and humidity with food available. The experiment was conducted in the light phase of the cycle. Mice were placed on a 23 hr water restriction schedule. All animals were maintained under ad lib food (standard chow, Harlan,US), and water was provided ad lib for 1 h per day. They were housed in a temperature and humidity controlled environment (22 degrees, 40-

60%) in the animal housing facility. All experiments were carried out in accordance to local and national rules, with appropriate project and personal license authority (Animals Scientific Procedures Act, 1986, PPL 40/2883).

4.2.2. Behavioural Testing

The LI protocol (Lubow 1959, 2010) comprised of six stages i.e. water restriction, pre-training, pre-exposure/non-pre-exposure, conditioning, re establishment of drinking, and testing.

WATER RESTRICTION: Day 1-7. Mice were placed on water deprivation schedule for 7 days prior to the pre training phase. In this period, the animals received water for 2 hours per day, every 23 hours. This regime was maintained throughout the experiment.

PRE-TRAINING: Day 8-13.Water was given in the test apparatus, in addition to the daily ration of 1 h delivered in the home cages. Mice were placed in the conditioning chambers and allowed to drink freely from a water sipper tube for 15 min. At the end of the session the animals received free access to water in their home cages.

PRE-EXPOSURE: Day 14. After the pretraining phase, mice were placed in the conditioning chambers with no water access and preexposed to the 85 dB, CS 60 PE's X 5 sec (high LI) tones with a 15 sec interstimulus interval. Non-pre-exposed control mice were placed in the chambers for the same amount of time but received no pre-exposures to the CS.

CONDITIONING: Day 15. Mice were trained with two pairings of the CS with a foot-shock as unconditioned stimulus (US; 0.38 mA, 1 s, 2.5 min inter stimulus interval) in the NPE (LI). After 5 minutes, two tone-foot-shock pairings were presented. Each tone was of 5 seconds duration, and followed by a .38 mA foot-shock.

Animals in the NC (non contingent) groups received unpaired tone and shocks (85 dB CS, US; .38 mA 1s). There was a 2.5 minute interval between the pairings in the NPE1 group and the unpaired stimuli in the NC group.

REBASELINE: Day 16-17. Mice were placed in the conditioning chambers for 15 minutes and given free access to the sipper tube to re-establish licking in the chamber prior to testing. Mice that did not lick consistently were omitted from the experiment at this stage.

TEST PHASE: Day 18. Mice were placed in the conditioning chambers with access to the sipper tube. A computer recorded the number of licks and the time to complete licks 80-90 (A) and 90-100 (B). After the first 90 licks, the tone conditioned stimulus (CS) was presented until the mouse reached lick 100. The measure of conditioned suppression was the time taken to complete licks 90-100 in the presence of the CS. A suppression ratio (SR) was calculated according to the formula A / (A + B) yielding a scale of 0 to 0.5. Low SR indicates good learning while high SR indicates poor learning of the association between the tone and foot-shock. LI is seen as higher SR in the PE group compared to the NPE group. See Fig 1c in introduction).

4.2.3 Apparatus

Training and testing was conducted in six identical light and sound attenuating conditioning chambers (21.6 cm X 17.8 cm X 12.7 cm, ENV-307W, MED Associates Inc., St. Albans, VT, USA). These were composed of Plexiglas walls in the front and back of the boxes, and two stainless steel sides. In addition to this, a metal floor grid was connected to a shock generator. Each box contained a ventilation fan, mounted the chambers to provide an inflow of air, and background noise (69 dB white noise). Moreover, a sonalert was mounted on the adjacent wall for delivering the CS (85 dB)

(ENV-323AW, Med Associates Inc., St. Albans, VT, USA). Each chamber was equipped with a removable drink spout located along the left wall. The lick spout was connected to a lickometer (ENV-250, Med Associates Inc., St. Albans, VT, USA) which recorded the number of licks made by the animal. The chambers were connected to a PC computer that employed MED-PC software (SOF-735, MED Associates Inc., St. Albans, VT, USA) to control stimulus presentation and record data.

4.2.3 Data Analysis

A suppression ratio (SR) was calculated, based on Time A and Time B. Time A consisted of the time taken to complete licks 80-90 (A) and time B was the time taken to complete licks 90-100 (B). After the first 90 licks, the tone Conditioned stimulus (CS) was presented until the mouse reached lick 100. The measure of conditioned suppression was the time taken to complete licks 90-100 in the presence of the CS. A suppression ratio (SR) was calculated according to the formula A / (A + B) yielding a scale of 0 to 0.5. A Low SR indicates good learning while high SR indicates poor learning of the association between the tone and foot-shock. LI is seen as higher SR in the PE group compared to the NPE group.

A one way ANOVA was conducted to determine whether there was a difference in learning (as indexed by a suppression of licking behaviour) in the PE, NPE (paired shock and tone) and NC (non contingent shock and tone) groups. Furthermore, post hoc tests were conducted to explore whether there was a difference in the SR in the PE vs the NPE group, and the PE vs the NC groups.

4.4 Results

Differences in Learning in the Pre-Exposed and Non PreExposed Group (paired) and Non Contingent Group2 (unpaired)

A one way ANOVA showed that there was a significant effect of exposure on the SR $[f(2,54)=11.961,p=.001]$.Furthermore, there was no significant main effect of genotype on SR $[(2,54)=3.038,p>0.05]$. There was a no significant main effect of sex on SR $[f(2,54)=1.533,p>0.05]$. There was also no genotype by exposure by sex interaction $[f(2,54)=.123,p>0.05]$. This indicates that both Nrg-1 WT and HET animals show intact LI. See Fig. 5.1.

Post Hoc tests (Fisher's LSD) indicated that there was a significant difference between the PE and NPE group (mean diff. $=.191,p=0.019$) and between NPE and NC groups (mean diff.$=.167,p=.029$), but not between the PE and NC groups (mean diff.$=.0235,p>0.05$). (See Fig 2.2)
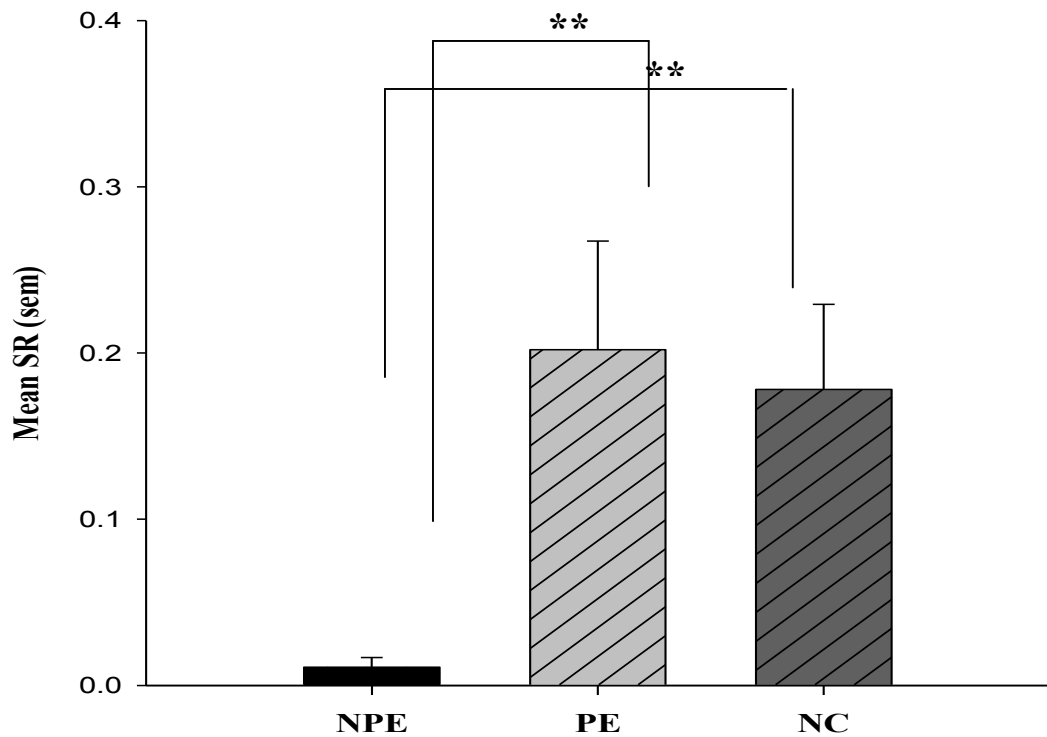
Fig.4.2 Mice show a robust LI effect, as indicated by the complete suppression of licking behaviour in the NPE group. Animals in the PE group do not learn the irrelevance of the tone presented, and thus do not show learning (p<0.05). LI is the difference in learning between the NPE group and PE. This is not an artefact of the aversive/novel properties of the stimulus alone, but of the CS-US association, as differences in SRs were also seen between the NPE and NC group (p<0.05), indicating that only the paired association of stimuli promoted associative learning.

4.5 Discussion

This data indicates that there is a difference in learning to suppress behaviour depending whether stimuli are paired or unpaired. In this experiment animals showed LI, as indicated by learning of the association to the CS-US contingent stimuli. No difference was seen between the PE and NC groups. However, a difference between the NC and NPE groups was seen, which jointly indicate that 1) the LI effect is a consequence of the learnt association between two paired stimuli, only and not due to the nature of the stimulus (unconditioned suppression) and 2) learning only occurred in the group that was presented with contingent stimuli and not non contingent stimuli, thus indicating LI is a consequence of learning to the CS- US relationship only. In our hands, pre exposure of 60X tone produced a robust LI effect as seen by a high suppression ratio in the NPE group and no suppression in the PE group. Our findings indicate that LI is a standalone phenomenon of associability that is not an artefact of the context or stimulus alone.

4.6 Conclusions

In this experiment animals showed LI, as indicated by learning of the association to the CS-US contingent stimuli only and not due to the nature of the stimulus (unconditioned suppression). Thus, LI is a robust phenomenon that relies on learning to make an association between the CS-US, rather than the aversive properties of the CS alone.

CHAPTER 5

LATENT INHIBITION IN NRG-1 WT AND HET

MICE

5.1 Introduction

Nrg-1 has been identified as a candidate gene for schizophrenia (Li, Collier et al., 2006). An association between the trans membrane domain Nrg-1 gene and schizophrenia has been identified (Walss-Bass, Liu et al.,2006) but the functional role of the TM-domain of the Nrg-1 gene in mediating cognitive disruptions in schizophrenia has not been extensively established. Nrg-1 mice show deficits in social interaction and social recognition memories, which are measures of the negative symptoms of schizophrenia pathophyisiology (O' Tuathaigh et al.,2007), but little is known about its contribution to positive or cognitive symptoms. We seek to investigate Nrg-1 as an endophenotype for Schizophrenia; whether it affects the entire schizophrenia syndrome or only distinct aspects of the schizophrenia phenotype. Using a similar behavioural approach to that used in D2R mutant mouse studies, we sought to investigate whether reduced function of the TM domain of the Nrg-1 gene, produces schizophrenia like cognitive impairments on measures of attention, sensorimotor gating and episodic memory. This experiment investigated the behavioural consequences of partially knocking down the Neuregulin-1 gene in producing disruptions on the LI task of conditioned inattention.

Nrg type 1 immunoglobulin-like (Ig) domain Heterozygous mice show deficits in LI compared to their wild type littermates on an ambulatory activity version of the LI task (Rimer, Barrett et al.,2005). The LI task consisted of 40 pre exposures to a tone, where LI was indexed by a measure of ambulatory activity. PE to a tone made the tone less likely to reduce ambulatory activity. However, this study did not have appropriate NPE controls and thus remains inconclusive. To date, studies investigating LI disruptions in TM domain Nrg-1 mutants (original strain was a 129 X C 57 cross) have not been carried out. Nrg-1 has been implicated as an at risk allele, that is associated with high risk for schizophrenia (Stefansson,2002), but studies have not investigated its role extensively in behavioural phenotypes relevant to schizophrenia. Furthermore, an LI paradigm that uses suppression of behaviour as an index of learning has not been employed in these mutant mice

LI is a good paradigm to model the disrupted attentional salience processes in schizophrenia, and has received robust validation as a pharmacological model in different mutant mouse models of schizophrenia (Bay-Richter et al.,2009; Clapcote et al.,2007). However, Neuregulin-1 has not been investigated in cognitive phenotypes relevant to schizophrenia pathophyisiology. Therefore we investigated whether disruption of the Nrg-1 gene, leads to disrupted LI, as a measure of attentional impairments seen in schizophrenia.

5.2 Materials and Methods

5.2.1 Animals

In the study, adult (8-12 weeks old) male and female WT (n=16) and HET (n=15) animals were used. Heterozygous Neuregulin 1 'knockout' mice were generated and imported from the Royal College of Surgeons, Dublin, Ireland. The original C57BL6

background strain mouse was backcrossed for 14 generations. These mice were originally generated at the Victor Chang Cardiac Institute, University of New South Wales, Australia, as described previously(Stefansson ,2002) and maintained on a C57BL6 background (14 backcrosses). Heterozygous mutants and wild type (WT) mice were generated from Heterozygous breeding pairs and the offspring were genotyped using polymerase chain reaction(O' Tuathaigh et al.,2007). Mice were housed into groups of three to five per cage and maintained on a standard 12 hour light: 12 hour dark cycle with ad libitum access to food and water. Mice were housed at constant temperature of 22 degrees in 45% humidity controlled environs. All experiments were carried out in accordance with and with appropriate personal and project licence authority under the Animals (Scientific Procedures) Act, UK 1986. PPL No: 40/2883.

5.2.2 Behavioural Testing
Refer to Chapter 4 (Section 4.2.2).

5.2.3 Apparatus
Refer to Chapter 4 (Section 4.2.3).

5.2.4 Data Analysis
Refer to Chapter 4 (Section 4.3).

5.5 Results

A one way ANOVA showed that there was a significant effect of exposure on the SR [f $(1,54)=11.961, p=.001$]. Furthermore, there was no significant main effect of genotype on SR [$(1,54)=3.038, p>0.05$] . There was a no significant main effect of sex on SR [f$(1,54)=1.533, p>0.05$]. There was also no genotype by exposure by sex interaction [f$(1,54)= .123, p>0.05$]. This indicates that both Nrg-1 WT and HET animals show intact LI. See Fig. 5.1.
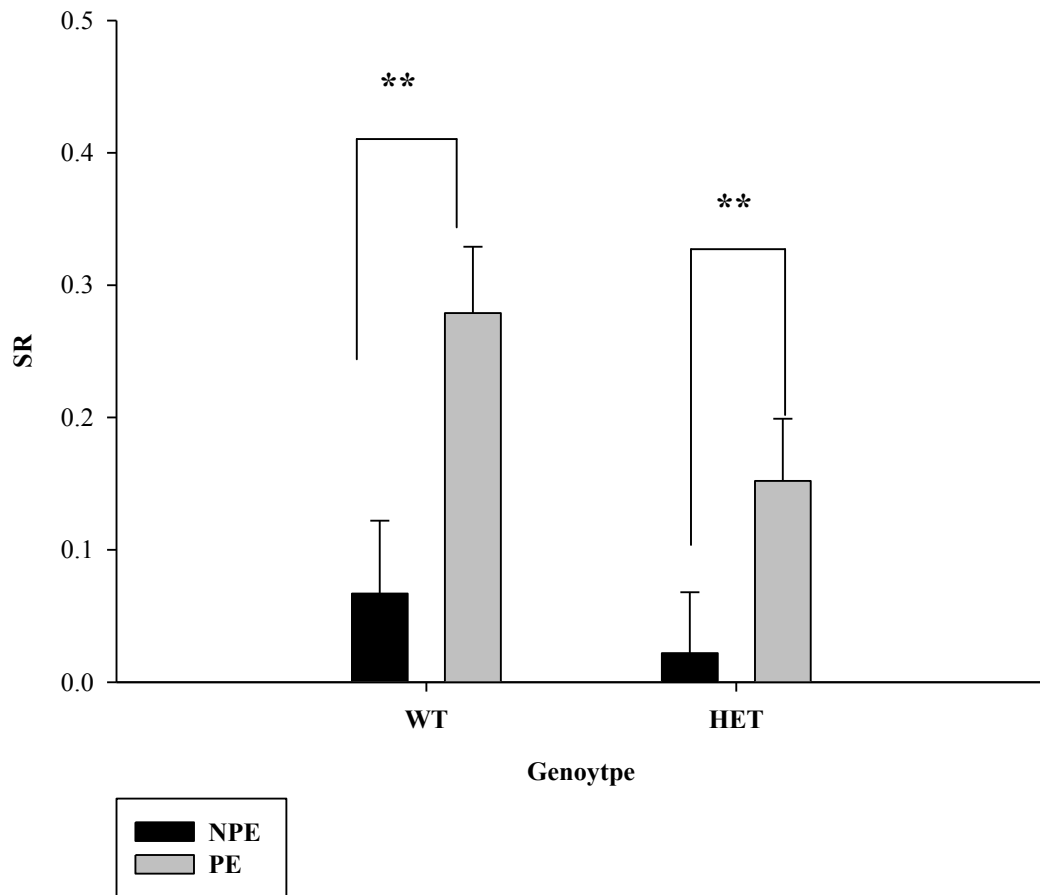
Figure 5.1 LI in Nrg-1 WT and HET animals. Both Nrg-1 WT and HET animal show LI at 60 PEs (p<0.05)

The data was also split by sex as sex specific cognitive deficits have been reported in previous studies in Nrg-1 mutants in phenotypes of relevance to schizophrenia (O'Tuathaigh, 2010). In female animals, a significant effect of exposure on the SR was seen [f(1,28)= 5.250, p=.03], indicating that there was a difference between the PE and NPE conditions (LI) in female animals. There was no significant effect of genotype on SR [f(1,28)= .191, p>0.05]. There was no genotype by exposure interaction [f(1,28)= .113, p>0.05]. Thus indicating that female HET animals did not exhibit differences in LI as compared to female WT animals. In male animals, a significant effect of exposure on the SR was seen [f (1,26)= 6.861, p=.015], indicating LI in male animals. In males there was a significant effect of genotype on SR [f(1,26)= 4.304, p=0.048]. There was no significant genotype by exposure interaction was seen [f(1,26)= .747, p>0.05] indicating that there was no impairemnet in LI in Nrg-1 Hets (Fig. 5.2).
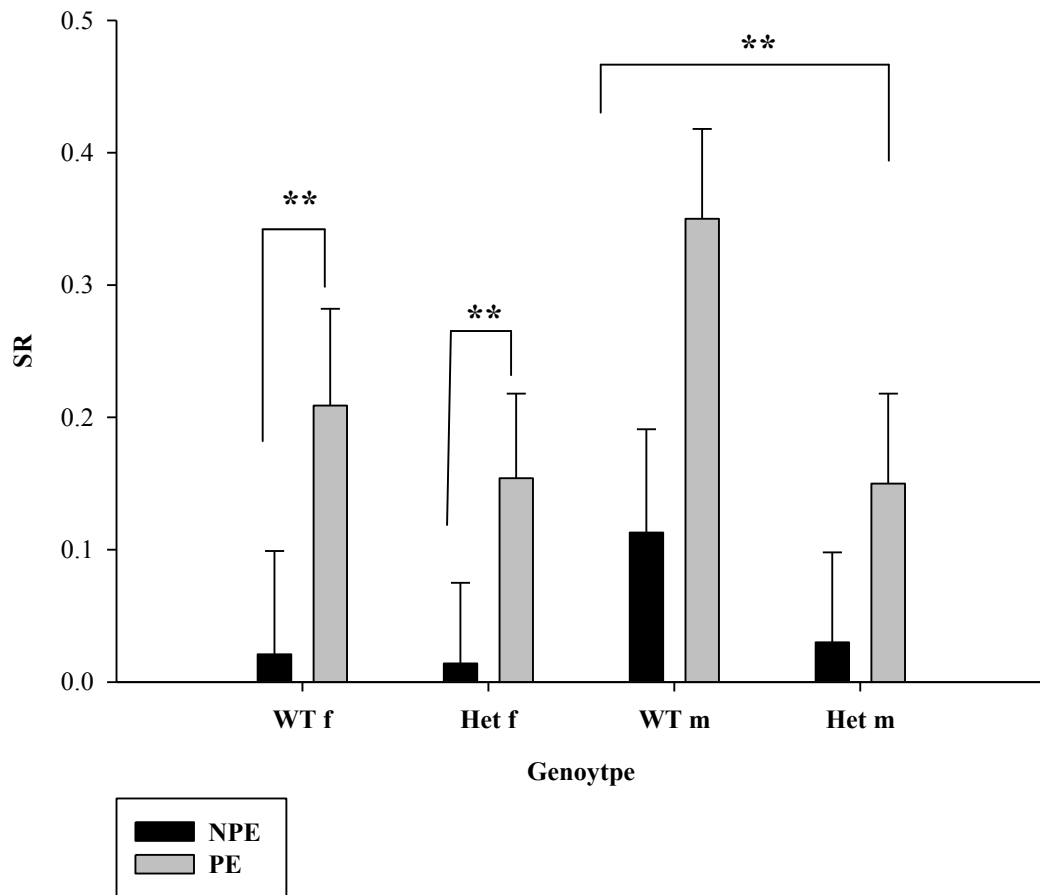
Figure 5.2 Graph expressing SR in the LI effect in Nrg-1 TM domain mutants. Split by sex, the analysis shows robust LI in WT male and female animals (p<0.05) and in HET female animals (p<0.05), but not in male HET animals (p>0.05).

An alternative way of measuring suppression of licking behaviours is to express it as the $\log_{10}$ of the T2 values based on time in seconds in NPE and PE conditions taken to resume licking after CS onset (Bay-Richter et al.,2009).

Using this measure a significant effect of exposure on the time taken to complete licks ($\log_{10}$ t times) was seen [$f(1,54)$= 11.643, p=.001] indicating that there was a difference in learning to lick in the PE vs. the NPE groups. There was no significant effect of sex [$f(1,54)$= 4.145, p=.05] or genotype [$f(1,54)$= 2.108, p>0.05] on $\log_{10}$ times. However, there was a significant genotype by sex interaction on $\log_{10}$ times [$f(1,54)$= 5.0343, p=.025]. No genotype by exposure by sex interaction was seen [$f(1,54)$= .878, p>0.05] indicating no differences in LI.
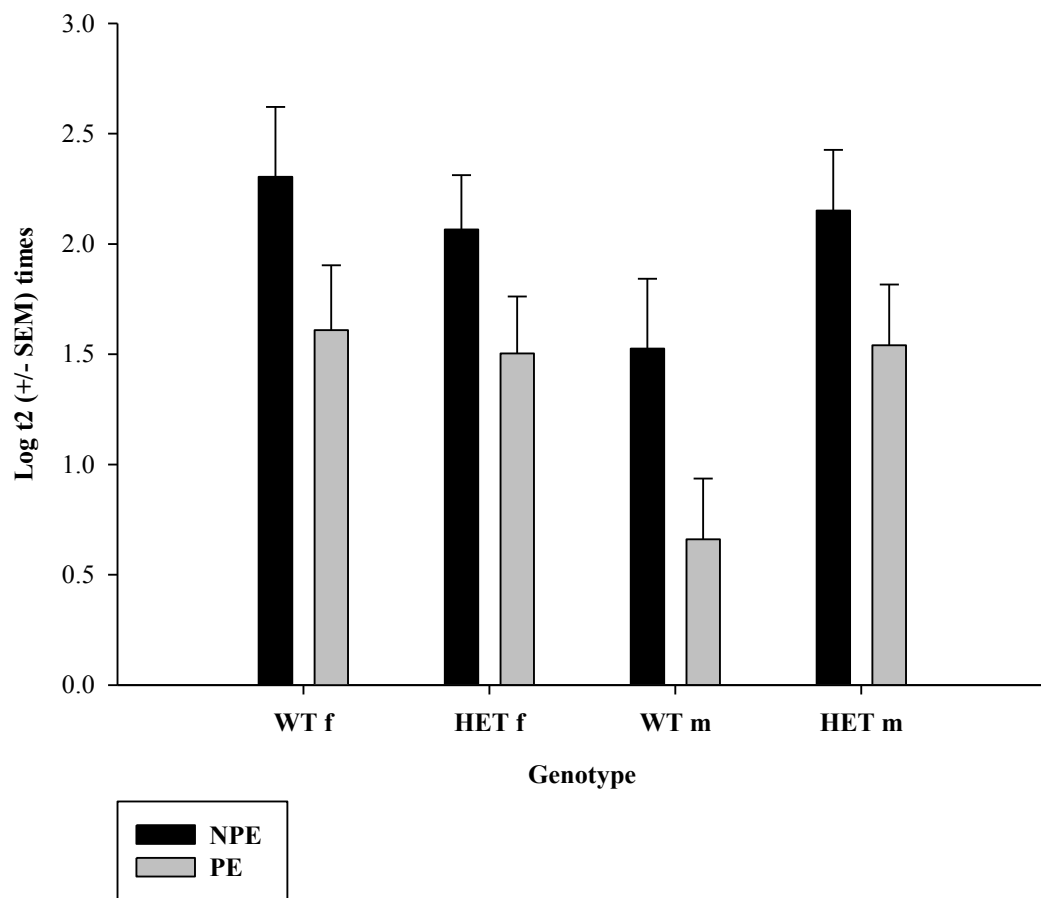
Fig 5.3 LI in Nrg-1 TM domain mutants as measured by $\log_{10}$ t2 values, indicating intact LI in female WT and HET animals (p<0.05).

5.6 Discussion

Our results indicate that overall both Nrg-1 WT and HET animals show LI,.Nrg-1 female WT and HET animals showed intact LI, as did male WT animals.

Previous studies have reported sex specific disruptions in TM domain male HET mutants. In the Barnes maze, learning is assessed by the latency to reach an escape hole and make errors in this process, and tests the ability of the mouse to encode information about the escape hole whilst it explores the maze. Male HET animals showed a disruption on this task; increased investigation of false escape holes, and a larger number of errors were made compared to female mutants(O' Tuathaigh et al.,2007). These were attributed to a disruption in attentional processes in the ability to encode information. These sex specific dissociations may reflect distinct information processing deficits that have been seen in schizophrenic males (Braff, Sedro et al., 1999; Roy, Maziade et al.,2001). Nrg-1 may be able to dissociate between sexes in behavioural phenotypes of schizophrenia, as has been seen consistently in tasks that measure the negative symptoms of schizophrenia.

The disruptive effects on cognitive tasks of relevance to schizophrenia by Nrg-1 may be governed differently by underlying neural mechanisms. The Nrg-1/ErbB4 receptor mediates the signalling of cortical GABA interneurons (Flames, Long et al., 2004), and is also involved in glutamatergic neurotransmission (Li B Woo,2007); where the frontal-cingulate cortical circuit is implicated in Nrg1 mediated function of attentional salience (Flames, Long et al.,2004) . It would be of interest to know whether other Nrg-1 isoforms inhibit GABAergic neurotransmission, as this could elucidate the mechanisms involved in aberrant Nrg-1 function. Alternately,

behavioural alterations on cognitive phenotypes of schizophrenia like behaviour by Nrg-1 isoforms have been attributed to mutations of targeted deletions at specific loci (Gerlai, Pisacane et al.,2000), but the exact mechanism by which Nrg1 contributes to disruptions in schizophrenia like behaviours is not clear.

In our study, dampened function of the Nrg-1 receptor leads to sex specific deficits in LI that suggests that Nrg1 may mediate cognitive behavioural phenotypes relevant to schizophrenia in a distinct sex specific manner.

5.7 Conclusions

Both WT and KO of the TM domain of Nrg-1 gene show intact LI.

CHAPTER 6

PREPULSE INHIBITION (PPI) IN NRG-1 WT and

HET mice

6.1 Introduction

This study carries on from our previous experiments that investigated role of the D2R in mediating sensorimotor gating in schizophrenia, as a cognitive endophenotype of schizophrenia like pathophyisiology. We investigated whether disruption of the TM domain Nrg-1 allele promotes deficits in a PPI task, as a cognitive phenotype of relevance to schizophrenia (Geyer et al.,2001).Nrg-1 has been associated with PPI. Reduced PPI has been reported in humans carrying the Rs3924999 mutation of this gene, and schizophrenic patients with abnormal PPI over express this mutation, compared to controls (Hong,Wonodiet al.,2008). Schizophrenia like phenotypes that are associated with the Nrg-1 gene vary depending on the Nrg-1 isoform that is disrupted. TM domain mice heterozygous for Nrg-1 show a hyperactive exploratory phenotype (Karl, Duffy et al.,2007)and Nrg-1 ErbB4 receptor knockout mice show hyperactivity and impairments in PPI (Wen, Lu et al.,2009). Alternately, TM domain Nrg-1 hypomorphs show deficits in PPI that are not reversed by clozapine (1 mg/kg) administration (Stefansson,2002), whereas Nrg-1 Ig domain mutants show a clozapine induced reversal of PPI deficits (Rimer, Barrett et al.,2005).

Schizophrenia relevant phenotypes are sex specifically mediated by the Nrg-1 gene; decreased PCP induced hyperlocomotion is seen in male mice Nrg-1 Het mice but not in females (O'Tuathaigh, Harte et al.,2010). Additionally, sub chronic treatment with either PCP or MK-801 results in pronounced sensitization in locomotor activity in female mutants only. Shifting of exploratory behaviour is also increased in female mutants but reductions in grooming behaviour are seen in the male mutants only (O'Tuathaigh, Harte et al.,2010). This suggests that the Nrg-1 TM domain allele may sex specifically dissociate for schizophrenia relevant phenotypes.

The role of the Nrg-1 TM domain gene in mediating PPI is inconclusive. It is suggested that PPI and PPI deficits may not be a robust replicable effect in Nrg-1 TM domain mutants. Rather deficits in PPI in Nrg-1 TM domain mutants are suggested to be protocol and site specific. No deficits in PPI were found in Nrg-1 HET mutants compared to their WT littermates; however reduced ASR in Nrg-1 HET animals was seen, but only at high levels of startle magnitude (110db, 115 db,120 db) (Karl, 2011). This disruption in PPI was specific to the intensity of prepulse magnitude used and the testing site, as different protocols at different testing sites did not establish replicable PPI or PPI disruptions in mutants Nrg-1 Het's. The PPI deficit that was seen in the original study TM domain Nrg-1 mutants (Stefansson,2002) has not been replicated (Boucher, Arnold et al., 2007, Van den Buuse, 2010).

We wanted to investigate whether the Nrg-1 knock-down mouse model shows schizophrenia relevant deficits on a sensorimotor gating task as a consequence of aberrant gene function, in mediating disruptions in cognitive phenotypes relevant to Schizophrenia. Following on from our Nrg-1 LI data, we also seek to determine whether sex specific dissociations are mediated by Nrg-1 on the PPI task of the gating of information.

6.2 Materials and Methods

Is the same as previous experiments in Chapter 2.

6.2.1 Animals

Animals are the same cohort as Chapter 5.

6.2.2. Behavioural Testing

Is the same as previous experiments that employed a two day paradigm in Experiment 2, in Chapter 2 (Section 2.5.1.2).

6.2.2.1 Apparatus

Is the same as previous experiments, refer to Chapter 2.

6.2.2.2 Acoustic Startle Session/ Prepulse Inhibition

Is the same as previous experiments, refer to Chapter 2.

6.2.2.3 Data Analysis

Is the same as previous experiments, refer to Chapter 2.

Percentage PPI was analyzed using a split plot ANOVA to compare differences in startle to prepulses in WT and HET animals. The computations were carried out using SPSS statistical software. Prepulse inhibition was the within subjects factor, with varying startle intensities (68 db, 72 db, 80 db and 90 db), with genotype (WT/ HET) as the between groups factor. Prepulses from the aforementioned subset of specific intensities were not administered in a particular sequence, but in a random order in the session.

6. 3 Results

% PPI in Nrg-1 WT and HET animals

A repeated measures ANOVA showed that there was a significant effect of prepulse intensities on % PPI [f(3,162)= 4.442,p=.005] . Furthermore, a significant main effect of genotype on % PPI was also seen [f(1,540= 5.722,p=.020]. Moreover, no genotype by PPI interaction was seen [f(3,162)= 1.683,p=.730] and no genotype by sex by PPI interaction [f(3,162)=.433,p=.730] was seen. See figure 6.1.

This indicates that % PPI was disrupted in Nrg-1 HET animals compared to their WT littermates.
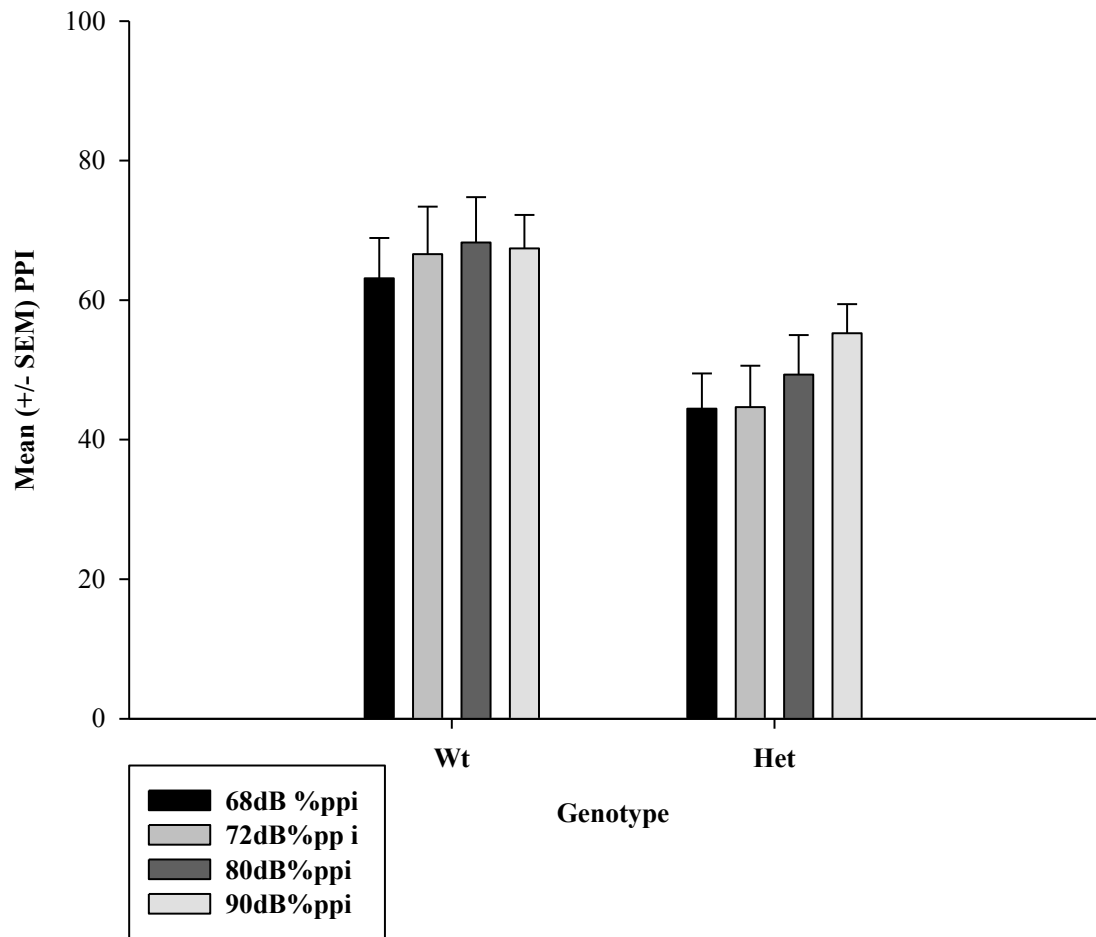
Figure 6.1 Diagram showing attenuated % PPI in Nrg-1 HET animals. A main effect of genotype was seen. % PPI was attenuated in Nrg-1 HET animals as compared to their WT littermates (p<0.05).

ASR to the 120 dB pulses in Nrg-1 WT and Het animals

A univariate ANOVA showed no significant main effect of genotype [$f(1,54)= 2.402, p>0.05$] or sex [$f(1,54)= 1.295, p>0.05$] on the startle to the 120 dB pulses. Furthermore, no genotype by sex interaction on the startle to the 120 dB pulses was seen [$f(1,54)= .242, p>0.05$].

ASR in prepulse+pulse trials in Nrg-1 WT and Het animals

A 2X4 repeated measures ANOVA showed that there was a significant main effect prepulse intensities [$f(3,162)= 7.867, p<0.001$. No significant effect of genotype on startle magnitude [$f(1,162= 1.085, p=.357$]was seen. Furthermore, no interaction of genotype and sex on startle magnitude [$f(3.162)=1.076, p=.361$] was seen. (Fig 6.3)
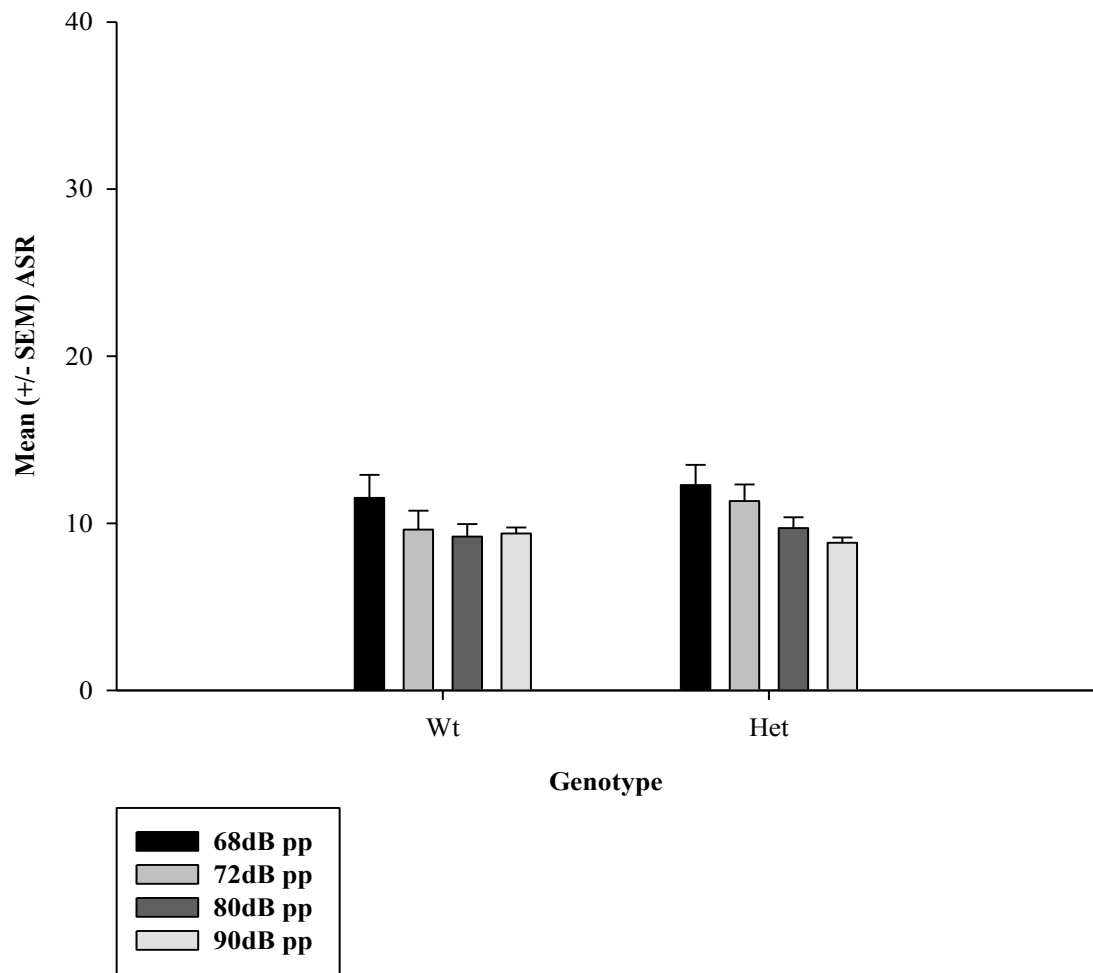
Figure 6.3 Graph showing ASR in Nrg-1 WT and Het animals. No significant main effects of genotype were seen on % PPI (p>0.05)

Habituation to the 120 dB pulses

A univariate ANOVA showed that there was no significant effect of genotype on % habituation [f(1,54)= 3.290,p=.075). Furthermore, no significant effect of sex on % habituation was seen [f(1,54)= 1.345, p=.251]. No interaction was seen of the effect of % habituation on genotype by sex [f(1,54)= 2.764, p=.102].When data was split by sex, no difference of the effect of genotype on PPI was seen in female [f(1,31)= .052, p>0.05] or male animals [f(1,23)= 2.667, p>0.05].See Figure 6.5.
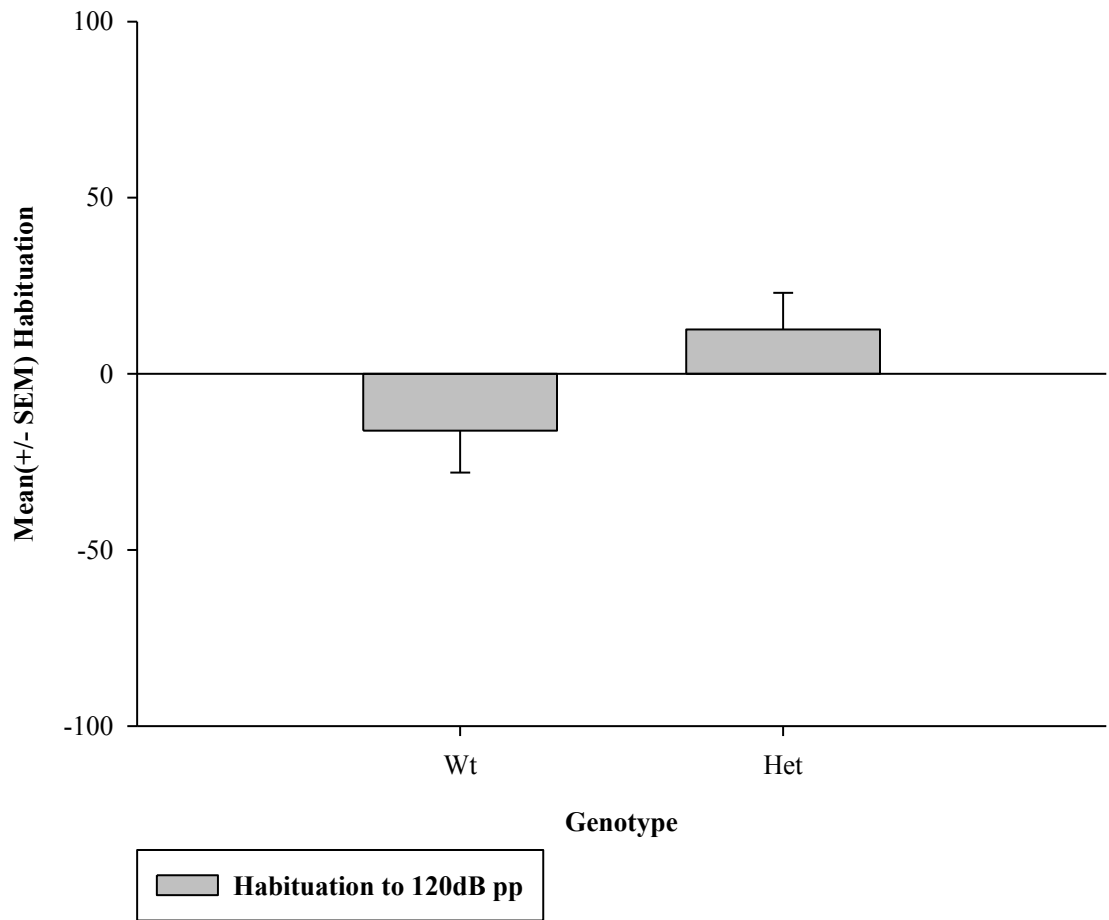
Figure 6.5 Graph showing habituation to the 120 dB pulses in Nrg-1 WT and HET animals.
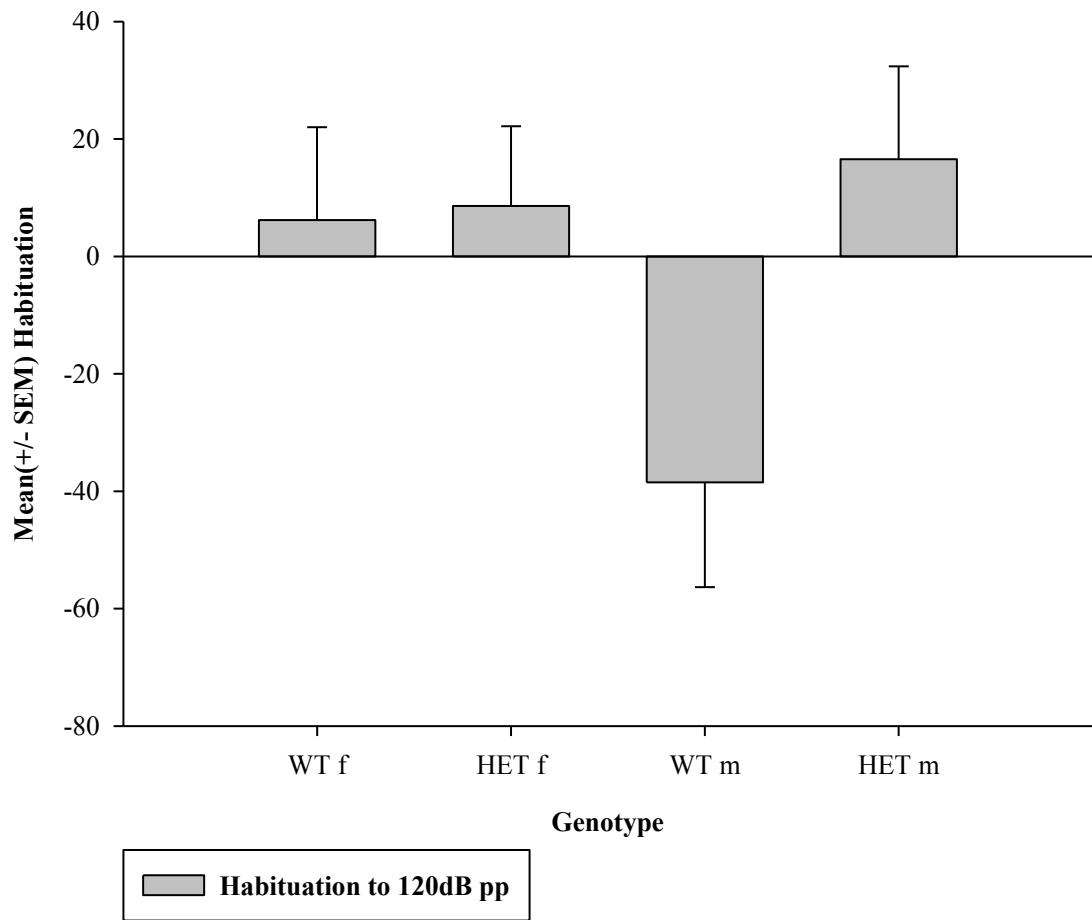
Figure 6.6 Graph showing habituation to the 120 dB pulses in Nrg-1 Wt and HET animals. No significant main effect of sex and no sex by genotype interaction are seen on habituation (p>0.05).

6.4 Discussion

Our results indicate that mutant mice Heterozygous for the Nrg-1 gene a) show deficits on the PPI task of sensorimotor gating compared to their WT littermates, and b) show robust baseline startle to the prepulses and c) no habituation or habituation deficits to the 120 dB pulses. More specifically, Nrg-1 HET mice show attenuated PPI as compared to their wild type littermates.

Previous studies that have looked at PPI in of Nrg-1 TM domain mice report mixed results. More specifically, no differences in the baseline startle response, PPI or startle habituation in Neuregulin-1 Het mutants has been reported (Van den Buuse 2009). Our results are consistent with other studies that have reported PPI disruptions in both TM domain and ErbB4 hypomorphs (Stefansson,2002).

The magnitude of PPI and disruptions in PPI are differently mediated depending on the protocol used. Different experimental protocols across experiments that produce weak or strong PPI, may not affect baseline startle (Swerdlow, Bakshi et al.,1996). In line with this, studies have found robust ASR but no PPI in Nrg-1 mutants (Karl 2011).Our study suggests PPI deficits at 68, 72 and 80 db prepulses in Nrg-1 mutants but not at a high intensity 90dB prepulse, and robust ASR.

Other studies suggest that apart from the requirement of prepulse intensity to be 5-10 dB above background noise to induce PPI and PPI disruptions (Sills et al.,1999), the inter trial interval between prepulses also mediates PPI. Female TM domain Nrg-1 animals show no alterations in PPI in a fixed inter trial stimulus paradigm, but in a variable ISI task they show enhanced PPI. Thus, TM domain Nrg-1 mice also show

sex specific dissociations, that are governed by the PPI protocol (Long, Chesworth et al.,2010).

Our results indicate that habituation to the 120 dB pulses in the Nrg-1 mutants is independent of PPI and ASR, as disruptions in habituation would also affect the magnitude of inhibition to the prepulses. This is corroborated by previous reports that indicated lack of habituation on a skin conductance behavioural response protocol that is associated with Nrg-1 phosphorylation AKT (marker for psychosis) was independent of PPI. In relation to schizophrenia symptoms, weak habituators show higher levels of delusions and anxiety as well as lower ratios of phosphorylated AKT, compared to strong habituators (Keri, Seres et al.,2011). This suggests that deficits in habituation may be associated with distinct positive symptom mediated phenotypes of schizophrenia like pathophysiology.

Our results indicate that disruptions in PPI are seen as a consequence of partially dampened Nrg1 functioning. Moreover, these disruptions may be mediated by the protocol employed. Nrg-1 may also in mediate distinct sex specific phenotypes of schizophrenia like symptoms on specific tasks of attentional salience and novelty ('O Tuathaigh, 2007) as previously reported, rather than sex specific phenotypes in all tasks of cognitive impairment in illness.

6.5 Conclusions

Nrg-1 TM domain mutants show deficits on the schizophrenia relevant PPI task of sensorimotor gating. Nrg-1 HET mutants show attenuated % PPI compared to their WT littermates, however, this may be subject to protocol specificity and prepulse intensities employed. This data suggests that partial deletion of Nrg-1 leads to disruption on the schizophrenia relevant phenotype of sensorimotor gating.

CHAPTER 7

EPISODIC MEMORY TASK IN NRG-1 WT AND

HET MICE

7.1 Introduction

This chapter explores the effect of reduced Nrg-1 function in an episodic memory task. The Nrg-1 gene is involved in the modulation of episodic memory in humans (Krug and Nöthen 2010) but has not been established in animal models assessing episodic memory. Spatial working memory is intact in Nrg-1 TM domain Het mutants as indexed by the Barnes and Y mazes (O' Tuathaigh et al.,,2007). Deficits in object recognition memory for a novel object have been seen in Nrg-1 TM domain Het mutants (Duffy, Cappas et al.,2010). These measures of novelty oriented memory and spatial memory suggest Nrg-1 involvement in memories for novelty driven memories but not in spatial object recognition. However, these tasks have not been extensively established in different Nrg-1 isoforms.

Nrg-1 mutants show certain sex specific behaviours; decreased PCP induced hyperlocomotion is seen in male HET mice but not in female Het mice (O'Tuathaigh, Harte et al.,2010). Locomotion, rearing and exploratory behaviour were also attenuated in both male and female Het mutants (O'Tuathaigh, Harte et al., 2010).

Although the neural circuitry involved in the modulation of memory tasks by the Nrg-1 gene are unknown, it is suggested that aberrant Nrg-1 functioning affects both

the dopaminergic and glutamate pathways in mediating memory disturbances. Nrg-1 mediates the consolidation and retrieval of memories in the hippocampus (Kempermann, Krebs et al.,2008) and the pre frontal cortex (Krug and Nöthen,2010). Consequently, it is suggested that dopamine neurotransmission in the mesolimbic regions may regulate the modulation of memory in the hippocampus (Dere, Pause et al., 2010), particularly if the episodic memory task encompasses a reward related/ novelty aspect. Thus, Nrg-1 mediates memories associated with novelty that may be affected by dopamine neurotransmission. Additionally, deficits in social learning, in Nrg-1 mutants also are attributed to NMDA receptor hypofunction in schizophrenia (Enomoto et al.,2007). Thus, Nrg-1 mediated disruptions in schizophrenia relevant phenotypes may be governed by complex interactions between dopamine and glutamate systems.

Following on from previous studies that report impairments in object recognition and intact spatial memory in Nrg-1 mutants, we seek to investigate whether disruption of the Nrg-1 gene leads to impairments on the recency mediated what-when and what-where object recognition components of an episodic memory task. It should be noted however, that the temporal element cannot be removed from the spatial memory aspect of this task, and this is not a standalone measure of spatial memory, but rather of spatio-temporal memory.

7.2 Materials and Methods
Same as previous experiment, refer to Chapter 3.

7.2.1 Animals

Same as previous experiment, refer to Chapter 3. Prior to this experiment, animals had undergone the PPI task 3 weeks after the LI study, and the episodic memory task 4-6 weeks after the PPI task.

7.2.2. Behavioural Testing
Same as previous experiment, refer to chapter 3.

7.2.3 Apparatus
Same as previous experiment, refer to chapter 3.

7.2.4 Data Analysis
Same as previous experiment, refer to chapter 3.

7.3 Results
Recency Discrimation: What when analysis using mean exploration times

A repeated measures ANOVA showed that there was a significant main effect of the familiarity of old and new objects in influencing the mean amount of time spent exploring the old familiar objects and the recent objects [$f(1,27)$= 28.905, $p<0.001$]. No significant main effect of genotype on the mean time spent exploring the old familiar objects and the recent objects [$f(1,27)$= .645, $p>0.05$] was seen. No genotype by sex interaction on mean amount of time spent exploring the old familiar objects and the recent objects [$f(1,27)$= .216, $p>0.05$]was seen.

To explore Nrg-1 mediated sex specific dissociations as informed by the previous literature (O' Tuathaigh et al.,2007,), on cognitive tasks as established in previous reports, two repeated measures ANOVA's split by sex were conducted. In female animals, a significant main effect of the familiarity of old and new objects in influencing the mean amount of time spent exploring the old familiar objects and the

recent objects [f(1,14)= 16.140, p=0.001]. No recency exploration (old vs new times) by genotype interaction on the mean amount of time spent exploring the old familiar objects and the recent objects [f(1,14)= 3.92, p>0.05] was seen. In male animals, a significant main effect of the familiarity of old and new objects in influencing the mean amount of time spent exploring the old familiar objects and the recent objects was found [f(1,14)= 13.559, p=0.002]. A recency exploration (old vs new times) by genotype interaction on the mean mean exploration time [f(1,14)= 5.729, p=.031] was seen reflecting memory impairment in the Het males.

Paired t tests split by sex were conducted. A significant difference in the amount of time male WT animals spent exploring the old objects as compared to the recent objects [t= 3.667,df=7, p<.0125] was seen. Furthermore, there was no significant difference in the amount of time male HET animals spent exploring the old objects as compared to the recent objects [t= 1.999,df=6, p>.0125]. Thus, indicating a disruption in the ability to explore the old vs recent objects in male HET animals as compared to their WT littermates. Figure 7.1
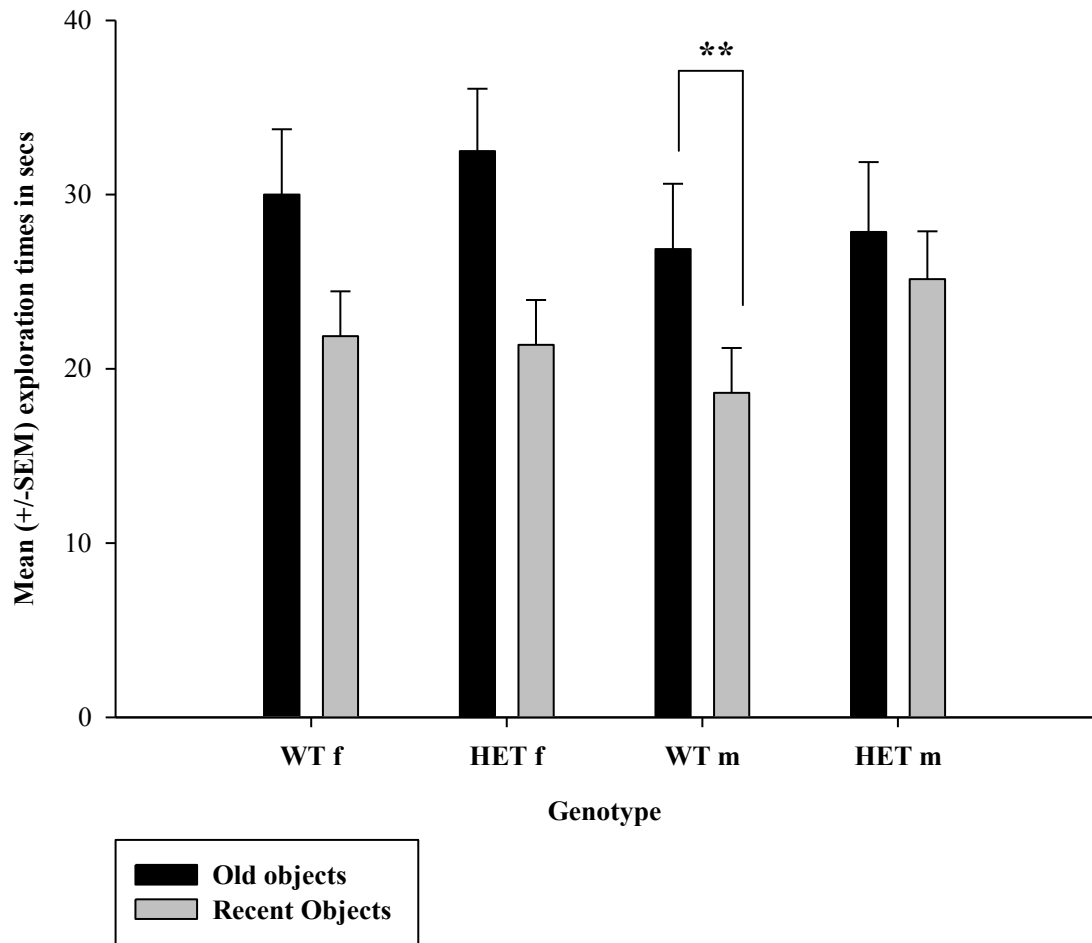
Figure 7.1 Graph showing exploration of old vs recently presented objects in Nrg-1 WT and HET animals. Male WT animals show differences in their preference for old or recent objects (p<0.05), but male HET animals do not indicate a preference for the old object or the recent object as compared to their WT littermates. Thus, indicating a subtle deficit in exploratory preferences for old vs recent objects in male HET animals. Female HET animals also show a enhanced preference in exploration of the old objects over the recent objects (p<0.05) in the 'when' component but we can't make any conclusions as no memory is seen in controls.

Recency Discrimation: What when analysis using mean discrimination ratios

A one way between groups ANOVA showed a genotype by sex interaction on the preference (discrimination ratios) for the old vs new object [f(1,27)= 4.796, p=.037].

The data was split by sex. There were no significant differences in female WT or HET animals in their preference for the old vs recent objects [f(1,14)= 1.417, p>0.05].However, a significant main effect of genotype was seen in male animals [f(1,13)= 5.745, p=.032] in their preference for an old vs new object. Thus, indicating that male HET animals differed in their preference of old vs new objects compared to male WT animals.

One sample t tests against the .5 chance level were conducted. They indicated that WT female animals did not show a preference for old vs new objects [t=.735, df=7,p>.0125]. However female Het animals did exhibit this preference [t= 5.026, df=7,p<.0125]. Male WT animals also indicated a preference of the old vs new objects [t= 3.680, df=7,p<.0125]. However, male HET animals did not show any preference [t= 2.286, df=6,p>.0125]. Thus, indicating that male HET animals have deficits in their memory for old vs new objects as compared to WT animals. See Fig 7.2
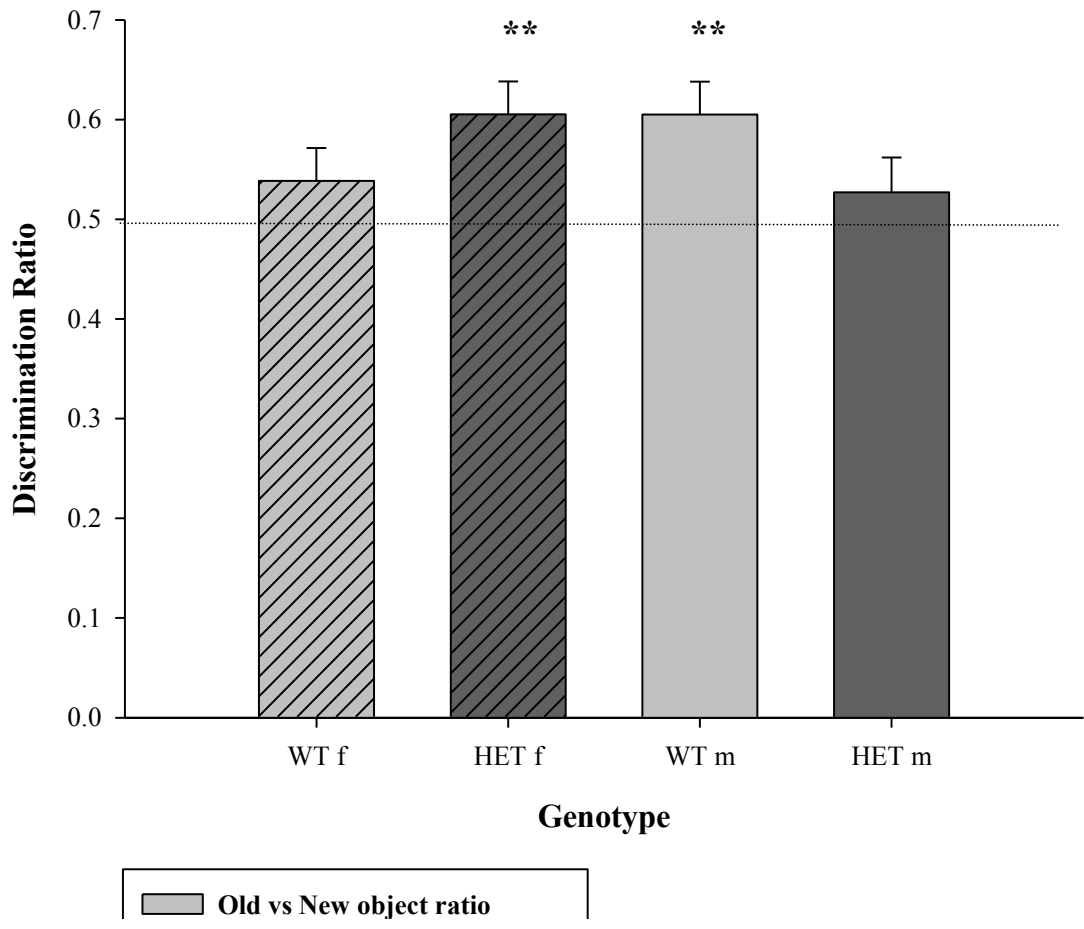
Figure 7.2 Graph showing discrimination ratios of old vs recently presented objects in Nrg-1 WT and HET animals. Male WT animals show a preference for old vs recent objects (p<0.05), male HET animals do not indicate a preference for the old object vs the recent object as compared to their WT littermates. Thus, indicating a subtle deficit in male HET animals. Female HET animals also show a trend for enhanced preference in exploration of the old objects over the recent objects (p<0.05) but no effect is seen in female WT animals.

Recency discrimination: What where using exploration times

A repeated measures ANOVA showed that there was no significant main effect of the displacement of old objects in influencing the mean amount of time spent exploring the old stationary objects and the displaced objects [$f(1,27)= 2.172$, $p>0.05$]. Furthermore, no significant main effect of genotype [$f(1,27)= .208$, $p>0.05$] or sex [$f(1,27)= 1.034$, $p>0.05$] on displacement was seen. No genotype by sex by displacement interaction [$f(1,27)= .247$, $p>0.05$]was seen (Fig 7.3).
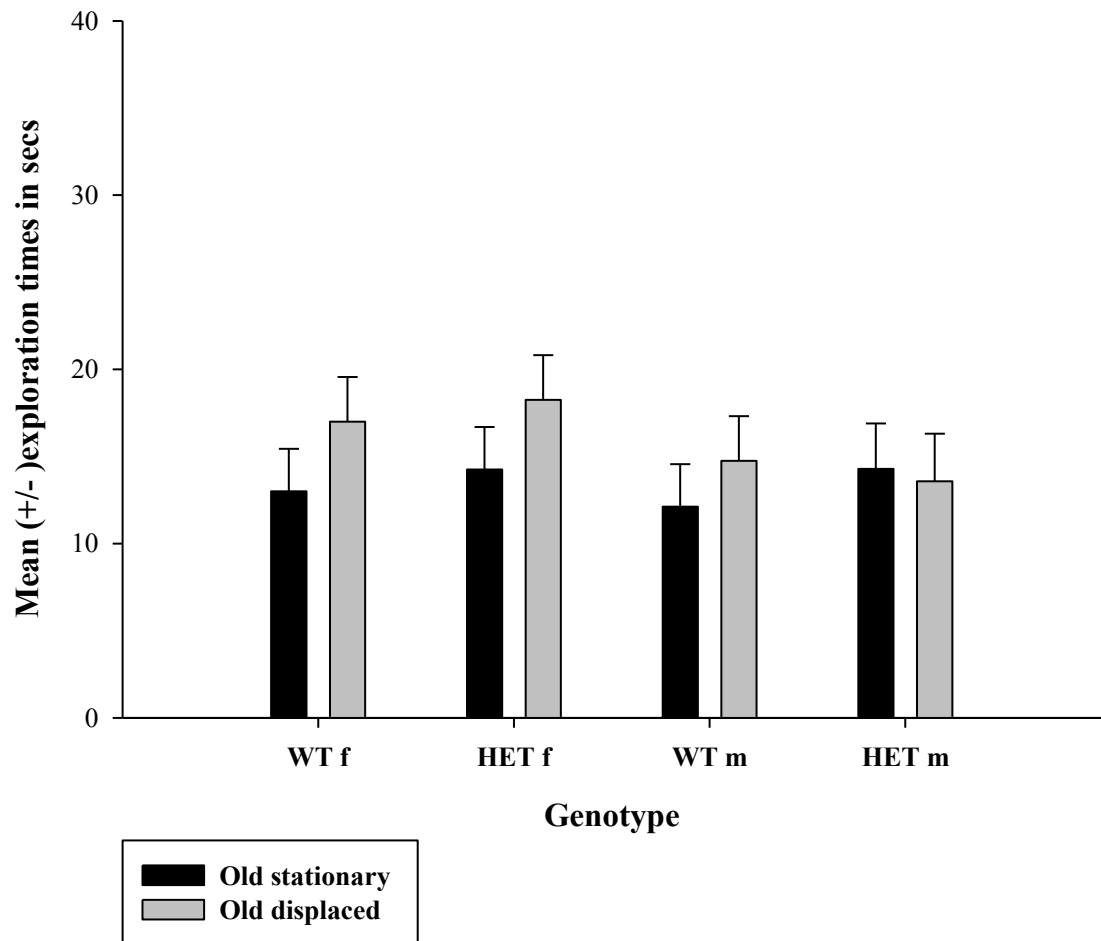
Figure 7.3 Graph showing exploration of stationary or displaced objects in Nrg-1 WT and HET animals. No significant differences in the exploration of stationary or displaced objects were seen in either Nrg-1 WT or HET animals. No sex specific differences were found in either genotype (p<0.05).

Recency discrimination: What where using discrimination ratios

One way between groups ANOVA showed no significant main effect of genotype [f(1,27)= .139, p>0.05] or sex[f(1,27)= .094, p>0.05] on the preference for stationary vs displaced objects. No genotype by sex interaction on the preference for stationary vs displaced objects [f(1,27)= .218, p>0.05] was seen. One sample t tests indicated that neither WT [t= -1.262, df=15,p>0.05] nor Het [t= -.684, df=14,p>.0125] animals showed an increased preference for the displaced vs stationary object (Fig. 7.4).
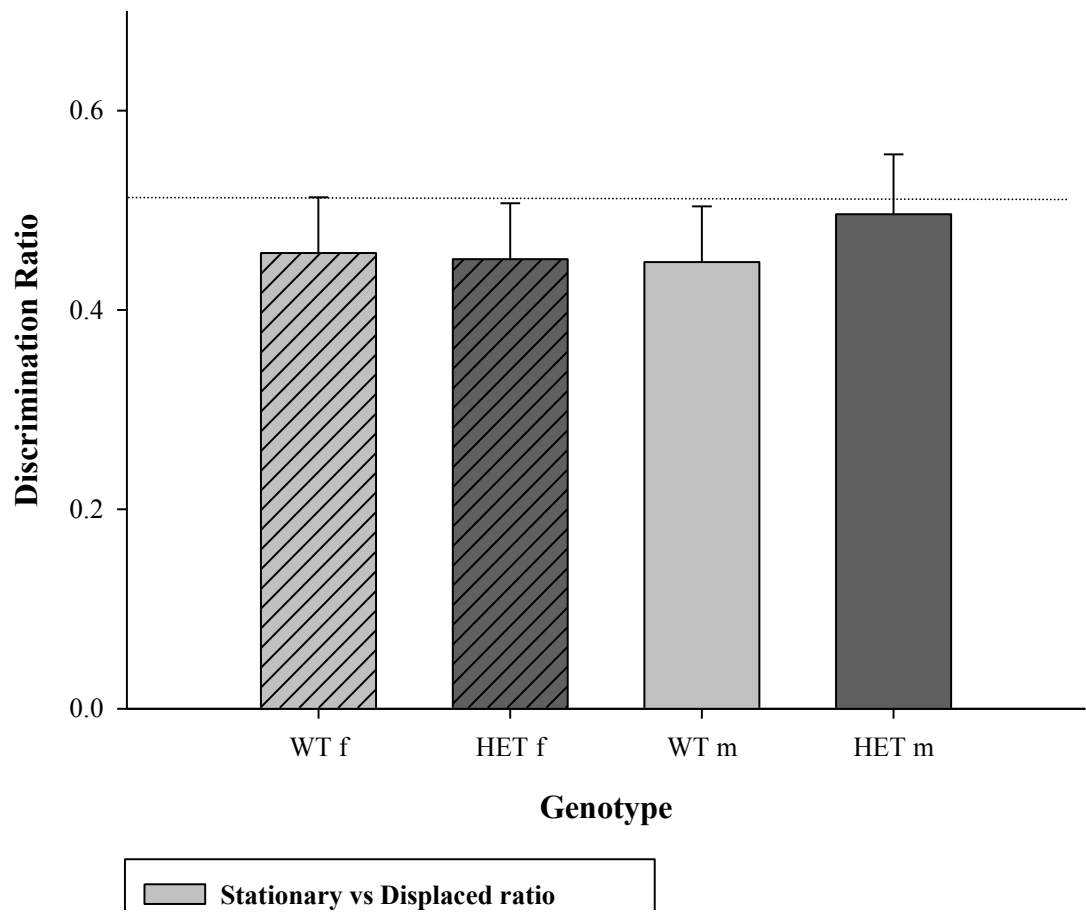
Fig 7.4 Graph showing discrimination ratios of stationary vs displaced objects in Nrg-1 WT and HET animals. No significant differences in the exploration of stationary vs displaced objects were seen in either Nrg-1 WT or HET animals. No sex specific differences in the discrimination for object displacement were seen in either genotype (p<0.05).

7.4 Discussion

Our results indicate that Nrg-1 Het animals show impairments in their memory for the what when component of the episodic memory task, which was marked by increased exploration for an old vs a recent object. No preferential discrimination of objects was seen in female WT animals. However, an enhancement for the exploration for old vs recent objects is seen in female HET animals, but we are unable to make a conclusion regarding this. It is to be noted, that the standard preference for the novel object was not observed in WT mice, as indicated in past literature (O' Tuathaigh et al.,2008).We cannot draw any conclusions with respect to the spatio temporal aspect of this task, as neither WT nor HET animals show any preference in exploration of the stationary vs displaced objects. There appears to be a trend toward intact spatio-temporal memory in Nrg-1 mutants and this is consistent with other studies (Duffy, Cappas et al.,2010), but not significant.

However, studies that report impairments in object recognition memory in TM domain mutants, do so on the rationale that increased exploration of the novel object reflects intact memory processes, where exploration of a less novel (old) object reflects an impairment in memory for the novel object (O' Tuathaigh 2007; Ennaceur 2010). However, our task adapted from Dere et al. (Dere, Huston et al.,2005) emphasizes on increased exploration of the old object based on recency. As the temporal component of recognition memory is always present in our task, it is not directly comparable to behaviours on a novel object recognition task, as both objects have been exposed prior to test, and are not 'novel', but defined in terms of their

recency of occurrence. Object exploration is thus defined in terms of recency discrimination for the objects, rather than a test of novelty.

However, it is suggested that novelty preference does not wane over multiple exposures or over time (Ennaceur and Delacour,1988; Ennaceur,2010) rather, the memory for a familiar stimulus decays over time, thus making the older stimulus look novel and facilitates the increased exploration of the older stimulus. Using an alternate 'which' contextual component, to mark a particular object at a particular point in time in an episode could be used in place of the 'when' component that measures object memory in terms of elapsed time (Ennaceur,2010). However, this is inaccurate as it still does not account for increases in exploration of an old object over a more recent one, and is not in line with novel object recognition. Additionally, the temporal occurrence of these objects may be confounded by place preference of the object, because it cannot be segregated from this analysis. Different objects (recent objects) are present in locations that were previously occupied by other objects (old objects); object B (recent object) occurs in the same place as object A (older object), at different points in time. This does not reflect a true, absolute temporal measure, or an absolute measure of place preference, as object occurrence at particular points in time are confounded by the different objects being present at the same locations.

The lack of exploration on the what where component, may be interpreted as a lack of learning in Nrg1 HET mutants (Dere, Huston et al.,2005). However, it has been proposed that equal or no differences in object exploration may not necessarily reflect that learning has not occurred; as it can be attributed to the temporary decay of memory or interest for a novel object, thus resulting in increased or equal amounts of exploration for an old object (refer to aforementioned comment about the waning

of a memory trace for the old object) (Ennaceur,2010). However, it is difficult to make a firm conclusion about the spatio temporal aspect as our control animals do not show significant exploration of displaced vs a stationary object. In the HET animals, these results may be confounded either the temporal point at which object occurred at object –place object was presented in isolation problem mentioned previously. However, there appears to be a trend toward for increased exploration of the displaced object as opposed to the stationary object in both WT and KO animals, which suggests intact spatio-temporal memory in these mutants.

7.5 Conclusions

These findings demonstrate that reduced function of TM domain of the Nrg-1 gene has sex-specific effects on episodic-like memory impairing the recency based discrimination in males and improving it in females. This suggests that the episodic memory model may have relevance for investigating memory dysfunction in schizophrenia, particularly in the context of sexually dimorphic memory impairment.

CHAPTER 8

GENERAL DISCUSSION

The original objective of this thesis was to investigate the effect of knocking out the dopamine D2R in mice on cognitive behavioural phenotypes relevant to schizophrenia. D-amphetamine induced disruptions on these cognitive tasks were also investigated in both D2R WT and KO animals. Furthermore, the role of another candidate gene that had been associated with schizophrenia i.e. the TM domain of the Nrg-1 gene was also investigated in cognitive phenotypes relevant to schizophrenia, in Nrg-1 Het mice. The dopamine D2R and Nrg-1 receptors are both susceptibility genes that have been associated with schizophrenia. Alterations in gene expression profiles manifest as deficits on behavioural tasks that are used as indices of schizophrenia like phenotypes. The cognitive tasks pertaining to attention i.e. Latent Inhibition and sensorimotor gating (PPI) and memory i.e. episodic memory were established in these mouse models as behavioural correlates of disrupted cognitive processes in schizophrenia. The results of this thesis1) demonstrate attenuated PPI in D2R KO mice as an attentionally mediated cognitive schizophrenia like phenotype; 2) show dissociable effects of one vs. two low doses of amphetamine in inducing PPI disruption in D2R KO and WT mice in a protocol dependent manner; 3) show deficits in D2R KO mice on the what-where component of the episodic memory task; 4) show intact LI in the TM-domain of the Nrg-1 HET mouse, with sex specific deficits in LI in male HET animals; 5) demonstrate attenuated PPI in Nrg-1 HET

animals; 6) show dissociable sex specific impairments in Nrg-1 HET animals on the what-when aspect of the episodic task.

8.1 One but not two injections of amphetamine disrupts PPI in D2R KO deficient mice

Two PPI protocols were used to investigate amphetamine mediated disruptions in D2R WT and KO mice. The first protocol consisted of a one day test session that investigated the effect of the administration of a single low dose injection of amphetamine in mediating disruptions in PPI. The second protocol consisted of a two day session; day 1 consisted of habituation to the chambers that were devoid of the stimuli, and day 2 consisted of the PPI test session. This protocol investigated the amphetamine induced disruption of PPI when a single low dose injection of amphetamine was administered (d1: saline, d2: amphetamine) as opposed to two single low dose injections (d1: amphetamine, d2: amphetamine). This single vs. two dose experiment was conducted to investigate whether low dose amphetamine mediated disruptions in D2R WT and KO mice are affected by the number of administrations of the drug, as seen in other schizophrenia relevant phenotypes.

As previously reported amphetamine induced disruptions in tasks of cognitive and attentional deficits in schizophrenia depend on the drug schedule adopted (Weiner, Lubow et al.,1988; Moran,1994). Thus, the one vs. two low dose injection experiment was conducted to elucidate whether amphetamine induced disruptions of PPI and LI are mediated similarly by the drug administration schedule adopted; to investigate whether two single low dose amphetamine injections administered 24h apart produce disruptions in PPI as they do in LI. In the first experiment, the protocol consisted of a one day test session, where animals were administered a single low dose of amphetamine prior to test. It was seen that D2R KO animals showed

attenuated % PPI compared to their WT littermates that precluded comparisons with treatment.

A trend toward amphetamine induced disruption of PPI was seen in the D2R KO animals, but this was not significant. In the two day paradigm, a single low dose of amphetamine disrupted % PPI in D2R KO animals, but two injections did not disrupt % PPI in either genotype. Conversely, two injections of amphetamine augmented % PPI in the D2R KO compared to animals treated with a single dose, but these findings were not significant against D2R KO saline treated controls.

These results suggest that amphetamine induced disruption of LI and PPI may be differentially mediated by the D2R KO depending on the injection schedule adopted. As previously mentioned, low doses of amphetamine (1mg/kg) disrupt latent inhibition and produce locomotor stimulation via the nucleus accumbens or ventral striatum (Weiner et al.,1988,Warburton et al.,1993.Bay-Richter et al. 2008: Gray et al. 1991). High doses (5 mg/kg) on the other hand, lead to stereotypy via the dorsal striatum and nucleus accumbens (Weiner et al.,1988, Joseph et al. 2000, Gray et al. 2005). Consequently, low dose amphetamine induced PPI disruption has been attributed to augmented dopaminergic activity in the mesolimbic regions (Swerdlow, Mansbach et al.,1990). High doses of amphetamine disrupt PPI in D2R WT mice only (Ralph, Varty et al.,1999). At low doses, amphetamine is coupled to impulse flow, and promotes augmented release of DA in the C57 mouse strain (Ventura et al., 2004). At high doses, amphetamine becomes uncoupled to impulse flow; and promotes preservative/restricted behaviours in this strain (Ralph et al., 2001). Consequently, dopamine depletion in the nucleus accumbens, olfactory tubercles and anterior striatum reverse amphetamine induced disruption on PPI. These studies

suggest that low and high doses of amphetamine interact with different neuronal mechanisms dose dependently to produce specific behavioural alterations.

A low dose of amphetamine was used in this study to avoid the effect of unknown compensatory mechanisms in mediating deficits in PPI in D2R KO animals and amphetamine's disruptive effects on PPI;. Activation of the serotonergic and adrenergic neurotransmitter systems by amphetamine augments motor activity and produces alterations in behaviours (Segal and Mandell,2002) at high doses. These behavioural alterations are governed by the drug administration schedule as well as the dose; accumulation of d-amphetamine in non adrenergic neurons following multiple amphetamine administrations promotes a reduction in brain norepinephine (adrenergic system neurotransmitter) levels and produces behavioural augmentation and stereotypy as a consequence of amphetamine accumulation in the brain (Browne and Segal,1977). However, it is suggested that repeated amphetamine administration induced behavioural responses may be subject to other factors during testing such as the housing environment and isolation rearing (Browne and Segal,1977). Rats were socially isolated by being individually housed in the experimental test chambers, and were placed in the chambers three days prior to 4 daily injections of saline or d-amphetamine (2.5 mg/kg). These were then followed by a single administration of amphetamine on the fifth injection day. Behavioural augmentation was seen after a single injection of d-amphetamine only, and no augmentations were seen in response to saline in the five days separating the first and second amphetamine injections.

No alterations in behaviour were seen in the amphetamine pre treated group compared to saline controls, indicating that social isolation factors, acclimatisation to test chambers or administration of injections do not affect amphetamine mediated augmentation of behaviours.

This lack of behavioural augmentation was attributed to state dependency and the same injection protocol was further assessed in rats housed in one of three different environments; singly housed in test chambers, singly housed in home cages or group housed in (6-8 per cage) in home cages. It was seen that pre treatment with amphetamine lead to a rapid onset of behavioural augmentation that was independent of the environment the animals were housed in, and indicates that behavioural augmentation is governed by the number of amphetamine administrations alone.

This corroborates our findings that suggest drug schedule dependent dissociations between one and two low dose mediated disruptive effects of amphetamine. Additionally, our findings suggest that prior conclusions about the requirement of the D2R for amphetamine's effects in PPI may not generalise to all doses, but rather, is dependent on the injection schedule adopted.

Alternately, it is suggested that amphetamine based disruptions on PPI are also governed by the PPI protocol employed. Amphetamine affects dopamine release in the mesolimbic region between a 1-3 hour time window (Gold, Swerdlow et al., 1988). Low dose amphetamine treatment (0, 0.3, 1, or 3 mg/kg) attenuates PPI when tested 10 minutes after a single administration, and when the prepulse stimuli are 5 dB above 65dB of background noise. These disruptions are seen within a narrow 10-40 min time when amphetamine is administered prior to test, but only high doses disrupt PPI when administered at delays of 40-70 minutes prior to test. Low or high doses of Amphetamine 60-90 min prior to test, do not disrupt PPI (Sills, Onalaja et al.,1998). Our results are consistent with the literature, as our protocol falls within this 10-40 min time window and produces PPI disruptions at a low dose in animals were tested 30 minutes after amphetamine treatment in both protocols, and where prepulses were 3 dB-25 dB, and therefore sufficiently over background noise. This

indicates that longer delays between injection and test prevent amphetamine induced disruptions in PPI. Additionally, Prepulse magnitudes are required to be sufficiently above background noise to influence amphetamine disruption of PPI. Thus, PPI disruptions by amphetamine are subject to the task paradigm as well as the injection schedule employed.

PPI studies in Nrg-1 mutants suggest that PPI is mediated by the protocol used (Karl et al.,2011). In our initial experiment, attenuated PPI was seen in drug naive D2R KO animals in the one day test protocol. However, in the two day protocol, PPI was not attenuated in D2R mutants, suggesting that habituation to the test chambers may influence PPI magnitude. This suggests that a longer habituation to the test chambers may produce more stable PPI. As attenuated PPI is only seen in the D2R mutants treated with a single low dose of amphetamine, the two day protocol and drug schedule together may mediate PPI and disruptions in PPI.

However, our analysis of the pulse alone trials suggests that we regard the interpretation of sensorimotor gating deficits with caution. Amphetamine treatment reduced startle reactivity to the 120 dB pulse alone trials, but left startle to the prepulse+pulse trials unaffected. It is suggested that this lack of reduction in the prepulse+pulse trials may be a floor effect, of the amphetamine induced disruption of the pulse alone trials (Swerdlow et al.,2000). The authors suggest that this floor effect may be absolute wherein, the startle magnitude is at its lowest to the pulse alone trials, thus leaving the prepulse+pulse trials unaffected; or relative wherein the magnitude in the lower range is more resistant to reduction relative to magnitude in the higher ranges (Swerdlow et al.,2000). In the one day protocol, amphetamine did not affect startle to the pulse alone trials, but there was a trend toward disruption of %PPI at the lower prepulse intensities, indicating that there may be a true disruption

toward sensorimotor gating. However, in the two day protocol, relative floor effects may be seen, as a consequence of attenuated startle reactivity to the 120 dB pulse and no change to the startle reactivity to the prepulse+pulse trials. However, different DA receptor subtype agonists predispose to genetically mediated differences in startle to the pulse alone and pre pule+pulse trials depending on the D1 or D2 receptor that is stimulated (Swerdlow et al.,2000) which may account for these amphetamine induced disruptions in startle reactivity in the pulse alone trials. However, the data must be interpreted with caution with regards to whether amphetamine truly disrupts sensorimotor gating or if these attenuations in % PPI may be attributed to relative floor effects.

Thus, amphetamine induced disruption of PPI by a single dose only may be a non D2R dependent phenomenon, but disruption of PPI by two doses may require the D2R. We conclude that these amphetamine mediated low dose disruptions in PPI are differentially mediated by the number of amphetamine administrations as well as the protocol employed.

8.2 D2R mutants show sex specific impairments in the 'what' and 'where' but not the 'what' and 'when' components of episodic memory

An episodic memory task that assessed the memory for object discrimination as indexed by the memory for 'what' the object was, 'where' it occurred and 'when' at a point in time it occurred, was conducted in D2R mutants. As previously mentioned, impairments in spatial and episodic memory have been well documented in schizophrenia patients (Park, Püschel et al.,1999; Tendolkar, Ruhrmann et al.,2002), and thus an episodic memory task was established in the D2R deficient mouse model, to investigate whether disrupted function of the D2R mediates memory deficits in this task.

In our experiment, male and female D2R WT animals did not show any preferential discrimination for recency mediated object recognition (what-when). Female KO animals show preferential exploration of old object, but no conclusions could be made due to a lack of discrimination for objects in D2R WTs. However, on the spatio-temporal component of this task, D2R WT animals discriminated between the old stationary vs. the old displaced objects; as did D2R WT and KO animals show intact memory for the displaced object, as seen by preferential discrimination of the displaced object. Thus, our analysis indicates that spatial memory is intact in both D2R WT and KO animals.

Our paradigm was based on the temporal element of memory that incorporates the relative order of recency at which the objects were seen (Dere, Huston et al.,2005) as opposed to memory for novelty on a one trial object recognition task. Animals are exposed an object (object B) in Sample 2, which is novel in terms of its recency to Object A presented in Sample 1. Thus, animals receive exposure to Object B which occurs at a more recent point in time, and is therefore not completely novel in test. It is suggested, that WT control animals would show increased preference of the older object that indicates the older object was remembered as having occurred at an earlier point in time (Dere, Huston et al., 2005; Dere, Kart-Teke et al.,2006).In this recency -oriented task, rats and mice spend a greater proportion of time exploring the old objects as compared to the recent objects, and also spend a greater amount of time exploring the spatially displaced object compared to the stationary object. In tasks that measure memory for objects based on their temporal recency, the more recent object is explored less than the less recent (old familiar) object, as a representation of the less recent object still exists in memory. This increased

exploration of less recent objects is not consistent with theories of novel object recognition, and is discussed in detail in the next section.

Secondly, female D2R KO animal show intact spatial memory, with a trend toward intact spatial memory in male D2R WT and KO mice indicating that this spatio temporal task is not impaired following D2R deletion, thus indicating that the consolidation of spatial memories may not be D2R dependent.

However, spatial memory deficits have been reported in rats administered dopamine D2R KO antagonist raclopride (Wilkerson and Levin,1999), and delay dependent intervals in spatial memory tasks promote disruptions in D2R KO mice (Glickstein, Hof et al., 2002). D2R KO mice show slower learning of place recognition and show partial alterations in their coding of spatial information to an open field. Consequently, marked decrements in signalling for reward as reflected by slower acquisition of place reward associations are also seen in D2R KO mice in comparison to their WT littermates (Tran, Tamura et al.,2003). This indicates that D2R blockade disrupts spatial memory tasks as well as spatial tasks that rely on place-appetitive reward stimulus properties.

However, in our task, it is impossible to separate the temporal aspect from the spatial task, and obtain an absolute measure of spatial memory, as memory consists of the consolidation of information about an object based on both its spatial properties and its recency. Furthermore, recent objects are present in locations that once contained the old objects, making it difficult to separate recency based discrimination of objects from place based preference. Thus, as the context-place association does not change, only the objects do, it is difficult to ascertain whether intact spatial memory is due to displacement of an old object, a consequence of

preferred exploration of old vs. recent objects; or if this reflects a preference in place due to increased exploration of the recent object vs. the old objects (Refer to Appendix 4).

Previous reports indicate that DA dissociates between novelty based object preference and place based preference for novel stimuli. D2R antagonist Eticlopride produces impairments in place related preference for a novel object, at a low dose that also impairs novelty based object preference. However, the D1R antagonist SCH 23390 induces impairments in place preference for the object only(Besheer, Jensen et al.,1999). These studies using D1 and D2R specific antagonists indicate dissociation between novelty preference and spatial discrimination mediated object discrimination memories. This suggests that in our task these D2R mediated dissociations may not be apparent due to the spatio-temporal nature of our task that involves both place and recency mediated memories, which make it difficult to separate dissociations in terms of spatial discrimination or recency preference alone.

Studies that investigate spatiotemporal memory with regard to the DA system suggest that the hippocampus and PFC are integral in the activation, processing and reconciliation of information about past and present environments (Wall and Messier, 2001). This activation and consolidation of memories may be mediated by D1R in the PFC (Glickstein and Schmauss,2002). Consequently, the PFC circuits are mediated by glutamatergic NMDA and AMPA channels that promote excitatory synaptic dopamine transmission, and mediate spatial memories (Tanaka,2002). This suggests differential modulation of spatio-temporal memories may not be limited to D1 and D2R KOs alone.

A lack of exploration on the what–when recency component in our task by D2R WT and KO animals may be a consequence of increased exploration of the more recent object as opposed to the old object as indicated by our secondary analysis that investigated exploration rates for the old stationary object only vs. the recent objects, to determine if displacement of the old objects (place) confounded exploration of the stationary old vs. recent objects (recency) (See Appendix 4).Thus, our results mimic the pattern proposed by (Ennaceur and Delacour,1988) where animals show increased preference for a more recent object, which is consistent with the novel object recognition literature. Alternatively, it is suggested that equal amounts of exploration for both the old and recent/new objects is not a failure in the ability to discriminate between objects (Ennaceur,2010). Rather, animals recognize both objects as familiar or explore both as novel (as object exploration for the less recent object wanes over time). Animals may show a short term preference for the more recent object, and then go back to the exploration of the old object, thus indicating increased preference of the old object. Consequently, the more recent object is explored less than the less recently encountered object (old object) because the representation of the former is still available in memory, and not because there is no memory for the recent object (Ennaceur,2010).

This suggests that in our task, animals show preferential exploration of the old object as they remember the occurrence of the old (less recent) and recent object. According to the literature for novelty, this is not possible as increased exploration of a less recent object reflects a weak or no memory of this object. It is suggested that object occurrence governed by recency is confused with the memory of an object at a particular place or time (Ennaceur,2010). This does not indicate that the memory for the recent object has been lost, and thus it is not possible to model the 'when' aspect.

Our results indicate that D2R KO's show no memory for what when and intact memory for what and where, on the episodic memory task. Episodic memory cannot be limited to the sum of "what", "where" and "when" that constitute an episode, as these can be still be indexed as individual components of memory at certain points of time in an episode, where past and present memories can be reconciled as they are reminiscent of an episode (Ennaceur,2010). In light of our results, this may be indicative of the D2R differentially mediating the what-where and what-when components of an episodic memory task; where mutants show intact memory on the spatio-temporal component of the task.

8.3 Nrg-1 mutants show no impairments in LI

LI is a model of learned inattention that is used to model schizophrenia like deficits governed by abnormal stimulus salience. LI is the difference in learning between the PE and NPE groups. As a model of attentional salience, the animal is required to ignore non reinforced stimuli, where pre-exposure to the stimulus retards learning (Lubow and Moore 1959). The suppression of drinking behaviour is an indicator of learning where NPE animals learn the tone-shock (CS-US) association and refrain from licking for water. However, animals pre exposed to the tone (PE group) do not make this differentiation. It is suggested that this suppression of behaviour in the NPE group may be due to the nature of the event itself (Estes and Skinner,1941). This is attributed to the presentation of a novel stimulus (tone), where animals in the NPE group suppress drinking behaviours in the test phase, owing to this stimulus novelty. Thus, leaving the group that had been pre exposed to the tone unaffected, and mimicking behaviour as seen in LI. This suggests that the nature of the stimulus itself, rather than learning to the tone-shock relationship would predispose to an LI like effect.

In order to differentiate between unconditioned suppression of behaviour and associative learning in LI, two sets of stimuli i.e. paired stimuli (contingent tone and shock) and unpaired stimuli (non contingent tone and shock) were used to show that the LI effect and the subsequent expression of behaviours was a consequence of this tone-shock association, rather than an artefact of behavioural suppression due to the aversive nature of the tone stimulus. LI is demonstrated as a difference between the PE and NPE groups only when the stimuli are paired, and the tone predicts the shock. Animals in the paired group learn to suppress licking behaviour, as a consequence of this association. The group that was exposed to non contingent stimuli however, does not differ from the PE group and did not show LI. Thus, animals presented with the non contingent stimuli did not show LI or suppress behaviour compared to animals that received contingent tone shock pairings.

These findings jointly suggest that the suppression of behaviour in LI is solely governed by the associative CS-US relationship. As suppression of behaviour was only seen on exposure to paired stimuli (tone-shock), and not as a response to the properties of the unpaired stimulus (stimulus 1: tone, stimulus 2: shock) itself. The difference in learning between the PE and NPE groups cannot be attributed to unconditioned learning to the aversive unpaired stimulus alone. If this were the case, and novelty to the tone did affect behaviour, animals in the contingent group would mimic behaviours of the non contingent group. These results suggest that attentive learning occurs as a consequence of the associative relationship between two paired stimuli, rather than the properties of the stimulus alone. This indicates that LI is a standalone phenomenon of associability that is not attributable to the nature of the stimulus alone. These findings thus replicate other studies that show LI to be a robust task of inattention.

Neuregulin-1 (NRG1) is an at risk haplotype for schizophrenia (Harrison and Law 2006). Mutant mice Heterozygous (Het) for the Nrg-1 gene or its receptor ErbB4 have been shown to display cognitive deficits reminiscent of those demonstrated in schizophrenia in tasks of social novelty and pre-pulse inhibition (O' Tuathaigh et al.,2007; Karl,2011). Like our previous studies in D2R mutants, we wanted to investigate whether dampened function of the Nrg-1 gene predisposes to schizophrenia like deficits on a task of learned inattention, as a measure of cognitive impairment in schizophrenia. The results from our experiment indicate that both Nrg-1 WT and Het animals show robust LI.

Previous reports indicate that Nrg-1 Ig domain mutants show impairments in LI (Rimer, Barrett et al.,2005). However, this study did not have an NPE control group, thus it is difficult to make a concrete conclusion regarding LI impairment. Behavioural alterations in Nrg-1 mutants are differentially mediated depending on the Nrg-1 isoform that is targeted. Targeted deletion of ErbB2 and ErbB3 receptors in Heregulin Het mutants spares disruptions on the T maze, and improves performance on the rotarod and locomotor acitivity in an open field, indicating that behavioural alterations in Nrg-1 mutants are a consequence of targeted deletions at specific loci (Gerlai, Pisacane et al.,2000).

Studies in TM domain mutants suggest sexually dimorphic effects in the exploration and habituation to a novel environment (O'Tuathaigh and Croke,2006; O' Tuathaigh, 2007), with impairments being seen in male Het animals only. Shifting of exploratory behaviour is increased in female mutants but reduced in male mutants (O'Tuathaigh, Harte et al.,2010). However, our study indicates intact behaviours in male and female mutants.

8.4 Nrg-1 Het mutants show impairments in Prepulse Inhibition

To investigate the role of the Nrg1 gene in other attentional salience mediated cognitive phenotypes in schizophrenia; we investigated the involvement of the TM domain of the Nrg-1 gene in mediating sensorimotor gating in a PPI task relevant to schizophrenia. Our results indicate that partial deletion of the TM domain of the Nrg-1 gene leads to attenuated PPI in Nrg-1 Het mutants compared to their WT littermates. No sex specific PPI disruptions were seen in Nrg-1 Het mutants.

It has been suggested that PPI and PPI deficits may not be a robust replicable effect in Nrg-1 TM domain mutants. Rather deficits in PPI in Nrg-1 TM domain mutants are suggested to be protocol and site specific (Karl,2011).The first PPI protocol employed in this study consisted of a fixed vs. variable ISI protocol in one phenotyping facility (i.e. Garvan) and a second, with variable ISI at a different facility (NeuRa). Garvan consisted of the ten 90 dB ASR trials, 18×120 dB ASR trials, two prepulse alone trials per prepulse intensity (i.e. 74/78/82/86 dB), eight PPI response trials per prepulse intensity (prepulse followed 80 ms later by a 120 dB startle pulse). NeuRA consisted of five 120 dB startle pulses after which four startle pulses (70/80/100/120 dB) were presented five times each in a pseudo-randomised order. After this, 75 PPI response trials (prepulse intensities of 74/82/86 dB followed by a 120 dB startle pulse) were presented five times in a quasi-randomised order employing five different inter stimulus intensities (ISI) (32/64/128/256/512 ms) followed by a final five 120 dB startle pulses. The fixed ISI protocol produced attenuated startle responses to a 120 dB tone in mutant Nrg-1 mice. However, in the PPI protocol with a variable ISI (Garvan/NeuRA) showed no differences in ASR to the 120 dB startle tone. Furthermore, no difference in PPI

between Nrg-1 WT and Het animals was seen. These findings strongly suggest that Nrg-1 mediates PPI in a protocol dependent- site specific manner.

Our protocol consisted of twelve 120-db pulses of broadband noise, in blocks of six at the beginning and end of the session. Prepulse Inhibition was based on acoustic prepulse intensities that consisted of noise prepulses (3db, 7db, 15db and 25db above 65 dB background noise) that were presented in a random order. Prepulse inhibition was measured as a magnitude of startle to the prepulse trial that consisted of a 20-ms noise prepulse, with a 100 ms delay which was then followed by 65 db of broadband noise, distributed at random throughout the task. Impairments in Nrg-1 Hets in baseline startle were seen in a third protocol (Karl, 2011) that was closest to our protocol in terms of the magnitude of the of prepulses used (2,4,8,16 dB above 70 dB background noise). The ASR was attenuated in Nrg-1 Het mutants compared to WTs, but no differences in % PPI were seen. Thus, indicating that PPI and PPI disruption in Nrg-1 mutants depends both on the protocol employed and the test site.

Previously reported Nrg-1 deficits in PPI (Stefansson H. 2002) have not been replicated. Moderate disruption of PPI in TM domain Nrg-1 mutants (Stefansson H. 2002) and no disruptions in other studies (Boucher, Arnold et al.,2007) have been attributed to differences in the magnitude of baseline responding, which may promote a floor effect. In Nrg-1 WTs when baseline % PPI was reported to be 60-65% in WTs, 50-55% PPI disruption was reported in Het mutants. Whereas when baseline inhibition was lower in controls, no disruptions in PPI were reported in Het mutants (van den Buuse 2010). These findings taken together confirm the suggestion that PPI and its disruption in Nrg-1 mutants depend on the protocol employed.

Nrg-1 WTs show a trend for habituation deficits, with a trend toward intact habituation in Nrg1 Hets. Heregulin (type 1 neuregulin with different n terminal) Het

mutants show intact behavioural processes (Gerlai, Pisacane et al.,2000), which suggests that Nrg-1 mutants show intact habituation and this allele may dissociate for habituation and PPI. Previous reports indicate deficits in the exploration and habituation to an open field in Nrg-1 TM domain mutants (Babovic, O'Tuathaigh et al.,2007). However, habituation to an open field is different from habituation to pulses, and it may not be possible to facilitate a direct comparison between the two, as the former measures habituation in an exploratory modality and the latter measures habituation from an attentional salience perspective. Intact LI in mutants from our previous study indicates that Het animals may show a spared disruption of habituation to stimuli of attentional salience, but previous reports have only indicated a role for Nrg-1 in novelty driven salience (O' Tuathaigh et al.,2007).

Overall, Nrg-1 mutants show robust impairments on %PPI that may be governed by the protocol used, whereas other behaviours such as habituation may be mediated distinctly by Nrg-1.

8.5 Nrg-1 Het mutants show sex specific dissociations on the 'what' and 'when', but not 'what' and 'where' components of episodic memory

People with schizophrenia have been shown to have disrupted episodic memory, which is defined as memory for items embedded in a spatiotemporal context (Leavitt and Goldberg 2009). In order to investigate the translational relevance of reduced function of TM domain Nrg-1 behaviourally, we investigated whether mice Heterozygous for the TM-domain Nrg-1 gene, would display impaired episodic memory in a task that requires simultaneous memory for "what", "when" and "where". Our data indicates that male Nrg-1 Het animals show disruptions in their memory for what and when as indicated by no preference for old vs. a recent objects as compared to their WT littermates. There was an intact preference for the old vs.

new object in female Het animals; however it is difficult to make a conclusion as WT female Nrg-1 animals did not show a significant preference for either object. Both Nrg-1 WT and Het animals failed to show preference in memory for old displaced vs. an old stationary object, reflecting no spatial memory for object displacement.

Deletion of the TM domain in Nrg-1 mutants affects Type III Nrg-1 signalling. Nrg-1 type III is defined by its cysteine-rich domain (CRD), which functions as a second transmembrane domain (Nave and Salzer,2006). CRD Nrg -1 Het mice show impairments in short term spatial memory on the T maze (Chen 2008), whereas TM domain Nrg-1 Het mutants show intact spatial memory(O' Tuathaigh et al.,2007). This may be due to Nrg1/ErbB receptor mediated function in CRD mutants, whereas TM domain mice are independent of ErbB signalling.

Studies that report impairments in object recognition memory in TM domain mutants are based on the hypothesis that posits that exploration of the novel object reflects intact memory processes, where exploration of a less novel (old) object reflects memory impairments (Ennaceur and Delacour 1988; O' Tuathaigh 2007; Ennaceur 2010). However, our task adapted from (Dere, Huston et al., 2005) emphasises increased exploration of the old object as opposed to the novel object, and these results may be subject to paradigmatic differences. As the temporal component of recognition memory is always present in our task, it is not directly comparable to behaviours on a novel object recognition task, as both objects have been exposed prior to test, and are not 'novel', but evaluated in terms of their relative recency, or on a spatial memory task, as place preference is always confounded by recency discrimination. Thus, reflecting object exploration in terms of recency discrimination for the objects, rather than a test of novelty based or spatial memories.

Episodic memory tested in a single day may be influenced by delays between sample and test phases. At short delays, it is suggested that memory for a familiar object is intact and at longer delays it becomes weak (Ennaceur,2010).The lack of exploratory preferences in the what-where component of the episodic task may be governed delays. Our task employed a long delay of 50 minutes. It is suggested that over a long delay an object loses its sense of familiarity and may appear to be less familiar or novel, reflecting a weakened memory for the old object, this would account for increased preference of an old vs. a recent object by Nrg-1 animals. The exploration of the more recent or 'novel' object depends on the consolidated memory for the familiar, old object, and therefore may promote increased exploration of the old object compared to the novel/recent object (Ennaceur,2010). Thus, increased exploration of the old object in our task may actually reflect weakened memory for that object. Additionally, equal or no differences in object exploration by Nrg-1 mutants may not necessarily reflect that learning has not occurred, as it may be a consequence of temporary decay of memory for an object as previously mentioned (Ennaceur,2010). No preference to an object indicates equal attention has been allocated to both the novel and familiar stimulus and is attributed to a weaker memory for the old object; in line with novel object recognition increased preference for an old object does not reflect memory for that object. In our task, even if the encoding and consolidation for the familiar/old object may have waned owing to a longer delay between the presentation of the old familiar object and test (50+50 minutes) vs. the presentation of the recent object and test (50 minutes), as it was seen at a further point in time compared to the recent object, and might be reflected by increased exploration of the old object.

The lack of exploration on the spatial aspect of the task may be attributed to a decline of interest for object exploration in this third phase (Sample1, Sample 2, and test) of object exposure. Rates of exploration for what where (stationary vs. displaced) objects is greatly reduced, compared to exploration rates for what when objects (old vs. recent). Exploration rates reflect no discrimination, as they do not surpass the .5 level of equal discrimination for either object. No preference to an object indicates equal attention has been allocated to both the novel and familiar stimulus. Alternatively, spatial discrimination memory may be subject to interaction with recency (Kart-Teke, De Souza Silva et al.,2006).

A revision of the protocol in mice (Dere, Huston et al.,2005) was created to investigate place and recency based interactions in episodic memory in rats, where rats were found to respond differently to the spatial displacement component of the task, depending on whether the old familiar or recent familiar objects were shifted to locations where they had not been previously encountered (Kart-Teke, De Souza Silva et al.,2006). Furthermore, rats were also allowed a 5 minutes cut off point of exploration per trial as opposed to the 10 minutes of exploration time allocated in the mouse trial. Two copies of the object from sample trial 1 (old familiar objects) and two copies of the object known from sample trial 2 (recent familiar objects) were present. Two of these objects were placed in random locations, which already contained objects during sample trial one (as seen in our design), while the remaining two objects were randomly placed in locations, which were not previously occupied by objects in the first sample trial. An old familiar object was kept in place (old familiar stationary object), while another was displaced to a novel location (old familiar displaced object). In contrast to this, the modified task did the same for the recent familiar objects presented in the second sample i.e. recent

familiar stationary object and recent familiar displaced object (Refer to Appendix 4). This revision was made to ascertain whether the exploration pattern exhibited by the rats would indicate an interaction between recency and spatial displacement.

It was seen, that consistent to the previous task in mice, rats made the same distinctions as mice in their preference for the old vs. recent objects and old displaced vs. old stationary objects (Dere, Huston et al.,2005). In the modified task however, rats preferred the displaced recent familiar object compared to the stationary recent familiar object. They also preferred the stationary old familiar object relative to the displaced old familiar object, indicating an interaction between recency and spatial displacement. This increased exploration of a newer, more recent displaced object over the old object is consistent with theories of novel object recognition. This indicates that the episodic memory task is subject to paradigmatic influences and indicates that the spatio-temporal interaction in the old protocol (Dere, Huston et al.,2005) may confound recency mediated memories.

Nrg-1 mutants show sex specific impairments on the what-when recency mediated component of the episodic memory task. This suggests that this Nrg-1 may have relevance for investigating memory dysfunction in schizophrenia, particularly in the context of sexually dimorphic memory impairment. Assessing Nrg-1 involvement in episodic memory in the revised protocol, as mentioned previously, would shed light whether Nrg-1 dissociates sex specifically for impairments in novelty and recency mediated memories.

8.6 Conclusions

- PPI is attenuated in D2R deficient mutants, in a one day sensorimotor gating task. Knocking out the D2R attenuates %PPI, and leaves ASR unaffected in both genotypes. A trend toward amphetamine disruption of PPI in D2R null mutants, in a one day, single low dose injection paradigm was seen, although this was non significant.

  d-Amphetamine induced disruption of LI administered prior to PE and COND 24 h apart disrupts LI in D2R KO (Weiner, Lubow et al.,1988; Weiner, Bernasconi et al.,1997;Bay-Richter et al.,2009). To investigate whether this drug administration dependent schedule of amphetamine disruption generalizes to cognitive phenotypes of relevance to schizophrenia, a one vs. two dose PPI protocol was used. A dissociation between a single and double dose of amphetamine exists with regard to D2R involvement in PPI. Amphetamine disrupts %PPI in D2R deficient mutants when administered as a single low dose injection, in a two day protocol. Two low dose injections of amphetamine however, do not disrupt PPI in D2R KO or their WT littermates. These findings demonstrate that prior conclusions about the requirement of the D2R for amphetamine effects in PPI does not generalise to all doses. Secondly, they suggest dissociation between one and two doses of amphetamine with respect to the D2R. Third they suggest the importance of protocol in phenotypic effects on PPI in mice; as the disruptive effects of amphetamine were only seen in a single low dose, two day (one injection prior to test only) protocol, and not in a double low dose protocol (prior to habituation and test).

- D2R WT and KO animals show equal exploration of in the recency mediated aspect of an episodic memory task, and female KO mutants show enhanced

memory for old vs. recent objects. Both D2R WT and KO mice show intact memory for displaced objects in the spatio-temporal aspect of the task. This indicates that absence of the D2R KO in these mice show intact memory for what and where on an episodic memory task.

- Nrg-1 TM domain mutants show no impairements onLI.

- Nrg-1 TM domain mutants show deficits on the schizophrenia relevant PPI task of sensorimotor gating. Nrg-1 Het mutants show attenuated % PPI compared to their WT littermates; however, this may be subject to protocol specificity and at certain prepulse intensities. A trend toward PPI disruptions in male animals was seen, suggesting that Nrg-1 mediates sex specific impairment in schizophrenia phenotypes, but this was not significant.

- Reduced function of TM-NRG1 gene has sex-specific effects on episodic-like memory impairing it in males and improving it in females. This suggests that this model may have relevance for investigating memory dysfunction in schizophrenia, particularly in the context of sexually dimorphic memory impairment. A revised episodic memory protocol(Kart-Teke, De Souza Silva et al.,2006) may help elucidate Nrg-1 involvement in novelty driven memories and recency.

FUTURE STUDIES SUGGESTED BY THIS WORK

- Future studies could involve dose response studies to investigate whether PPI disruption in the D2R KO as mediated by protocol is by low doses of amphetamine only, or whether these disruptions by the low dose are mediated differently by protocol when amphetamine is administered at a high dose. A replication of the episodic memory task in both models is

warranted, to demonstrate intact memory for what-where-when. Additionally, a modification of the task is required to determine whether mice do show the non- standard preference for the old objects and its implications for memory impairment in the knock out. Pharmacological models investigating the effect of amphetamine in disruption of episodic memory could also then be conducted in the D2R, in the new modified version of the task. Replicability of the schizophrenia relevant disruption in these three tasks in the Nrg-1 model is warranted, to determine whether disruptions on schizophrenia relevant phenotypes can be consistently reproduced in this mutant model. If PPI disruptions are protocol specific in Nrg-1 mutants, a one vs. two day PPI task would help elucidate this stance in the current literature. Additionally, the effects of a low dose of amphetamine in the disruption of these schizophrenia relevant phenotypes and the reversal of these disruptions by antipsychotic drugs could also be established in Nrg-1 mutants. This would help elucidate whether the disruptions in these behavioural phenotypes by dampened Nrg-1 function are mediated differently by aberrant dopamine neurotransmission, and have predictive validity for pharmacological models of schizophrenia relevant phenotypes.

REFERENCES

Abel, T., Nguyen, et al., (1997). Genetic Demonstration of a Role for PKA in the Late Phase of LTP and in Hippocampus-Based Long-Term Memory. Cell 88(5): 615-626.

Abi-Dargham, A. (2004). Do we still believe in the dopamine hypothesis? New data bring new evidence. The International Journal of Neuropsychopharmacology 7(SupplementS1): S1-S5.

Abi-Dargham, A., Rodenhiser, J. et al., (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 97(14): 8104-8109.

Abi-Dargham, A., Mawlawi, O. et al., (2002). Prefrontal Dopamine D1 Receptors and Working Memory in Schizophrenia. The Journal of Neuroscience 22(9): 3708-3719.

Adams, A. L., Hudson, A. et al., (2008). Effects of estrous stage and time of day on prepulse inhibition in female rats. Journal of Neuroscience Methods 173(2): 295-298.

Adcock, R. A., Thangavel, A. et al., (2006). Reward-Motivated Learning: Mesolimbic Activation Precedes Memory Formation. Neuron 50(3): 507-517.

Alexander, G. E., DeLong, M. R. et al., (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annual Review of Neuroscience VOL. 9: 357-381.

Alexander, R. C., Wright, R. et al., (1996). Quantitative trait loci contributing to phencyclidine-induced and ampHetamineinduced locomotor behavior in inbred mice. Neuropsychopharmacology 15(5): 484-490.

Andreasen, N. (1995). Symptoms, signs, and diagnosis of schizophrenia. The Lancet 346(8973): 477-481.

Andreasen, N. C., Flashman, vet al., (1994). Regional Brain Abnormalities in Schizophrenia Measured With Magnetic Resonance Imaging. JAMA: The Journal of the American Medical Association 272(22): 1763-1769.

Andreasen, N. C., Olsen, S. A. et al., (1982). Ventricular enlargement in schizophrenia: relationship to positive and negative symptoms. Am J Psychiatry 139(3): 297-302.

Angrist, B. M. and Gershon, S. (1970). The phenomenology of experimentally induced ampHetamine psychosis: Preliminary observations. Biological Psychiatry 2(2): 95-107.

Angrist, B., Rotrosen,v et al., (1980). Differential effects of ampHetamine and neuroleptics on negative Vs. positive symptoms in schizophrenia. Psychopharmacology 72(1): 17-19.

Arco, A. and Mora, F. (2009). Neurotransmitters and prefrontal cortico limbic system interactions: implications for plasticity and psychiatric disorders. Journal of Neural Transmission 116(8): 941-952.

Arwas, S., Rolnick, A. et al., (1989). Conditioned Taste Aversion in humans using motion- induced sickness as the US. Behaviour Research and Therapy 27(3): 295-301.

Azrin, N. H. and Hake, D. F.(1969). Positive conditioned suppression: conditioned suppression using positive reinforcers as the unconditioned stimuli1.

Babovic, D., O'Tuathaigh, C. M. et al., (2007). Exploratory and habituation phenotype of Heterozygous and homozygous COMT knockout mice. Behavioural Brain Research 183(2): 236-239.

Barr, A. M., Lehmann-Masten, V. et al., (2004). The Selective Serotonin-2A Receptor Antagonist M100907 Reverses Behavioral Deficits in Dopamine Transporter Knockout Mice. Neuropsychopharmacology 29(2): 221-228.

Bay-Richter, O'Tuathaigh, C. M. P. et al., (2009). Enhanced latent inhibition in dopamine receptor-deficient mice is sex-specific for the D1 but not D2 receptor subtype: implications for antipsychotic drug action. The International Journal of Neuropsychopharmacology 12(03): 403-414.

Berridge, K. C. and Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews 28(3): 309-369.

Besheer, J., Jensen, H. C. et al., (1999). Dopamine antagonism in a novel-object recognition and a novel-object place conditioning preparation with rats. Behavioural Brain Research 103(1): 35-44.

Bevins, R. A. and Besheer, J. (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat. Protocols 1(3): 1306-1311.

Bia, Grazyna, et al., (2007). Amphetamine-induced anxiety-related behavior in animal models., Polish Academy of Sciences, Institute of Pharmacology.Biochem Soc Trans37, 308-12.

Bisagno, V., Ferguson,v et al., (2003). Chronic -ampHetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. Pharmacology Biochemistry and Behavior 74(4): 859-867.

Bleuler, E. (1911). Dementia Praecox or the Group of schizophrenias. Oxford, England: International Universities Press; 548 pp.

Bonner-Jackson, A., Haut, K. et al., (2005). The Influence of Encoding Strategy on Episodic Memory and Cortical Activity in Schizophrenia. Biological Psychiatry 58(1): 47-55.

Boucher, A., Arnold, J. et al., (2007). Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of tetrahydrocannabinol. Psychopharmacology 192(3): 325-336.

Braff, D. L. (1993). Information Processing and Attention Dysfunctions in Schizophrenia. Schizophrenia Bulletin 19(2): 233-259.

Braff, D. L., Swerdlow,v et al., (1999). Symptom Correlates of Prepulse Inhibition Deficits in Male Schizophrenic Patients. Am J Psychiatry 156(4): 596-602.

Braff, L. David, et al., (2001). Human studies of prepulse inhibition of startle; normal subjects, patient groups, and pharmacological studies. Berlin, ALLEMAGNE, Springer.

Brand, M. (2008). The role of the prefrontal cortex in episodic memory. Handbook of Episodic Memory 18.

Braun, A. R. and Chase, T. N. (1986). Obligatory D-1/D-2 receptor interaction in the generation of dopamine agonist related behaviors. European Journal of Pharmacology 131(2-3): 301-306.

Breier, A., Su, T. P. et al., (1997). Schizophrenia is associated with elevated ampHetamine-induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography f??method. Proceedings of the National Academy of Sciences of the United States of America 94(6): 2569-2574.

Brinkmann, B. G., Agarwal, A. et al., (2008). Neuregulin-1/ErbB Signaling Serves Distinct Functions in Myelination of the Peripheral and Central Nervous System. Neuron 59(4): 581-595.

Brody, S. A., Conquet, F. et al., (2003). Disruption of prepulse inhibition in mice lacking mGluR1. European Journal of Neuroscience 18(12): 3361-3366.

Browne, R. G. and Segal, D. S. (1977). Metabolic and experiential factors in the behavioral response to repeated ampHetamine. Pharmacology Biochemistry and Behavior 6(5): 545-552.

Burgess, N., Maguire, E. A. et al., (2002). The Human Hippocampus and Spatial and Episodic Memory. Neuron 35(4): 625-641.

Bussey, T. J., Muir, J. L. et al., (1999). Functionally Dissociating Aspects of Event Memory: the Effects of Combined Perirhinal and Postrhinal Cortex Lesions on Object and Place Memory in the Rat. J. Neurosci. 19(1): 495-502.

Butterfield,D. and Galvan,V. et al., (2010).In vivo oxidative stress in brain of Alzheimer disease transgenic mice: Requirement for methionine 35 in amyloid β-peptide of APP. Free Radical Biology and Medicine 48(1), 136-144.

Cannon, T. D. and Keller, M. C. (2006). Endophenotypes in the Genetic Analyses of Mental Disorders. Annual Review of Clinical Psychology 2(1): 267-290.

Cardno, A. G. and Gottesman, I. I. (2000). Twin studies of schizophrenia: From bow-and-arrow concordances to Star Wars Mx and functional genomics. American Journal of Medical Genetics 97(1): 12-17.

Carlsson, A. and Lindqvist, M. (1963). Effect of Chlorpromazzie or Haloperdol on formation of 3-Methoxytyramine and normetanephrene in mouse. Acta Pharmacologica Et Toxicologica 20(2): 140-&.

Carter CS, Ganguli R, et al., (1998). Functional hypofrontality and working memory dysfunction in

Castner, S. A. and Goldman-Rakic, P. S. (2003). AmpHetamine sensitization of hallucinatory-like behaviors is dependent on prefrontal cortex in nonhuman primates. Biological Psychiatry 54(2): 105-110.

Castner, S. A., Goldman-Rakic, P. S. et al., (2004). Animal models of working memory: insights for targeting cognitive dysfunction in schizophrenia. Psychopharmacology (Berl) 174(1): 111-125.

Bonner-Jackson, A., Haut, K. et al., (2005). The Influence of Encoding Strategy on Episodic Memory and Cortical Activity in Schizophrenia. Biological Psychiatry 58(1): 47-55.

Centonze, D., Picconi, B. et al., (2001). Dopaminergic control of synaptic plasticity in the dorsal striatum. European Journal of Neuroscience 13(6): 1071-1077.

Centonze, D., Picconi, B. et al., (2002). Cocaine and AmpHetamine Depress Striatal GABAergic Synaptic Transmission through D2 Dopamine Receptors. Neuropsychopharmacology 26(2): 164-175.

Cervenka, S., Eckman, L. B et al., (2008). Associations between dopamine D2-receptor binding and cognitive performance indicate functional compartmentalization of the human striatum. Neuroimage 40(3): 1287-1295.

Chang, T., Meyer, U. et al., (2007). Disruption of the US pre-exposure effect and latent inhibition in two-way active avoidance by systemic ampHetamine in C57BL/6 mice. Psychopharmacology 191(2): 211-221.

Chavez, C., Gogos, A. et al., (2009). Psychotropic drug-induced locomotor hyperactivity and prepulse inhibition regulation in male and female aromatase knockout (ArKO) mice: role of dopamine D1 and D2 receptors and dopamine transporters. Psychopharmacology (Berl) 206(2): 267-79.

Chen, P. S., Kuang Yang, et al., (2005). Correlation between different memory systems and striatal dopamine D2/D3 receptor density: a single photon emission computed tomography study. Psychological Medicine 35(02): 197-204.

Chen, Y. J., Johnson,M.A., et al. (2008). Type III Neuregulin-1 is required for normal sensorimotor gating, memory related behaviors and cortico-striatal circuit components. J Neurosci. 28(27): 6872?6883.

Clapcote, S., Lipina, T. et al., (2007). Behavioral Phenotypes of Disc1 Missense Mutations in Mice. Neuron 54(3): 387-402.

Clapcote, S.J., Lipina, T.V.,. et al., (2007)Behavioral Phenotypes of Disc 1Missense Mutations in Mice. Neuron 54(3); 387-402.

Clayton, N. S. and Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. Nature 395(6699): 272-274.

Clea Warburton, Joseph, E., M. et al., (1994). Antagonism of ampHetamine-induced disruption of latent inhibition in rats by haloperidol and ondansetron: Implications for a possible antipsychotic action of ondansetron. Psychopharmacology 114(4): 657-664.

Corfas, C., Roy, K. and Buxbaum J.D. (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. Nature Neuroscience 7: 575-580.

Corfas, G., Roy, K. et al., (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. Nature Neuroscience 7(6): 575-580.

Coyle, J. T. (2004). The GABA-glutamate connection in schizophrenia: which is the proximate cause? Biochemical Pharmacology 68(8): 1507-1514.

Csernansky, J. G., Kerr, S. et al., (1988). Mesolimbic dopamine receptor increases two weeks following hippocampal kindling. Brain Research 449(1-2): 357-360.

Csomor, P. A., Vollenweider, F. X. et al., (2008). On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behavioral Neuroscience 122(4): 885-900.

Davis, H. (1972). Conditioned suppression of an avoidance response by a stimulus paired with food. J Exp Anal Behav. 1972 March;

Davis, K. L., Kahn, R. S. et al., (1991). Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 148(11): 1474-1486.

De la Casa, L. G., Ruiz, G. et al., (1993). AmpHetamine-produced attenuation of latent inhibition is modulated by stimulus preexposure duration: Implications for schizophrenia. Biological Psychiatry 33(10): 707-711.

De Leonibus, E., M. Verheij, M. M. et al., (2006). Distinct kinds of novelty processing differentially increase extracellular dopamine in different brain regions. European Journal of Neuroscience 23(5): 1332-1340.

Dere, E., B. Pause, M. et al., (2010). Emotion and episodic memory in neuropsychiatric disorders. Behavioural Brain Research 215(2): 162-171.

Dere, E., Kart-Teke, E. et al., (2006). The case for episodic memory in animals. Neuroscience and Biobehavioral Reviews 30(8): 1206-1224.

Dere, E., Huston, J. P. et al., (2005). Episodic-like memory in mice: Simultaneous assessment of object, place and temporal order memory. Brain Research Protocols 16(1-3): 10-19.

Dere, E., Huston, J. P. et al., (2005). Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. Neurobiology of Learning and Memory 84(3): 214-221.

Desbonnet L, , O'Tuathaigh CM. (2009). Mutant models for genes associated with schizophrenia.

Deutch, A. Y. (1992). The regulation of subcortical dopamine systems by the prefrontal cortex:

Deutch, A. Y., Tam, S.Y.,. et al. (1987). Mesolimbic and mesocortical dopamine activation induced by phencyclidine: Contrasting response to striatal response. Eur J Pharmacol 134,: pp. 257?264.

Di Paolo, (1994). Modulation of Brain Dopamine Transmission by Sex Steroids. Reviews in the Neurosciences 5(1): 27-42.

Dietrich, A. (1998). Functional dissociation of the prefrontal cortex and teh hippocampus in timing behaviour. Nat Neurosci. 1(4): 266-7.

Dix, S.L. and Aggleton J.P. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behavioural Brain Research

Doherty, J. M., Masten, V. L. et al., (2008). Contributions of dopamine D1, D2, and D3 receptor subtypes to the disruptive effects of cocaine on prepulse inhibition in mice. Neuropsychopharmacology 33(11): 2648-2656.

Dubertret, C., Gouya, L. et al., (2004). The 3' region of the DRD2 gene is involved in genetic susceptibility to schizophrenia. Schizophrenia Research 67(1): 75-85.

Duffy, L., Cappas, E. et al., (2008). Behavioral profile of a Heterozygous mutant mouse model for EGF-like domain neuregulin 1. Behavioral Neuroscience 122(4): 748-759.

Duffy, L., Cappas, E. et al., (2010). Cognition in transmembrane domain neuregulin 1 mutant mice. Neuroscience In Press, Corrected Proof.

Dulawa, S. C. and Geyer, M. A. (2000). Effects of strain and serotonergic agents on prepulse inhibition and habituation in mice. Neuropharmacology 39(11): 2170-2179.

Eacott, M. J. and Easton, A. (2010). Episodic memory in animals: Remembering which occasion. Neuropsychologia 48(8): 2273-2280.

Ehrlichman, R. S., Luminais,S.N et al., (2009). Neuregulin 1 transgenic mice display reduced mismatch negativity, contextual fear conditioning and social interactions. Brain Research 1294: 116-127.

Eichenbaum, H. (2004). Hippocampus: Cognitive Processes and Neural Representations that Underlie Declarative Memory. Neuron 44(1): 109-120.

Elvevag, B. and Goldberg, P.D. (2000). Cognitive Impairment in Schizophrenia Is the Core of the Disorder. 14(1): 21.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. Behavioural Brain Research 215(2): 244-254

Ennaceur, A. and Delacour, J. (1988). A new one- trial test for neurobiological studies of memory in rats. Behavioural Brain Research 31(1): 47-59.

Ennaceur, A., Neave, N. et al., (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Experimental Brain Research 113(3): 509-519.

Estes, W. K. and Skinner, B. F. (1941). Some quantitative properties of anxiety. Journal of Experimental Psychology 29(5): 390-400.

Enomoto T, Noda,Y. amd Nabeshima T (2007). Phencyclidine and genetic animal models of schizophrenia developed in relation to the glutamate hypothesis. Methods Find Exp Clin Pharmacol.29(4):291-301.

Farh, K. K., Grimson, A., C., et al.,(2005). The widespread impact of mammalian MicroRNAs on mRNA repression and evolution,. Science 310: pp. 1817?1821. .

Featherstone, R. E., Kapur, S. et al., (2007). The ampHetamine-induced sensitized state as a model of schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 31(8): 1556-1571.

Feldon, J., Weiner, I. et al., (1991). The latent inhibition model of schizophrenic attention disorder. Haloperidol and sulpiride enhance rats' ability to ignore irrelevant stimuli. Biological Psychiatry 29(7).

Feldon, J., Barkai, R. et al., (1990). Latent Inhibition and its Modulation by AmpHetamine in rats screened for high and low spontaneous activity. Journal of Psychopharmacology 4(4): 283.

Fienberg, A. A., Hiroi, N. et al., (1998). DARPP-32: Regulator of the Efficacy of Dopaminergic Neurotransmission. Science 281(5378): 838-842.

Flames, N., Long, J. E. et al., (2004). Short- and Long-Range Attraction of Cortical GABAergic Interneurons by Neuregulin-1. Neuron 44(2): 251-261.

Foong, J., Symms, M. R. et al., (2001). Neuropathological abnormalities in schizophrenia: evidence from magnetization transfer imaging. Brain 124(5): 882-892.

Fornito, A., Yucel, M. et al., (2009). Mapping grey matter reductions in schizophrenia: An anatomical likelihood estimation analysis of voxel-based morphometry studies. Schizophrenia Research 108(1): 104-113.

Forwood, S. E., Winters, B. D. et al., (2005). Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. Hippocampus 15(3): 347-355.

Gainetdinov, R. R., Mohn A.R. and Caron M.G. (2001). Genetic animal models: focus on

Gajendran N, K. J., Lain E, et al., (2009). Neuregulin signaling is dispensable for NMDA- and GABA(A)-receptor expression in the cerebellum in vivo. J Neurosci. 29(8): 2404-13.

Gal, G., Schiller, D. et al., (2005). Latent inhibition is disrupted by nucleus accumbens shell lesion but is abnormally persistent following entire nucleus accumbens lesion: The neural site controlling the expression and disruption of the stimulus preexposure effect. Behavioural Brain Research 162(2): 246-255.

Gerlai, R., Pisacane, P. et al., (2000). Heregulin, but not ErbB2 or ErbB3, Heterozygous mutant mice exhibit hyperactivity in multiple behavioral tasks. Behavioural Brain Research 109(2): 219-227.

Geyer, A. Mark, et al., (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia : a decade in review. Berlin, ALLEMAGNE, Springer.

Geyer, M. A. (1999). Assessing prepulse inhibition of startle in wild-type and knockout mice. Psychopharmacology 147(1): 11-13.

Geyer, M. A.. (1987). Startle habituation and sensorimotor

Geyer, M. A., Russo, P. V. et al., (1987). Effects of apomorphine and ampHetamine on patterns of locomotor and investigatory behavior in rats. Pharmacology Biochemistry and Behavior 28(3): 393-399.

Geyer, M.A., Krebs-Thomson, et al.,.(2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: A decade in review.Psychopharmacology, Vol 156(2-3), 117-154.

Glatt, S. J., Faraone, S. V. et al., (2003). Meta-analysis identifies an association between the dopamine D2 receptor gene and schizophrenia. Mol Psychiatry 8(11): 911-915.

Glickstein, S. B., Hoff, P.R. and Schmauss, C. (2002). Mice Lacking Dopamine D2 and D3 Receptors Have Spatial Working Memory Deficits The Journal of Neuroscience

Goff, D. C. and Coyle, J. T. (2001). The Emerging Role of Glutamate in the Pathophysiology and Treatment of Schizophrenia. Am J Psychiatry 158(9): 1367-1377.

Gogos A,. (2009). Gender differences in prepulse inhibition (PPI) in bipolar disorder: men have reduced PPI, women have increased PPI. Neurpsychopharmacology 12(9): 1249-59.

Gold, L. H., Swerdlow, N. R. et al., (1988). The role of mesolimbic dopamine in conditioned locomotion produced by ampHetamine. Behavioral Neuroscience 102(4): 544-552.

Gottesman, I. I. and Gould, T. D. G., (2003). The endophenotype concept in psychiatry: etymology and strategic intentions, . Am. J. Psychiatry 160: pp. 636?645.

Gottesman, I. I. and Erlenmeyer-Kimling,L. (2001). Family and twin strategies as a head start in defining prodromes and endophenotypes for hypotHetical early-interventions in schizophrenia. Schizophrenia Research 51(1): 93-102.

Gould, T. D. and Gottesman, I. I. (2006). Psychiatric endophenotypes and the development of valid animal models. Genes, Brain and Behavior 5(2): 113-119.

Golub, M.S. et al. Behavioral characteristics of a nervous system-specific erbB4 knock-out mouse. Behav. Brain Res., 153, pp. 159–170.

Granon, S., F. Passetti, et al., (2000). Enhanced and impaired attentional performance after infusion of the D1 dopaminergic receptor agents into rat prefrontal cortex. The Journal of Neuroscience 20(3): 1208-1215.

Gray J.A., F. J., Rawlins J.N.P., et al., (1991). The neuropsychology of schizophrenia. Behavioural and Brain Sciences(14): 1?84.

Gray, J. A., M. H. Joseph, et al., (1995). The role of mesolimbic dopaminergic and retrohippocampal afferents to the nucleus accumbens in latent inhibition: implications for schizophrenia. Behavioural Brain Research 71(1-2): 19-31.

Gray, N., A. Pickering, et al., (1992). Abolition of latent inhibition by a single 5 mg dose-ampHetamine in man. Psychopharmacology 107(2): 425-430.

Greicius, M. D., B. Krasnow, et al., (2003). Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. Hippocampus 13(1): 164-174.

Groves, P. M. and Rebec,G.V. (1976). Biochemistry and Behavior: Some Central Actions of AmpHetamine and Antipsychotic Drugs. Annual Review of Psychology 27(1): 91-127.

Hahn, C.-G., and Wang, H.-Y. et al., (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nat Med 12(7): 824-828.

Hall, J. et al.(2006) A neuregulin 1 variant associated with abnormal cortical function and psychotic symptoms. Nat. Neurosci., 9 pp. 1477–1478.

Hampson, R. E. and S. A. Deadwyler (1998). Role of Cannabinoid Receptors in Memory Storage. Neurobiology of Disease 5(6): 474-482.

Hampton, R. R., B. M. Hampstead, et al., (2005). Rhesus monkeys (Macaca mulatta) demonstrate robust memory for what and where, but not when, in an open-field test of memory. Learning and Motivation 36(2): 245-259.

Hannesson, D. K., J. G. Howland, et al., (2004). Interaction between Perirhinal and Medial Prefrontal Cortex Is Required for Temporal Order But Not Recognition Memory for Objects in Rats. J. Neurosci. 24(19): 4596-4604.

Harrison, P. J. and A. J. Law (2006). Neuregulin 1 and Schizophrenia: Genetics, Gene Expression, and Neurobiology. Biological Psychiatry 60(2): 132-140.

Harrison, P. J. and D. R. Weinberger (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence (vol 10, pg 420, 2005). Molecular Psychiatry 10(8): 804-804.

Hashimoto.K., Shimizu E., and Iyo M. (2004). Glutamate Hypothesis of Schizophrenia and Approach for Possible Therapeutic Drugs. Current Medicinal Chemistry - Central Nervous System Agents 4: 147-154.

Heckers S, R. S., Goff D, Savage CR, Schacter DL, Fischman AJ, Alpert NM. (1998). Impaired recruitment of the hippocampus during conscious recollection in

Heckers, S., T. Curran, et al., (2000). Abnormalities in the thalamus and prefrontal cortex during episodic object recognition in schizophrenia. Biological Psychiatry 48(7): 651-657.

Hemsley, D. R. (1993). A Simple or (simplistic-questionable) SIMPLE (OR SIMPLISTIC-cognitive model for schizoprenia. Behaviour Research and Therapy 31(7): 633-645.

Henry, S. A., Lehmann-Masten, V. et al., (2002). The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. Neuropharmacology 43(8): 1199-1209.

Hitzemann,R.(2000). Animal Models of psychiatric Disorders and their relevance to Alcoholism. Alcohol Research and Health, 24(3), pp 149-158.

Hirsch, S. R., I. Das, et al., (1997). A Pivotal Role for Glutamate in the Pathogenesis of Schizophrenia, and Its Cognitive Dysfunction. Pharmacology Biochemistry and Behavior 56(4): 797-802.

Ho, B.C., Wassink,T.H. et al. (2005). Catechol-*O*-methyl transferase Val[158]Met gene polymorphism in schizophrenia: working memory, frontal lobe MRI morphology and frontal cerebral blood flow. *Molecular Psychiatry* 10, 287–298.

Hoff, A. L., M. Wieneke, et al., (1998). Sex Differences in Neuropsychological Functioning of First-Episode and Chronically Ill Schizophrenic Patients. Am J Psychiatry 155(10): 1437-1439.

Hoffman, D. C. and Donovan,H. (1994). D-1 AND D-2 Dopamine-receptor antagonists reverse prepuse inhibition deficits in an animal-model of schizophrenia. Psychopharmacology 115(4): 447-453.

Holmes, A., Lachowicz, J. E. et al., (2004). Phenotypic analysis of dopamine receptor knockout mice; recent insights into the functional specificity of dopamine receptor subtypes. Neuropharmacology 47(8): 1117-1134.

Holmes, A., Hollon, T. R. et al., (2001). Behavioral Characterization of Dopamine D5 Receptor Null Mutant Mice. Behavioral Neuroscience 115(5): 1129-1144.

Honack, D. and W. Loscher (1993). Sex differences in NMDA receptor mediated responses in rats. Brain Research 620(1): 167-170.

Hong, C.J., Huo, S.J. et al., (2004). Case-control and family-based association studies between the neuregulin 1 (Arg38Gln) polymorphism and schizophrenia. Neuroscience Letters 366(2): 158-161.

Hong, L. E., Wonodi, I. et al., (2008). Evidence of Missense Mutations on the Neuregulin 1 Gene Affecting Function of Prepulse Inhibition. Biological Psychiatry 63(1): 17-23.

Hotte, M., Naudon, L. et al., (2005). Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. Neurobiology of Learning and Memory 84(2): 85-92.

Howes, O.D. and Kapur,S. The Dopamine hypothesis of schizophrenia: Version III-The Final common Pathway. Schizophrenia Bulletin, 3593); 549-562.

Hutchison, K. E. and Swift, R. (1999). Effect of d-amphetamine on prepulse inhibition of the startle reflex in humans. Psychopharmacology 143(4): 394-400.

Hutton, S. B., B. K. Puri, et al., (1998). Executive function in first-episode schizophrenia. Psychological Medicine 28(02): 463-473.

Impey, S., Mark, M., et al., (1996). Induction of CRE-Mediated Gene Expression by Stimuli That Generate Long-Lasting LTP in Area CA1 of the Hippocampus. Neuron 16(5): 973-982.

Inta, D., Monyer, H. , et al., (2009). Mice with genetically altered glutamate receptors as models of schizophrenia: A comprehensive review. Neuroscience and Biobehavioral Reviews 34(3): 285-294.

Ishihara,T., Hong,M. et al. (1999). Age-Dependent Emergence and Progression of a Tauopathy in Transgenic Mice Overexpressing the Shortest Human Tau Isoform. **81,** 127-7.

Israel SL,, Black M.L., Brewer J.B. (2010). Going their separate ways: dissociation of hippocampal and dorsolateral prefrontal activation during episodic retrieval and post-retrieval processing. J Cogn Neurosci. 22(3): 513-25.

Jackson, D. M. and Westlinddanielsson, A. (1994). Dopamine-Receptors – Molecular Biology, Biochemistry and behavioral aspects. Pharmacology & Therapeutics 64(2): 291-370.

Javitt, D. C. and Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148(10): 1301-1308.

Jeanblanc, J., Hoeltzel, A. et al., (2002). Dissociation in the involvement of dopaminergic neurons innervating the core and shell subregions of the nucleus accumbens in latent inhibition and affective perception. Neuroscience 111(2): 315-323.

Jentsch, J. D. and Roth, R. H. (1999). The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20(3): 201-225.

Jentsch, J. D., Redmond, D. E. et al., (1997). Enduring Cognitive Deficits and Cortical Dopamine Dysfunction in Monkeys After Long-Term Administration of Phencyclidine. Science 277(5328): 953-955.

Joseph, M. H., Peters, S. L. et al., (2000). Modulation of latent inhibition in the rat by altered dopamine transmission in the nucleus accumbens at the time of conditioning. Neuroscience 101(4): 921-930.

Joyce, E. M., Collinson, S. L. et al., (1996). Verbal fluency in schizophrenia: relationship with executive function, semantic memory and clinical alogia. Psychological Medicine 26(01): 39-49.

Kallmann, F. J. (1946). The Genetic Theory of Schizophrenia: An Analysis of 691 Schizophrenic Twin Index Families. Am J Psychiatry 103(3): 309-322.

Kandel, E. R., Schwartz J.H. and Jessell T.M. (1991). Principles of Neural Science. 854-867.

Kapur, S. (2003). Psychosis as a State of Aberrant Salience: A Framework Linking Biology, Phenomenology, and Pharmacology in Schizophrenia. Am J Psychiatry 160(1): 13-23.

Kapur, S. and Mamo, D. (2003). Half a century of antipsychotics and still a central role for dopamine D-2 receptors. Progress in Neuro-Psychopharmacology & Biological Psychiatry 27(7): 1081-1090.

Kapur, S., Mizrahi, R. et al., (2005). From dopamine to salience to psychosis - linking biology, pharmacology and phenomenology of psychosis. Schizophrenia Research 79(1): 59-68.

Karl, T. (2011). Do transmembrane domain neuregulin 1 mutant mice: Exhibit a reliable sensorimotor gating deficit? Behav Brain Res. Behav Brain Res.

Karl, T., Duffy, L. et al., (2007). Altered motor activity, exploration and anxiety in Heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes, Brain and Behavior 6(7): 677-687.

Karl, T., Duffy, L. et al., (2007). Altered motor activity, exploration and anxiety in Heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes, Brain and Behavior 6(7): 677-687.

Kart-Teke, E., De Souza Silva, M. A. et al., (2006). Wistar rats show episodic-like memory for unique experiences. Neurobiology of Learning and Memory 85(2): 173-182.

Kato, T., Abe, Y. et al., (2010). Transient exposure of neonatal mice to neuregulin-1 results in hyperdopaminergic states in adulthood: implication in neurodevelopmental hypothesis for schizophrenia. Mol Psychiatry.

Kegeles, L. S., Abi-Dargham, A. et al., (2010). Increased Synaptic Dopamine Function in Associative Regions of the Striatum in Schizophrenia. Arch Gen Psychiatry 67(3): 231-239.

Kellendonk, C., Simpson, E. H. et al., (2006). Transient and Selective Overexpression of Dopamine D2 Receptors in the Striatum Causes Persistent Abnormalities in Prefrontal Cortex Functioning. Neuron 49(4): 603-615.

Kellendonk,C., Simpsn,E.H. et al. (2009) Modelling cognitive endophenotypes of schizophrenia in mice. Trends in Neurosciences,32 (6), 347-358.

Kelly M.A., et al. (1997) Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron* 19(1):103-113.

Kelly, M. A., Rubinstein, M. et al., (1998). Locomotor Activity in D2 Dopamine Receptor-Deficient Mice Is Determined by Gene Dosage, Genetic Background, and Developmental Adaptations. J. Neurosci. 18(9): 3470-3479.

Kempermann, G., Krebs, J. et al., (2008). The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. Current Opinion in Psychiatry 21(3): 290-295 10.1097/YCO.0b013e3282fad375.

Keri, S., Seres,I et al., (2011). The Relationship Among Neuregulin 1 Stimulated Phosphorylation of AKT, Psychosis Proneness, and Habituation of Arousal in Nonclinical Individuals. Schizophrenia Bulletin 37(1): 141-147.

Killcross, A., Dickinson, A. et al., (1994). AmpHetamine-induced disruptions of latent inhibition are reinforcer mediated: implications for animal models of schizophrenic attentional dysfunction. Psychopharmacology 115(1): 185-195.

Kirby, B. P.,. Waddington, J. L et al., (2009). Advancing a functional genomics for schizophrenia: Psychopathological and cognitive phenotypes in mutants with gene disruption. Brain Research Bulletin 83(3-4): 162-176.

Koike, H., Arguello, P. A. et al., (2006). Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice. Proceedings of the National Academy of Sciences of the United States of America 103(10): 3693-3697.

Kokkinidis, L. (1986). Sensitization to ampHetamine and tolerance to cocaine and phencyclidine stimulation in mice. Pharmacology Biochemistry and Behavior 25(6): 1175-1180.

Kraeplin, E. (1919). Dementia Praecox and Paraphrenia E&S Livingstone.

Krug A, M. V., Krach S, et al. (2010). The effect of Neuregulin 1 on neural correlates of episodic memory encoding and

Kuczenski, R., Segal, v et al., (1991). AmpHetamine, cocaine, and fencamfamine: relationship between locomotor and stereotypy response profiles and caudate and accumbens dopamine dynamics. The Journal of Neuroscience 11(9): 2703-2712.

Kumari, V., Gray, J. A. et al., (2003). Sex differences in prepulse inhibition of the acoustic startle response. Personality and Individual Differences 35(4): 733-742.

Kwon, O. B., Paredes,v et al., (2008). Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. Proceedings of the National Academy of Sciences 105(40): 15587-15592.

Kwon, O.-B., Longart, M., et al. (2005). Neuregulin-1 Reverses Long-Term Potentiation at CA1

Laruelle, M., Abi-Dargham, v et al., (1996). Single photon emission computerized tomography imaging of ampHetamine-induced dopamine release

in drug-free schizophrenic subjects. Proceedings of the National Academy of Sciences of the United States of America 93(17): 9235-9240.

Laruelle, M., Kegeles,v et al., (2003). Glutamate, Dopamine, and Schizophrenia. Annals of the New York Academy of Sciences 1003(1): 138-158.

Lawford, B. R., Young, R. M. et al., (2005). The C/C genotype of the C957T polymorphism of the dopamine D2 receptor is associated with schizophrenia. Schizophrenia Research 73(1): 31-37.

Leavitt, V. and Goldberg, T. (2009). Episodic Memory in Schizophrenia. Neuropsychology Review 19(3): 312-323.

Lemmens, K., Doggen, K. et al., (2007). Role of Neuregulin-1/ErbB Signaling in Cardiovascular Physiology and Disease: Implications for Therapy of Heart Failure. Circulation 116(8): 954-960.

Lennox, B. R., Park,S.B.G. et al., (2000). The functional anatomy of auditory hallucinations in schizophrenia. Psychiatry Research: Neuroimaging 100(1): 13-20.

Leung ,D. A. and Chue D.P. Psych (2000). Sex differences in schizophrenia, a review of the literature. Acta Psychiatrica Scandinavica 101(401): 3-38.

Lewine, R. R., Walker,R.F. et al., (1996). Sex differences in neuropsychological functioning among schizophrenic patients. Am J Psychiatry 153(9): 1178-1184.

Lewis, D. A. and Levitt,V. (2002). Schizophrenia as a disorder of neurodevelopment. Annual Review of Neuroscience 25(1): 409-432.

Li B Woo, R. and Malinow R. (2007). The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. Neuron. 2007 54(4)(583-97).

Li, D., Collier,D.A. et al., (2006). Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. Human Molecular Genetics 15(12): 1995-2002.

Li, W., Zhou,Y. et al., (2007). Specific developmental disruption of disrupted-in-schizophrenia-1 function results in schizophrenia-related phenotypes in mice. Proceedings of the National Academy of Sciences 104(46): 18280-18285.

Lieberman, J. A., Kinon, B. J. et al., (1990). Dopaminergic Mechanisms in Idiopathic and Drug-induced Psychoses. Schizophrenia Bulletin 16(1): 97-110.

Lind, N. M., Arnfred, S. M. et al., (2004). Prepulse inhibition of the acoustic startle reflex in pigs and its disruption by D-ampHetamine. Behavioural Brain Research 155(2): 217-222.

Lipina, T., Weiss, K. et al., (2007). The ampakine CX546 restores the prepulse inhibition and latent inhibition deficits in mGluR5-deficient mice. Neuropsychopharmacology 32(4): 745-756.

Lipska, B. K., Jaskiw, G. E. et al., (1993). Postpubertal emergence of hyperresponsiveness to stress and to ampHetamine after neonatal excitotoxic hippocampal damage: A potential animal model of schizophrenia. Neuropsychopharmacology 9(1): 67-75.

Lok, J., Hui Wang, Murata, Y., et al., (2007). Effect of Neuregulin-1 on Histopathological and Functional Outcome after Controlled Cortical Impact in Mice. Journal of Neurotrauma. 24(12): 1817-1822.

Long, L., R. Chesworth, et al., (2010). A follow-up study: acute behavioural effects of THC in female Heterozygous & transmembrane domain mutant mice. Psychopharmacology 211(3): 277-289.

Lubow, R. E. (1989). Latent Inhibition and conditioned Attention theory. Cambridge, Cambridge University Press.

Lubow, R. E. (2010). Latent Inhibition, John Wiley & Sons, Inc.

Lubow, R.E., and Moore, A.U. (1959). Latent inhibition: The effect of nonreinforced pre-exposure to the conditional stimulus. Journal of Comparative and Physiological Psychology 52(4): 415-419.

Luciana, M., R. A. Depue, et al., (1992). Facilitation of working memory in humans by a dopamine receptor agonist. Journal of Cognitive Neuroscience 4(1): 58-68.

Ludewig, K., Geyer, M.A., et al. (2003). Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. Biological Psychiatry, 54(2): 121-128

Malenka, R. C. and Bear, M. F. (2004). LTP and LTD: An Embarrassment of Riches. Neuron 44(1): 5-21.

Mansbach, R. S., Geyer, M.A.et al., (1988). Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 94(4): 507-514.

McAllister, K. H. (1997). A single administration of d-ampHetamine prior to stimulus pre-exposure and conditioning attenuates latent inhibition. Psychopharmacology 130(2): 79-84.

Meck, W. H. and Benson, A. M. (2002). Dissecting the Brain's Internal Clock: How Frontal-Striatal Circuitry Keeps Time and Shifts Attention. Brain and Cognition 48(1): 195-211.

Mei, L. and W.-C. Xiong (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 9(6): 437-452.

Meltzer, H. Y. and Stahl, S. M. (1976). The dopamine hypothesis of schizophrenia. Schizophrenia Bulletin 2(1): 19-76.

Meltzer, H. Y., Park, S. et al., (1999). Cognition, schizophrenia, and the atypical antipsychotic drugs. Proceedings of the National Academy of Sciences of the United States of America 96(24): 13591-13593.

Meyer, U., Feldon, J. et al., (2005). Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. Neuroscience & Biobehavioral Reviews 29(6): 913-947.

Meziane, H., A. M. Ouagazzal, et al., (2007). Estrous cycle effects on behavior of C57BL/6J and BALB/cByJ female mice: implications for phenotyping strategies. Genes Brain and Behavior 6(2): 192-200.

Mishara, A. L. and Goldberg, T. E. (2004). A meta-analysis and critical review of the effects of conventional neuroleptic treatment on cognition in schizophrenia: Opening a closed book. Biological Psychiatry 55(10): 1013-1022.

Moghaddam, B. (2003). Bringing Order to the Glutamate Chaos in Schizophrenia. Neuron 40(5): 881-884.

Moghaddam, B., Adams, B., et al. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci(17,): pp. 2921?2927.

Mohn A.R.,. Caron M.G. and Koller B.H. (1999). Mice with Reduced NMDA Receptor Expression Display Behaviors Related to Schizophrenia. Cell(98).

Mohn, A. R., Gainetdinov, R. R. et al., (1999). Mice with Reduced NMDA Receptor Expression Display Behaviors Related to Schizophrenia. Cell 98(4): 427-436.

Mohr, E., Fabbrini, G. et al., (1989). Dopamine and memory function in Parkinson's disease. Movement Disorders 4(2): 113-120.

Monchi, O., Petrides, M. et al., (2001). Wisconsin Card Sorting Revisited: Distinct Neural Circuits Participating in Different Stages of the Task Identified by Event-Related Functional Magnetic Resonance Imaging. J. Neurosci. 21(19): 7733-7741.

Moran, P. M., Fischer, T. R. et al., (1996). Effects of clozapine on latent inhibition in the rat. Behavioural Pharmacology 7(1): 42-48.

Moran, P. M., Young, A.M.J., Gray,J.A., M.H. Joseph (1994). A single ampHetamine treatment is able to disrupt latent inhibition in the rat by an action at conditioning alone. Behav. Neurosci. 108: 645?649. .

Morey, R. A., Inan, S. et al., (2005). Imaging Frontostriatal Function in Ultra-High-Risk, Early, and Chronic Schizophrenia During Executive Processing. Arch Gen Psychiatry 62(3): 254-262.

Moser, P. C., Hitchcock, J. M. et al., (2000). The pharmacology of latent inhibition as an animal model of schizophrenia. Brain Research Reviews 33(2-3): 275-307.

Moy, S., H. Troy Ghashghaei, et al., (2009). Deficient NRG1-ERBB signaling alters social approach: relevance to genetic mouse models of schizophrenia. Journal of Neurodevelopmental Disorders 1(4): 302-312.

Morrissette,D., Parachikova, A. et al. (2009) Relevance of Transgenic Mouse Models to Human Alzheimer Disease. The Journal of Biological Chemistry, 284, 6033-6037

Mumby DG, G. S., Glenn MJ, et al. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects. Learning and Memory 9, 47-59.

Nakajima, S., H. Ka, et al., (1999). Summation of Overshadowing and Latent Inhibition in Rats‾ Conditioned Taste Aversion: Scapegoat Technique Works for Familiar Meals. Appetite 33(3): 299-307.

Nave, K.-A. and J. L. Salzer (2006). Axonal regulation of myelination by neuregulin 1. Current Opinion in Neurobiology 16(5): 492-500.

Neckelmann G, S. K., Lund A, et al. (2006). Mr morphometry analysis of grey matter volume reduction in schizophrenia:

Newcomer, W. J, et al., (1999). Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Basingstoke, ROYAUME-UNI, Nature Publishing Group.

Niwa, M., A. Kamiya, et al., (2010). Knockdown of DISC1 by In Utero Gene Transfer Disturbs Postnatal Dopaminergic Maturation in the Frontal Cortex and Leads to Adult Behavioral Deficits. Neuron 65(4): 480-489.

Nyberg,L., Marklund,P et al. (2002). Common prefrontal activations during working memory, episodic memory and semantic memory. Neuropsychologica,41(3): 371-377.

O' Tuathaigh, B., D., O' sullivan G.J., Clifford J.J, Tighe, O., Croke, D.T., Harvey,R. and Waddington J.L. (2007). Phenotypic characterization of spatial

cognition and social behaviour in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. Neuroscience 147: 18-27.

O'Tuathaigh, C. M. P., M. Harte, et al., (2010). Schizophrenia-related endophenotypes in Heterozygous neuregulin-1 knockout mice. European Journal of Neuroscience 31(2): 349-358.

O'Tuathaigh, C. M. P., Salum, C., et al. (2003). The effect of ampHetamine on Kamin blocking and overshadowing,. Behav Pharmacol 14: pp. 315?322.

O'Tuathaigh, C. M. P., D. Babovic, et al., (2007). Susceptibility genes for schizophrenia: Characterisation of mutant mouse models at the level of phenotypic behaviour. Neuroscience and Biobehavioral Reviews 31(1): 60-78.

O'Tuathaigh, M. Colm, et al., (2006). Sexually dimorphic changes in the exploratory and habituation profiles of Heterozygous neuregulin-I knockout mice. Hagerstown, MD, ETATS-UNIS, Lippincott Williams and Wilkins.

Oades, D. R, et al., (1997). The problems of inattention : methods and interpretations. Shannon, IRLANDE, Elsevier.

Oie,M. and Rund, B.R. (1999). Neuropsychological Deficits in Adolescent-Onset Schizophrenia Compared With Attention Deficit Hyperactivity Disorder. Am J Psychiatry 156(8): 1216-1222.

O'Leary, D. S., Andreasen, N. C. et al., (1996). Auditory Attentional Deficits in Patients With Schizophrenia: A Positron Emission Tomography Study. Arch Gen Psychiatry 53(7): 633-641.

Olney, J. W. and N. B. Farber (1995). Glutamate Receptor Dysfunction and Schizophrenia. Arch Gen Psychiatry 52(12): 998-1007.

Ongur, D.,. Cullen, T. J et al., (2006). The Neural Basis of Relational Memory Deficits in Schizophrenia. Arch Gen Psychiatry 63(4): 356-365.

O'Tuathaigh C.M.P, and Waddington, J.L. (2009). Neuregulin-1 signalling in schizophrenia: 'Jack of all trades' or master of some. Expert Rev. Neurother. 9(1).

Pantelis, C., Stuart, et al., G. W. (2001). Spatial Working Memory Deficits in Schizophrenia: Relationship With Tardive Dyskinesia and Negative Symptoms. Am J Psychiatry 158(8): 1276-1285.

Pantelis, C., Barnes, T. R. et al., (1997). Frontal-striatal cognitive deficits in patients with chronic schizophrenia. Brain 120(10): 1823-1843.

Park, S., Schel, J. P et al., (1999). Spatial working memory deficits and clinical symptoms in schizophrenia: a 4-month follow-up study. Biological Psychiatry 46(3): 392-400.

Park, S., Schel, J. P et al., (2003). Visual object working memory function and clinical symptoms in schizophrenia. Schizophrenia Research 59(2-3): 261-268.

Parkinson, J. A., Olmstead, M. C. et al., (1999). Dissociation in Effects of Lesions of the Nucleus Accumbens Core and Shell on Appetitive Pavlovian Approach Behavior and the Potentiation of Conditioned Reinforcement and Locomotor Activity byd-AmpHetamine. The Journal of Neuroscience 19(6): 2401-2411.

Parwani, A., Duncan, E. J. et al., (2000). Impaired prepulse inhibition of acoustic startle in schizophrenia. Biological Psychiatry 47(7): 662-669.

Paylor, R. and Crawley, J. N. (1997). Inbred strain differences in prepulse inhibition of the mouse startle response. Psychopharmacology 132(2): 169-180.

Peleg-Raibstein, D., Sydekum, E. et al., (2006). Withdrawal from repeated ampHetamine administration leads to disruption of prepulse inhibition but not to disruption of latent inhibition. Journal of Neural Transmission 113(9): 1323-1336.

Perkins, D. O., Jeffries,C.D., et al.(2007). MicroRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder, . Genome Biol. 8.

Perlstein, W. M., Carter, C. S. et al., (2001). Relation of Prefrontal Cortex Dysfunction to Working Memory and Symptoms in Schizophrenia. Am J Psychiatry 158(7): 1105-1113.

Petryshen, T. L., Middleton, F. A. et al., Support for involvement of neuregulin 1 in schizophrenia pathophysiology. Mol Psychiatry 10(4): 328-328.

Powell, C. M. and Miyakawa, T. (2006). Schizophrenia-Relevant Behavioral Testing in Rodent Models: A Uniquely Human Disorder? Biological Psychiatry 59(12): 1198-1207.

Powell, S. B., Zhou, X. J. et al., (2009). Prepulse inhibition and genetic mouse models of schizophrenia. Behavioural Brain Research 204(2): 282-294.

Ragland, J. D., Gur, R. C. et al., (2001). Effect of Schizophrenia on Frontotemporal Activity During Word Encoding and Recognition: A PET Cerebral Blood Flow Study. Am J Psychiatry 158(7): 1114-1125.

Rajah, M. N. and D'Esposito, M. (2005). Region-specific changes in prefrontal function with age: a review of PET and fMRI studies on working and episodic memory. Brain 128(9): 1964-1983.

Ralph, R. J. and Caine, S. B. (2005). Dopamine D1 and D2 agonist effects on prepulse inhibition and locomotion: Comparison of Sprague-Dawley rats to Swiss-Webster, 129X1/SvJ, C57BL/6J, and DBA/2J mice. Journal of Pharmacology and Experimental Therapeutics 312(2): 733-741.

Ralph, R. J., Varty, G. B. et al., (1999). The dopamine D-2, but not D-3 or D-4, receptor subtype is essential for the disruption of prepulse inhibition produced by ampHetamine in mice. Journal of Neuroscience 19(11): 4627-4633.

Ralph, R. J., Paulus, M. P. et al., (2001). Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: Differential effects of D1 and D2 receptor antagonists. Journal of Neuroscience 21(1): 305-313.

Ralph, R. J., Paulus, M. P. et al., (2001). Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: Differential effects of D1 and D2 receptor antagonists. Journal of Neuroscience 21(1): 305-313.

Ralph-Williams, R. J., Lehmann-Masten, V. et al., (2002). Differential effects of direct and indirect dopamine agonists on prepulse inhibition: A study in D1 and D2 receptor knock-out mice. Journal of Neuroscience 22(21): 9604-9611.

Ramonet,D., . Daher,J.P.L. (2011). Dopaminergic Neuronal Loss, Reduced Neurite Complexity and Autophagic Abnormalities in Transgenic Mice Expressing G2019S Mutant LRRK2. PLoS ONE 6(4): e18568. doi:10.1371/journal.pone.0018568.

Rascle, C., Mazas, O. et al., (2001). Clinical features of latent inhibition in schizophrenia. Schizophrenia Research 51(2): 149-161.

Rico,B.and Marin,O. (2011). Neuregulin signalling, cortical circuitary development and schizophrenia. Current opinion in Genetics and Development 21; 262-270.

Rimer, M., Barrett, D. W. et al., (2005). Neuregulin-1 immunoglobulin-like domain mutant mice: clozapine sensitivity and impaired latent inhibition. Neuroreport 16(3): 271-275.

Robbins TW, G. V., Jones GH, Reading P, Sahakian BJ. (1990). Effects of dopamine depletion from the caudate-putamen and nucleus accumbens septi on the acquisition and performance of a conditional discrimination task. Behav Brain Res. 38((3)): 243-61.

Robbins, T. W. and Everitt, B. J. (1992). Functions of dopamine in the dorsal and ventral striatum. Seminars in Neuroscience 4(2): 119-127.

Roy, M.-A., Maziade, M. et al., (2001). Male gender is associated with deficit schizophrenia: a meta-analysis. Schizophrenia Research 47(2-3): 141-147.

Ruob, C., Elsner, J. et al., (1997). AmpHetamine-induced disruption and haloperidol-induced potentiation of latent inhibition depend on the nature of the stimulus. Behavioural Brain Research 88(1): 35-41.

Rushe, T. M., Woodruff, P. W. R. et al., (1999). Episodic memory and learning in patients with chronic schizophrenia. Schizophrenia Research 35(1): 85-96.

Russig, H., A. Durrer, et al., (2003). The acquisition, retention and reversal of spatial learning in the morris water maze task following withdrawal from an escalating dosage schedule of ampHetamine in wistar rats. Neuroscience 119(1): 167-179.

Salimi, K., L. F. Jarskog, et al., (2009). Antipsychotic Drugs for First-Episode Schizophrenia A Comparative Review. Cns Drugs 23(10): 837-855.

Sanchez-Soria, P. and Camenisch, T. D. (2010). ErbB signaling in cardiac development and disease. Seminars in Cell & Developmental Biology 21(9): 929-935.

Sawaguchi, T. and Goldman-Rakic, P. S. (1991). D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251(4996): 947-950.

Schott, B. H. (2008). The multiple roles of dopaminergic neurotransmission in episodic memory. Handbook of Episodic Memory 18.

Schultz, W. (2004). Neural coding of basic reward terms in animal learning theory,game theory, microeconomics and behavioural ecology. Curr.Opinion. neurobiol. 14: 139-147.

Seamans, J. K., Durstewitz, D. et al., (2001). Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. Proceedings of the National Academy of Sciences 98(1): 301-306.

Seeman, P. (1987). Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1(2): 133-152.

Seeman, P., Schwarz,v et al., (2006). Psychosis pathways converge via D2high dopamine receptors. Synapse 60: 319 - 346.

Segal, D. D., Weinberger, S.B., et al., (1996). Multiple Daily AmpHetamine Administration: Behavioral and Neurochemical Alterations. Neuroscience

Segal, D. S. and Mandell, A. J. (1974). Long-term administration of d-ampHetamine: Progressive augmentation of motor activity and stereotypy. Pharmacology Biochemistry and Behavior 2(2): 249-255.

Segal, D. S. and Mandell, A. J. (2002). Long-term administration of d-ampHetamine: Progressive augmentation of motor activity and stereotypy. Pharmacology Biochemistry and Behavior 2(2): 249-255.

Seiden, L. S., Sabol, K. E. et al., (1993). AmpHetamine: Effects on Catecholamine Systems and Behavior. Annual Review of Pharmacology and Toxicology 33(1): 639-676.

Shadach, E., Gaisler,v et al., (2000). The Latent Inhibition Model Dissociates between Clozapine, Haloperidol, and Ritanserin. Neuropsychopharmacology 23(2): 151-161.

Shen, S., Lang,v et al., (2008). Schizophrenia-Related Neural and Behavioral Phenotypes in Transgenic Mice Expressing Truncated Disc1. J. Neurosci. 28(43): 10893-10904.

Shifman, S., Bronstein,M., et al. (2002). A Highly Significant Association between a COMT Haplotype and Schizophrenia. Am J Hum Genet 71(6).

Sibley, D. R. (1999). New insights into dopaminergic receptor function using antisense and genetically altered animals. Annual Review of Pharmacology and Toxicology 39: 313-341.

Sills, T. L. (1999). Amphetamine dose dependently disrupts prepulse inhibition of the acoustic startle response in rats within a narrow time window. Brain Research Bulletin 48(4): 445-448.

Sills, T. L., Onalaja, A. O. et al., (1998). Mesolimbic dopaminergic mechanisms underlying individual differences in sugar consumption and ampHetamine hyperlocomotion in Wistar rats. European Journal of Neuroscience 10(5): 1895-1902.

Sircar, R. (2003). Postnatal phencyclidine-induced deficit in adult water maze performance is associated with N-methyl--aspartate receptor upregulation. International Journal of Developmental Neuroscience 21(3): 159-167.

Skarsfeldt, T (1996).Differential effect of antipsychotics on place navigation of rats in the Morris water maze: A comparative study between novel and reference antipsychotics. Psychopharmacology. Volume 124, Numbers 1-2,pp.126-133.

Solomon, P. R. and et al., (1981). Disrupted latent inhibition in the rat with chronic ampHetamine or haloperidol-induced supersensitivity: Relationship to schizophrenic attention disorder. Biological Psychiatry 16(6): 519-537.

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. Psychological Review 99(2): 195-231.

Steele, R. J. and Morris,v (1999). Delay-dependent impairment of a matching-to-place task with chronic and intrahippocampal infusion of the NMDA-antagonist D-AP5. Hippocampus 9(2): 118-136.

Stefani, M. R. and Moghaddam,v (2002). Effects of repeated treatment with ampHetamine or phencyclidine on working memory in the rat. Behavioural Brain Research 134(1-2): 267-274.

Stefansson H., Steinhorsdottir et al. S. E., (2002). Neuregulin 1 and Susceptibility to Schizophrenia. Am. J. Hum.Gen 71: 877-892.

Sullivan E.V, Zipursky RB, etal., (1994). A deficit profile of executive, memory, and motor functions in schizophrenia. Biol Psychiatry. 36(10): 641-53.

Swerdlow, M., Braff D. et al. (1994). Assessing the Validity of an Animal Model of Deficient Sensorimotor Gating in Schizophrenic Patients. Arch Gen Psychiatry 2(51): 139-154. .

Swerdlow, N. R., Braff, D. L. et al., (1990). GABAergic projection from nucleus accumbers to ventral pallidum mediates dopamine-induced sensorimotor gating deficits of acoustic startle in rats. Brain Research 532(1-2): 146-150.

Swerdlow, N. R., Geyer, M. A. et al., (2001). Neural circuit regulation of prepulse inhibition of startle in the rat: Current knowledge and future challenges. Psychopharmacology 156(2-3): 194-215.

Swerdlow, N. R., Bakshi, V. et al., (1996). Seroquel restores sensorimotor gating in phencyclidine-treated rats. Journal of Pharmacology and Experimental Therapeutics 279(3): 1290-1299.

Swerdlow, N., Mansbach, R. et al., (1990). AmpHetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology 100(3): 413-416.

Swerdlow, N., Mansbach, R. et al., (1990). AmpHetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology 100(3): 413-416.

Swerdlow, N., Weber, M., et al. (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology 199: 331-388.

Tanaka, S. (2002). Dopamine controls fundamental cognitive operations of multi-target spatial working memory. Neural Networks 15(4-6): 573-582.

Tendolkar, I., Ruhrmann, S. et al., (2002). Remembering or knowing: electrophysiological evidence for an episodic memory deficit in schizophrenia. Psychological Medicine 32(07): 1261-1271.

Tenn, C. V., Kapur, S. et al., (2005). Sensitization to ampHetamine, but not phencyclidine, disrupts prepulse inhibition and latent inhibition. Psychopharmacology 180(2): 366-376.

Terry E. Goldberg,T.E. Goldman, R.S., Burdick, K.E. et al.ÿ(2007). Cognitive Improvement After Treatment With Second-Generation Antipsychotic Medications in First-Episode Schizophrenia. Is It a Practice Effect? Arch Gen Psychiatry.ÿ2007;64(10):1115-1122.

Thaden, E., Rhinewine, J. P. et al., (2006). Early-onset schizophrenia is associated with impaired adolescent development of attentional capacity using the identical pairs continuous performance test. Schizophrenia Research 81(2-3): 157-166.

Thompson, P. M., Vidal, C. et al., (2001). Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 98(20): 11650-11655.

Thomsen, P. H. (1996). Schizophrenia with childhood and adolescent onset ? a nationwide register-based study. Acta Psychiatrica Scandinavica 94(3): 187-193.

Tosato, S., Dazzan, P. et al., (2005). Association Between the Neuregulin 1 Gene and Schizophrenia: A Systematic Review. Schizophrenia Bulletin 31(3): 613-617.

Toulopoulou, T., Rabe-Hesketh, S. et al., (2003). Episodic memory in schizophrenic patients and their relatives. Schizophrenia Research 63(3): 261-271.

Tran, A. H., Tamura, R. et al., (2003). Dopamine D2 receptor-knockout changed accumbens neural response to prediction of reward associated with place in mice. International Congress Series 1250: 493-508.

Tran, A. H., Tamura, R. et al., (2005). Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place. Proceedings of the National Academy of Sciences of the United States of America 102(6): 2117-2122.

Trimble, K. M., Bell, R. and King,D.J. (1997). Enhancement of latent inhibitionin the rat by the atypical antipsychotic agent remoxipride. Pharmacol. Biochem. Behav 56: 809?816.

Tseng, K.-Y., Lewis, B. L. et al., (2007). Post-Pubertal Disruption of Medial Prefrontal Cortical Dopamine-Glutamate Interactions in a Developmental Animal Model of Schizophrenia. Biological Psychiatry 62(7): 730-738.

Turgeon, S. M., Auerbach, E. A. et al., (1998). The delayed effects of phencyclidine (PCP) disrupt latent inhibition in a conditioned taste aversion paradigm. Pharmacology Biochemistry and Behavior 60(2): 553-558.

van den Buuse M, Lee R.X., et al., (2009). Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition regulation. Int J Neuropsychopharmacol. 12(10): 1383-93.

Van den Buuse, M. (2010). Modeling the Positive Symptoms of Schizophrenia in Genetically Modified Mice: Pharmacology and Methodology Aspects. Schizophrenia Bulletin 36(2): 246-270.

Van den Buuse, M., Wischhof, L., et al., (2009). Neuregulin 1 hypomorphic mutant mice: enhanced baseline locomotor activity but normal psychotropic drug-induced hyperlocomotion and prepulse inhibition

Vanderschuren, L. J. J., Schmidt, E.D.,et al.,(1999). A Single Exposure to AmpHetamine Is Sufficient to Induce Long-Term Behavioral, Neuroendocrine, and Neurochemical Sensitization in Rats The Journal of Neuroscience 19(21): 9579-9586.

Varty, G. B., N. Walters, et al., (2001). Comparison of apomorphine, ampHetamine and dizocilpine disruptions of prepulse inhibition in inbred and outbred mice strains. European Journal of Pharmacology 424(1): 27-36.

Venables, P. H. (1960). The effect of auditory and visuval stimulation on the skin potential response of schizophrenics. Brain 83(1): 77-92.

Waddington, J. L., Corvin, A. P. et al., (2007). Functional Genomics and Schizophrenia: Endophenotypes and Mutant Models. The Psychiatric clinics of North America 30(3): 365-399.

Waddington, J. L., O'Tuathaigh, C. et al., (2005). Phenotypic studies on dopamine receptor subtype and associated signal transduction mutants: insights and challenges from 10 years at the psychopharmacology-molecular biology interface. Psychopharmacology 181(4): 611-638.

Wall, P. M. and Messier, C. (2001). The hippocampal formation -- orbitomedial prefrontal cortex circuit in the attentional control of active memory. Behavioural Brain Research 127(1-2): 99-117.

Walsh, T., McClellan, J. M. et al., (2008). Rare Structural Variants Disrupt Multiple Genes in Neurodevelopmental Pathways in Schizophrenia. Science 320(5875): 539-543.

Walss-Bass, C., Liu, W. et al., (2006). A Novel Missense Mutation in the Transmembrane Domain of Neuregulin 1 is Associated with Schizophrenia. Biological Psychiatry 60(6): 548-553.

Wang, M., Pei,v et al., (2010). Schizophrenia, ampHetamine-induced sensitized state and acute ampHetamine exposure all show a common alteration: increased dopamine D2 receptor dimerization. Molecular Brain 3(1): 25.

Warburton, E. C., Joseph, M.H. , et al., (1996). Antagonism of ampHetamine-induced disruption of latent inhibition in rats by haloperidol and ondansetron: implications for a possible antipsychotic action of ondansetron

Weickert, C. S., .Rothmond, D.A., et al., (2008). Reduced DTNBP1 (dysbindin-1) mRNA in the hippocampal formation of schizophrenia patients. Schizophr. Res. 98: pp. 105?110.

Weinberger, D. R. (1988). Schizophrenia and the frontal lobe. Trends in Neurosciences 11(8): 367-370.

Weinberger, D. R. (1993). A connectionist approach to the prefrontal cortex. J Neuropsychiatry Clin Neurosci 5(3): 241-253.

Weinberger, D. R., Berman, K. F. et al., (1986). Physiologic Dysfunction of Dorsolateral Prefrontal Cortex in Schizophrenia: I. Regional Cerebral Blood Flow Evidence. Arch Gen Psychiatry 43(2): 114-124.

Weinberger, D. R., K. F. Berman, et al., (1992). Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: a magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. Am J Psychiatry 149(7): 890-897.

Weiner, I. (2003). The 'two-headed' latent inhibition model of schizophrenia: modeling positive and negative symptoms and their treatment. Psychopharmacology 169(3-4): 257-297.

Weiner, I. and Feldon, J. (1987). Facilitation of latent inhibition by haloperidol in rats. Psychopharmacology 91(2): 248-253.

Weiner, I. and Feldon, J. (1992). Phencyclidine does not disrupt Latent Inhibition in rats -Implications for animal-models of schizophrenia. Pharmacology Biochemistry and Behavior 42(4): 625-631.

Weiner, I., Feldon, J. . (1987). Latent inhibition is not affected by acute or chronic administration of 6 mg/kg dlampHetamine. Psychopharmacology (Berlin) 91

Weiner, I., Izraeli-Telerant, A. et al., (1987). Latent inhibition is not affected by acute or chronic administration of 6 mg/kg mpHetamine. Psychopharmacology 91(3): 345-351.

Weiner, I., Bernasconi, E. et al., (1997). AmpHetamine-induced disruption of latent inhibition depends on the nature of the stimulus. Behavioural Pharmacology 8(5): 442-457.

Weiner, I., Shadach, E. et al., (1996). The latent inhibition model of schizophrenia: Further validation using the atypical neuroleptic, clozapine. Biological Psychiatry 40(9): 834-843.

Weiner, I., Feldon,J et al., (1987). Facilitation of the expression but not the acquisition of latent inhibition by haloperidol in rats. Pharmacology Biochemistry and Behavior 26(2): 241-246.

Weiner, I., Lubow, R. E. et al., (1988). Disruption of latent inhibition by acute administration of low doses of ampHetamine. Pharmacology Biochemistry and Behavior 30(4): 871-878.

Wen, L., Lu, Y.-S. et al., (2009). Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. Proceedings of the National Academy of Sciences 107(3): 1211-1216.

Wheeler, M. A.,. Stuss, D. T et al., (1997). Toward a theory of episodic memory: The frontal lobes and autonoetic consciousness. Psychological Bulletin 121(3): 331-354.

Wilkerson, A. and Levin, E. D. (1999). Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. Neuroscience 89(3): 743-749.

Wilkerson, A. and Levin, E. D. (1999). Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. Neuroscience 89(3): 743-749.

Williams N.M, Williams H, et al.(2003). A systematic genomewide linkage study in 353 sib pairs with

Williams, R. J., Masten, V.L. and Geyer,M.A. (2002). Dopamine D1 Rather than D2 Receptor Agonists Disrupt Prepulse Inhibition of Startle in Mice. Neuropsychopharmacology 28: 108?118.

Wittmann, B. C., Schott, B. H. et al., (2005). Reward-Related fMRI Activation of Dopaminergic Midbrain Is Associated with Enhanced Hippocampus-Dependent Long-Term Memory Formation. Neuron 45(3): 459-467.

Yee, B. K., Chang D.L., and Feldon, J. (2004). The Effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. Neuropsychopharmacology 29(10): 1865-77.

Young, A. M. J., Gray,J.A., and Joseph M.H. (1992). Dopamine function in selective attention and conditioning: a microdialysis study using latent inhibition. J. Psychopharmacol 6.

Young, A. M. J., Moran, P. M. et al., (2005). The role of dopamine in conditioning and latent inhibition: What, when, where and how? Neuroscience and Biobehavioral Reviews 29(6): 963-976.

Zahrt, J., Taylor, J. R. et al., (1997). Supranormal Stimulation of D1 Dopamine Receptors in the Rodent Prefrontal Cortex Impairs Spatial Working Memory Performance. The Journal of Neuroscience 17(21): 8528-8535.

Zetterstrm, T., Sharp, T. et al., (1986). Further evaluation of the mechanism by which ampHetamine reduces striatal dopamine metabolism: a brain dialysis study. European Journal of Pharmacology 132(1): 1-9.

Zhuang, X., Oosting, R. S. et al., (2001). Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proceedings of the National Academy of Sciences of the United States of America 98(4): 1982-1987.

APPENDIX

1.1 Appendix 1: Data and analysis showing no effect of experiment in the AmpHetamine disruption of PPI in D2R WT and KO animals from two separate cohorts

Repeated measures ANOVA indicating no effect of experiment on the dataset, thus permitting pooling data from two separate cohorts to show sufficient genotype and treatment matched animal cohorts. Between subjects effects in the ANOVA (Appendix 1.1) showing main effects of Experiment, Genotype and Treatment in Chapter 2.

Between Subjects Effects

Source	Type III Sum of Squares	df	Mean Sq.	f	Sig.
	17.742	1	396583.547	249.681	.000
Genotype	10685.281	1	17.742	.011	.916
Treatment	41.775	1	10685.281	6.727	.012
Experiment*Genotype	26.035	1	41.775	.026	.872
Experiment *	2492.721	1	26.035	.016	.899

Source		Type III	df	Mean	F	Sig.
Treatment						
Genotype* Treatment	1640.436		1	2492.721	1.569	.216
Experiment*Genotype* Treatment	.000		1	1640.436	1.033	.314
	77829.639		0	.	.	.
Error			49	1588.360		

Table 1.1 (Appendix 1.1) Table showing results of ANOVAs between subjects differences of the effects of experiment, genotype and treatment on PPI in Chapter 2.

1.1.1. Repeated measures ANOVA, split by experiment (Experiment 1 vs Experiment 2) showing raw data each experiment.

The ANOVA was also split by experiment to look at group differences within each experiment. An absence of a control saline KO group and inadequate ampHetamine treated D2R WTs (n=3) in experiment 1, do not permit conclusive results. See Table 1.2

Within subjects Effects

Experiment 1

Source		Type III	df	Mean	F	Sig.

		Sum of Squares		Sq.		
PPI	Sphericity Assumed	719.036	3	239.679	5.869	.001
	Greenhouse-Geisser	719.036	2.420	297.114	5.869	.003
	Huynh-Feldt	719.036	3.000	239.679	5.869	.001
	Lower-bound	719.036	1.000	719.036	5.869	.025
PPI * Genotype	Sphericity Assumed	150.348	3	50.116	1.227	.307
	Greenhouse-Geisser	150.348	2.420	62.126	1.227	.306
	Huynh-Feldt	150.348	3.000	50.116	1.227	.307
	Lower-bound	150.348	1.000	150.348	1.227	.280
PPI* treatment	Sphericity Assumed	36.448	3	12.149	.297	.827
	Greenhouse-Geisser	36.448	2.420	15.061	.297	.784
	Huynh-Feldt	36.448	3.000	12.149	.297	.827
	Lower-bound	36.448	1.000	36.448	.297	.591

PPI*Genotype* Treatment	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
Error(ppi)	Sphericity Assumed	2572.898	63	40.840		

	Greenhouse-Geisser	2572.898	50.821	50.626		
	Huynh-Feldt	2572.898	63.000	40.840		
	Lower-bound	2572.898	21.000	122.519		

Table 1.2 (Appendix 1.1) Table showing results of within subjects effects of the repeated measures ANOVA as split by Experiment. PPI is the repeated measures factor and genotype and treatment are the between subjects factors.

Between Subjects Effects

Experiment 1

Source	Type III Sum of Squares	df	Mean Sq.	f	Sig.
Intercept	138336.719	1	138336.719	98.806	.000
Genotype	3795.046	1	3795.046	2.711	.115
Treatment	1689.506	1	1689.506	1.207	.284
Genotype * Treatment	.000	0	.	.	.
Error	29401.757	21	1400.084		

Table 1.3 (Appendix 1.1) Table showing results of ANOVAs between subjects differences of the effects of experiment, genotype and treatment on PPI in Chapter 2, as split by experiment.

The ANOVA split by experiment shows results of experiment 2. For within subjects effects in experiment 2, see Table 1.4. For between subjects effects in experiment 2, see Table1.5.

Experiment 2

Source		Type III Sum of Squares	df	Mean Sq.	F	Sig.
PPI	Sphericity Assumed	2476.489	3	825.496	8.919	.000
	Greenhouse-Geisser	2476.489	2.088	1186.295	8.919	.000
	Huynh-Feldt	2476.489	2.501	990.266	8.919	.000
	Lower-bound	2476.489	1.000	2476.489	8.919	.006
PPI * Genotype	Sphericity Assumed	389.930	3	129.977	1.404	.247
	Greenhouse-Geisser	389.930	2.088	186.786	1.404	.254
	Huynh-Feldt	389.930	2.501	155.920	1.404	.251
	Lower-bound	389.930	1.000	389.930	1.404	.246
PPI* treatment	Sphericity	275.233	3	91.744	.991	.401

	Assumed					
	Greenhouse-Geisser	275.233	2.088	131.843	.991	.380
	Huynh-Feldt	275.233	2.501	110.056	.991	.391
	Lower-bound	275.233	1.000	275.233	.991	.328
PPI*Genotype* Treatment	Sphericity Assumed	112.774	3	37.591	.406	.749
	Greenhouse-Geisser	112.774	2.088	54.021	.406	.677
	Huynh-Feldt	112.774	2.501	45.095	.406	.713
	Lower-bound	112.774	1.000	112.774	.406	.529
Error(ppi)	Sphericity Assumed	7774.797	84	92.557		
	Greenhouse-Geisser	7774.797	58.452	133.011		
	Huynh-Feldt	7774.797	70.023	111.032		
	Lower-bound	7774.797	28.000	277.671		

Table 1.4(Appendix 1.1) Table showing results of within subjects effects of the repeated measures ANOVA as split by Experiment. PPI is the

repeated measures factor and genotype and treatment are the between subjects factors.

Between Groups Effects

Experiment 2

Source	Type III Sum of Squares	df	Mean Sq.	f	Sig.
Intercept	324728.293	1	324728.293	187.751	.000
Genotype	15283.712	1	15283.712	8.837	.006
Treatment	6656.345	1	6656.345	3.849	.060
Genotype * Treatment	1640.436	1	1640.436	.948	.338
Error	48427.882	28	1729.567		

Table 1.5 (Appendix 1.1) Table showing results of ANOVAs between subjects differences of the effects of experiment , genotype and treatment on PPI in Chapter 4, as split by experiment.

1.1.2 Appendix 2: Data and analysis showing outliers removed from the AmpHetamine disruption of PPI in D2R WT and KO animals from two cohorts in Chapter 2

Outliers pertaining to the pooled data set consisting of two cohorts; Subject 48B1.7 (D2R KO, saline treated) was not included in the data set, owing to very low levels of % PPI (See Fig. 1a-d). Studies that have used prepulse intensities, similar to ours above 65 dB background noise; 69 dB, 73 dB and 81 db show upto 45% PPI in D2R WT and 40 % PPI in D2R KO administered saline (Ralph, Varty et al., 1999; Ralph-Williams, Lehmann-Masten et al., 2002). Neither of these studies show % PPI at or below 0 on the scale, in either D2R KO or WT mutants.
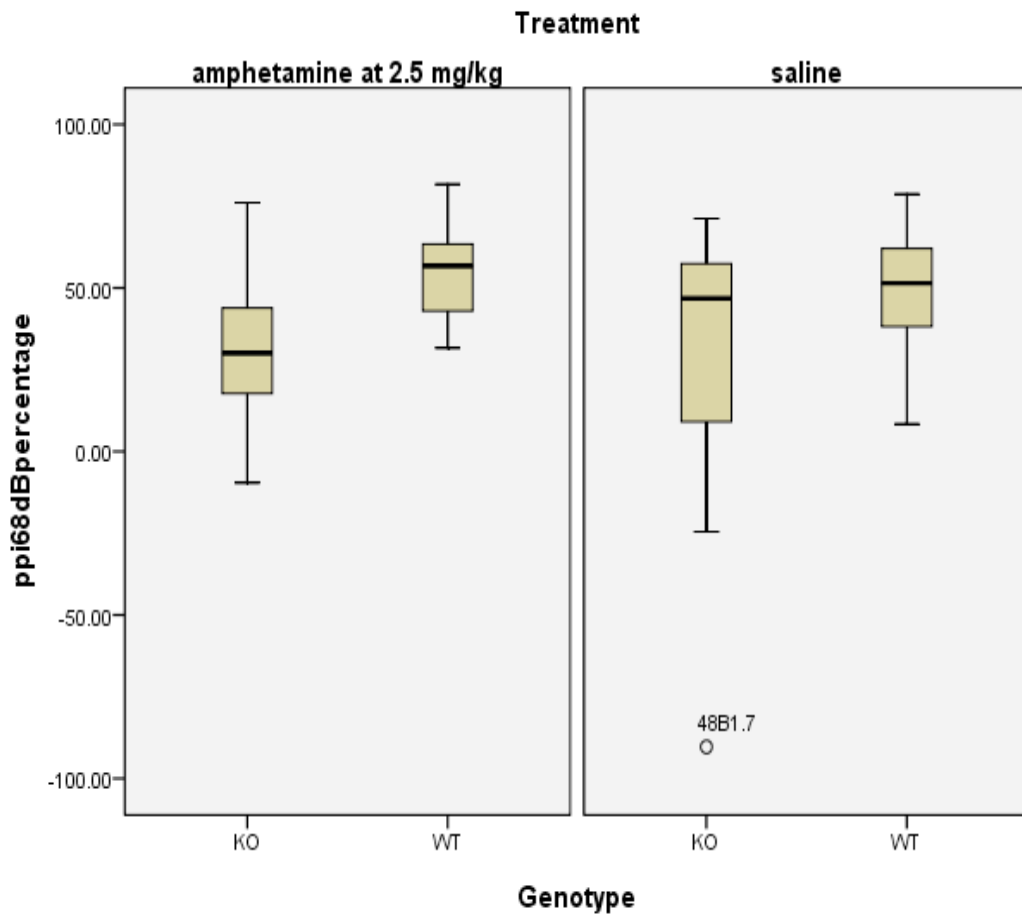
Figure 1a % PPI in D2R WT and KO animals, at the 68 dB prepulse intensity, split by treatment. Outliers are indicated below 0 level of PPI responding, with 0 being the maximum % inhibition toward the prepulse.
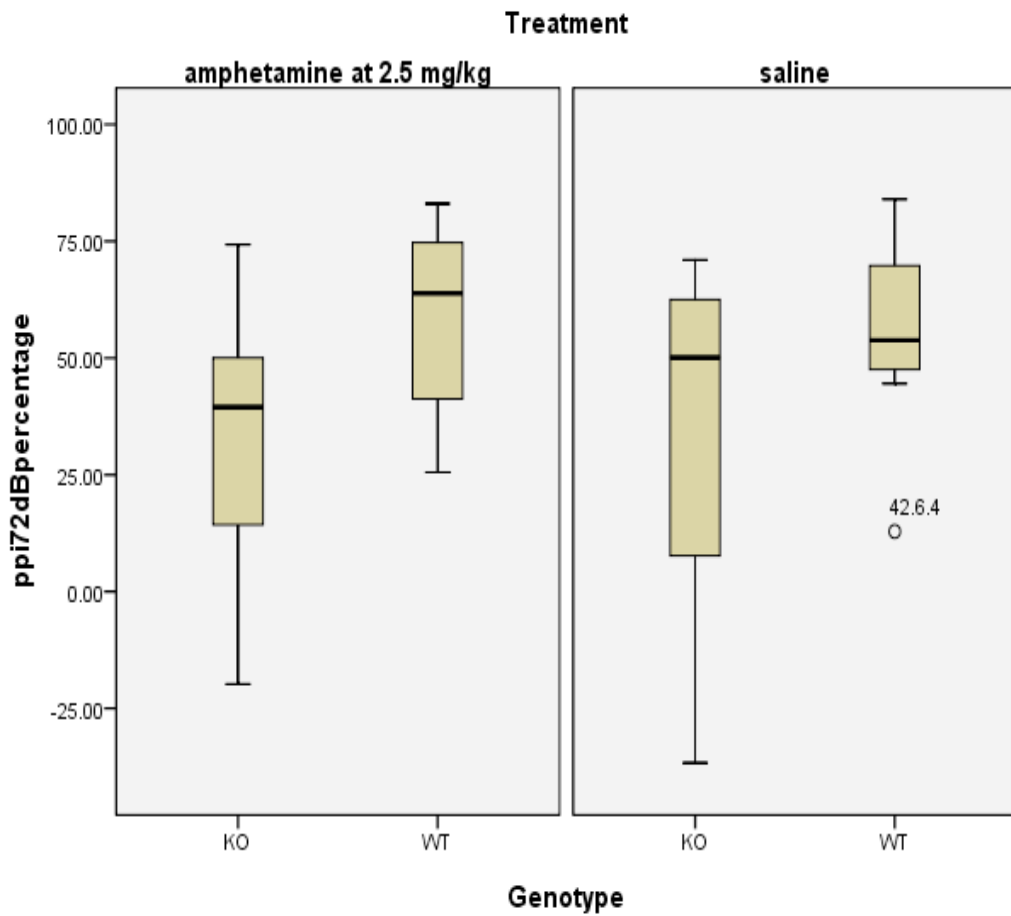
Figure 1b % PPI in D2R WT and KO animals, at the 72 dB prepulse intensity, split by treatment. Outliers are indicated below 0 level of PPI responding, with 0 being the maximum % inhibition toward the prepulse.

Figure 1c % PPI in D2R WT and KO animals, at the 80 dB prepulse intensity, split by treatment. Outliers are indicated below 0 level of PPI responding, with 0 being the maximum % inhibition toward the prepulse.

Figure 1d % PPI in D2R WT and KO animals, at the 68 dB prepulse intensity, split by treatment. Outliers are indicated below 0 level of PPI responding, with 0 being the maximum % inhibition toward the prepulse.
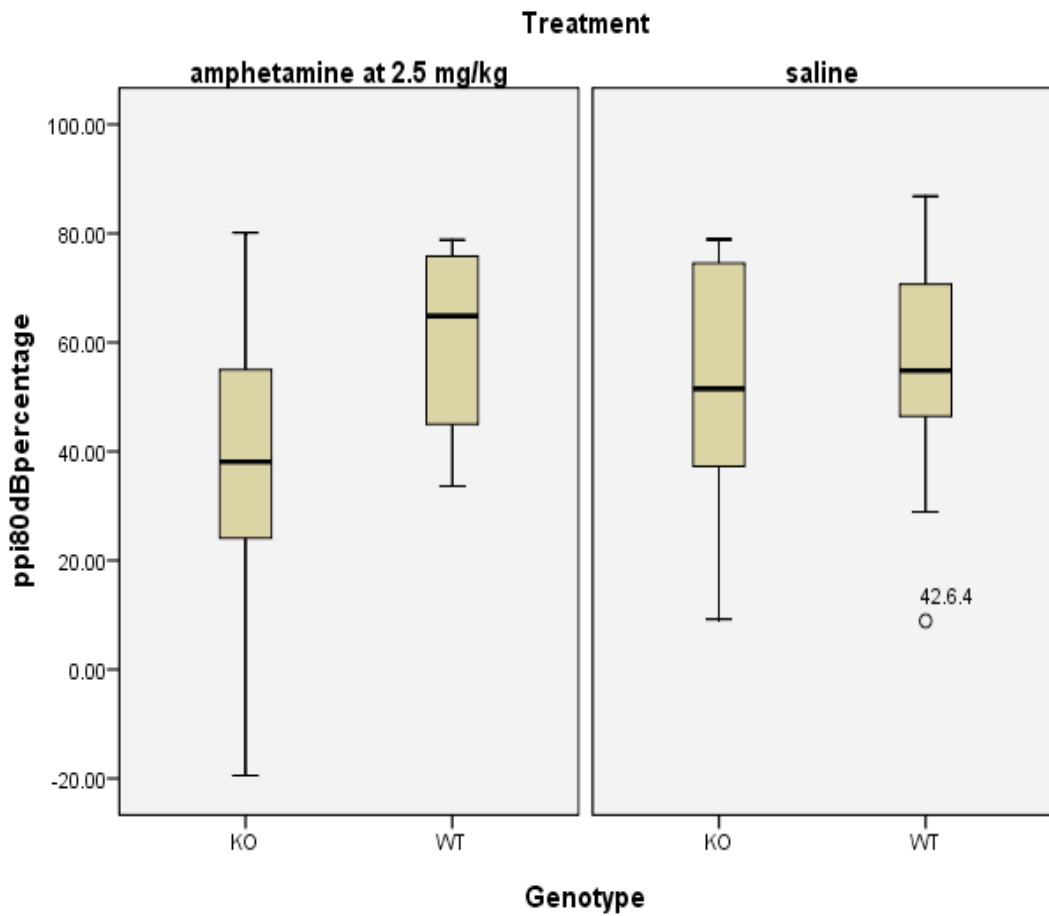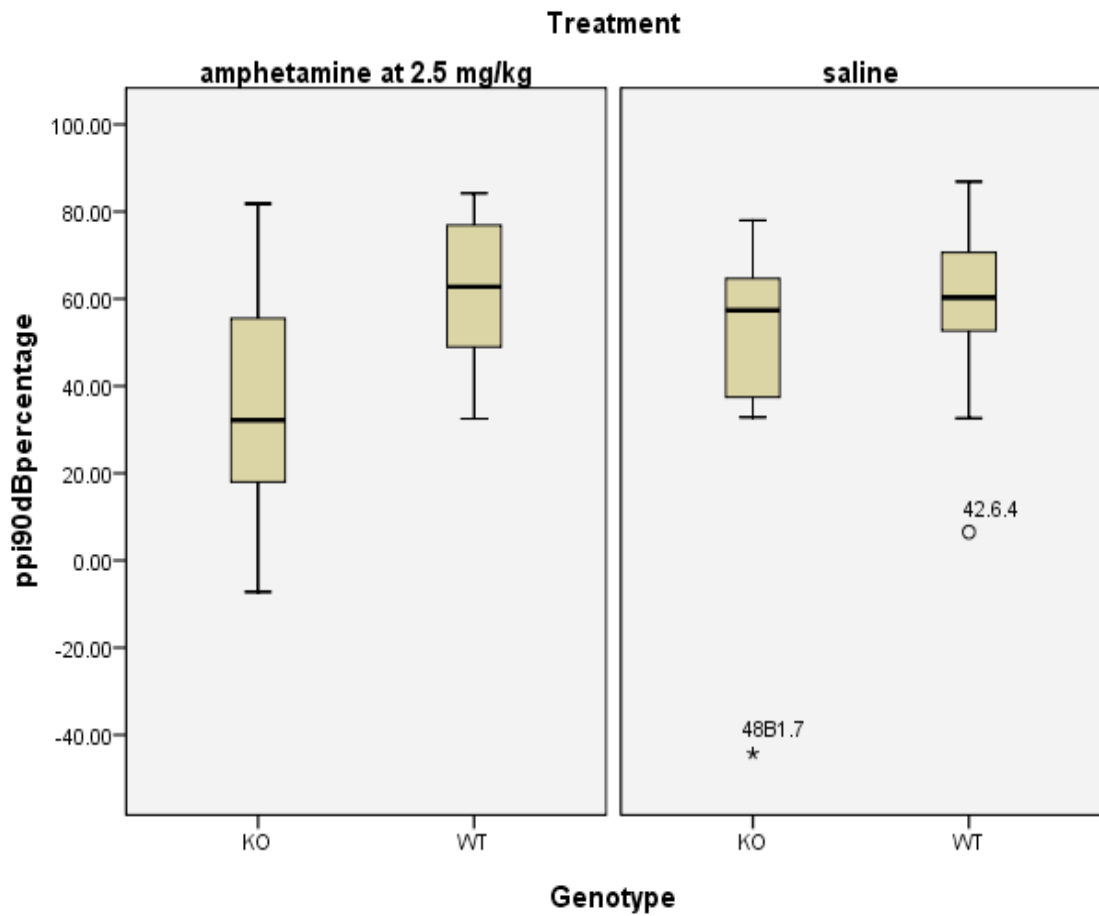
2.1 Appendix 3: Data and analysis showing D2R WT and KO sample exploration times for animals removed from the analysis in the episodic memory task in Chapter 3

Animal Id 100b1.4 showed little exploration in object A i.e. bottles as reflected by exploration time in sample 1. However, in test, it was seen when object A was displaced, exploration times increased significantly for the object displaced, and also for the object that occurred in the same position in the sample and test phases (See Table 1.6) that suggests a preference for place may interact with temporal memory for when the object occurred. Exploration times are dramatically increased for the old object, which in the sample stage had very little exploration. This makes it difficult to ascertain wHether increased exploration in test is due to the object being displaced, due to increased exploration of the more recent object, or that it is present in the location that was previously also occupied by this object in sample 1, See Table 1.8 and Figure 1e and Fig 1f.

Subject	Sample Stage	Object	Total Exploration times in sample	Test object	Total Exploration in test	Location in test	Location in Sample
100b1.4	1	Bottle	6	Bottle displaced	39	Top right corner (NE)	Bottom right (SE)
				Bottle stationary	31	**Bottom left (SW)**	**Bottom left (SW)**
	2	Golf Ball	36		51	**Top right (NW) and bottom right (SE)**	**Top right (NW) and bottom right (SE)**
99b4.1	1	Bottle	30	Bottle displaced	50	Top right corner (NE)	bottom right (SE)
				Bottle	41	Bottom	Bottom

				stationary		left (SW)	left (SW)
	2	Golf Ball	71		48	**bottom right (SE)**	**bottom right (SE)**
99b4.2	1	Bottle		Bottle displaced	40	Top right corner (NE)	bottom right (SE)
				Bottle stationary	45	**Bottom left (SW)**	**Bottom left (SW)**
	2	Golf Ball	24		42	**bottom right (SE)**	**bottom right (SE)**

Table 1.6 Table showing raw data for exploration times in animals that were removed from the analysis.

The same pattern is seen in subject 99b4.1 and 99b4.2. It is difficult to ascertain wHether exploration occurred independently of a spatial- temporal interaction between object, as the objects in the sample were placed in the same locations as they were in the test. The sample stages indicate preferences for the golf ball, but the test stages indicate preferences for the bottle. Here it is difficult to ascertain wHether object preference is due to the nature of the object (as reflected by exploration in sample stages), or due displacement of objects, as these objects were not explored to the same extent in sample 1, or due to objects being presented in the same locations in sample and test, thus reflecting a place preference that is independent of object exploration.
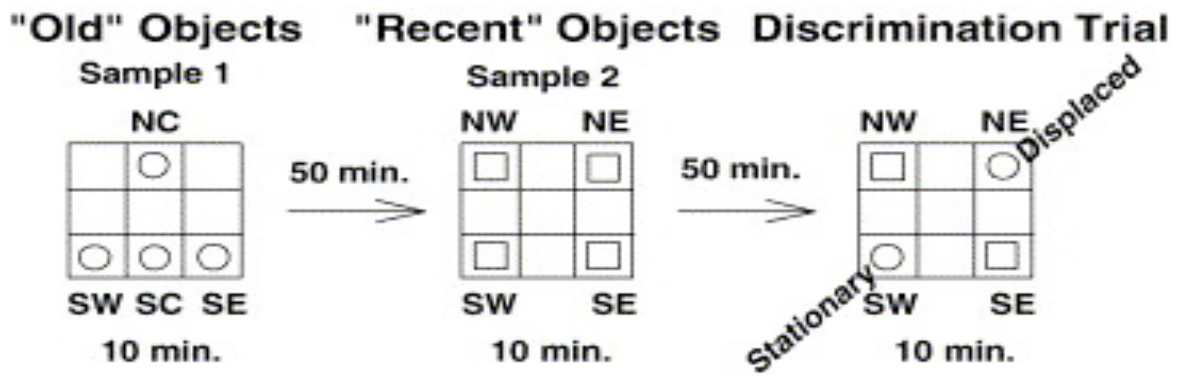
"Old" Objects   "Recent" Objects   Discrimination Trial

Figure 1e Schematic drawing of the what, where , when object exploration task. The mice received three 10-min trials with a 50-min inter-trial interval. On sample trial 1, 4 novel objects were presented arranged in a triangle spatial configuration. On sample trial 2, another 4 novel objects were presented in a different spatial arrangement. During the test trial, two "old familiar" and two "recent familiar" objects known from the sample trials were presented as depicted. Circles and squares represent equal objects presented initially in the first and second sample trial respectively. Object locations: NW = north-west, NC = north-center,   NE = north-east,   SW = south-west,   SC = south-center, SE = south-east.

2.1.1 Appendix 4: Analysis showing Recency Discrimination for old stationary object only vs recent stationary objects in D2R WT and KO animals in the episodic memory task in Chapter 3

A secondary analysis investigating discrimination of the recent objects vs the older stationary object only was conducted in order to explore the exploration of the old vs recent objects, and ascertain that increased exploration of the old objects was not due to one of the old objects being displaced, but a true measure of memory for the old object in a particular temporal context (sample 1) that occurred before the recent object (sample 2) (Dere, Huston et al., 2005).This indicates that animals genuinely prefer to explore the old object as they remember it occurred in sample 1, rather than because this object was simply displaced. However, our analysis shows that this was only true for female wt animals. All other animals show increased preference of the more recent objects in their original positions as in sample 2. However, this does not necessarily reflect object exploration due to recency alone, increased exploration of Recent object 2 at fixed locations, rather than equal exploration of recent objects suggests a place preference associated with the recent objects. Therefore, making it difficult to arrive at a conclusion about object exploration preference based on recency alone. See Table 1.6-1.7 and Figure 1f.

Source		Type III Sum of Squares	df	Mean Sq.	F	Sig.
Recency Discrimination	Sphericity Assumed	1923.875	1	1923.875	61.382	.000
	Greenhouse Geisser	1923.875	1.000	1923.875	61.382	.000
	Huynh-Feldt	1923.875	1.000	1923.875	61.382	.000
	Lower-bound	1923.875	1.000	1923.875	61.382	.000
Recency discrimination *Sex	Sphericity Assumed	244.994	1	244.994	7.817	.009
	Greenhouse-Geisser	244.994	1.000	244.994	7.817	.009
	Huynh-	244.994	1.000	244.994	7.817	.009

	Feldt				
	Lower-bound	244.994	1.000	244.994	7.817	.009
Recency discrimination*Genoytpe	Sphericity Assumed	11.907	1	11.907	.380	.543
	Greenhouse-Geisser	11.907	1.000	11.907	.380	.543
	Huynh-Feldt	11.907	1.000	11.907	.380	.543
	Lower-bound	11.907	1.000	11.907	.380	.543
Recency discrimination *Sex*genotype	Sphericity Assumed	149.750	1	149.750	4.778	.037
	Greenhouse-Geisser	149.750	1.000	149.750	4.778	.037

	Huynh-Feldt	149.750	1.000	149.750	4.778	.037
	Lower-bound	149.750	1.000	149.750	4.778	.037
Error(ppi)	Sphericity Assumed	877.589	28	31.342		
	Greenhouse-Geisser	877.589	28.000	31.342		
	Huynh-Feldt	877.589	28.000	31.342		
	Lower-bound	877.589	28.000	31.342		

Table 1.6 (Appendix 4) Results of repeated measures ANOVA for recency, showing Within subjects effects of the discrimination of the old stationary objects vs the recent objects

Source	Type III Sum of Squares	df	Mean Sq.	f	Sig.
Intercept	14863.022	1	14863.022	103.626	.000
Sex	225.478	1	225.478	1.572	.220
genotype	.156	1	.156	.001	.974
Sex * genotype	301.226	1	301.226	2.100	.158
Error	4016.034	28	143.430		

Table 1.7 (Appendix 4) Results of repeated measures ANOVA for recency showing the between groups effects of the discrimination of the old stationary objects vs the recent objects
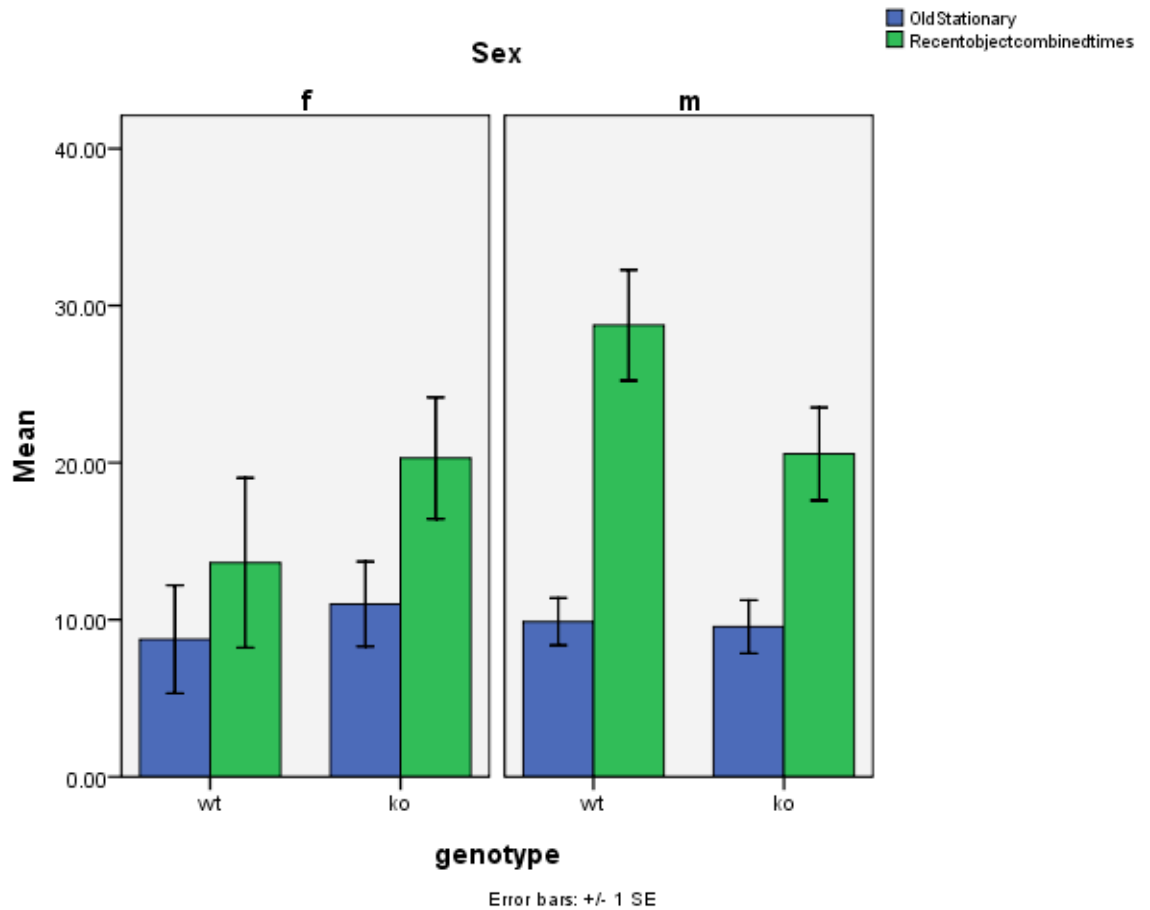
Figure 1f Graph showing raw means of exploration in D2R WT and KO of old stationary vs recent stationary objects only. Animals show increased exploration of the recent objects, when the raw exploration of old displaced objects is removed.
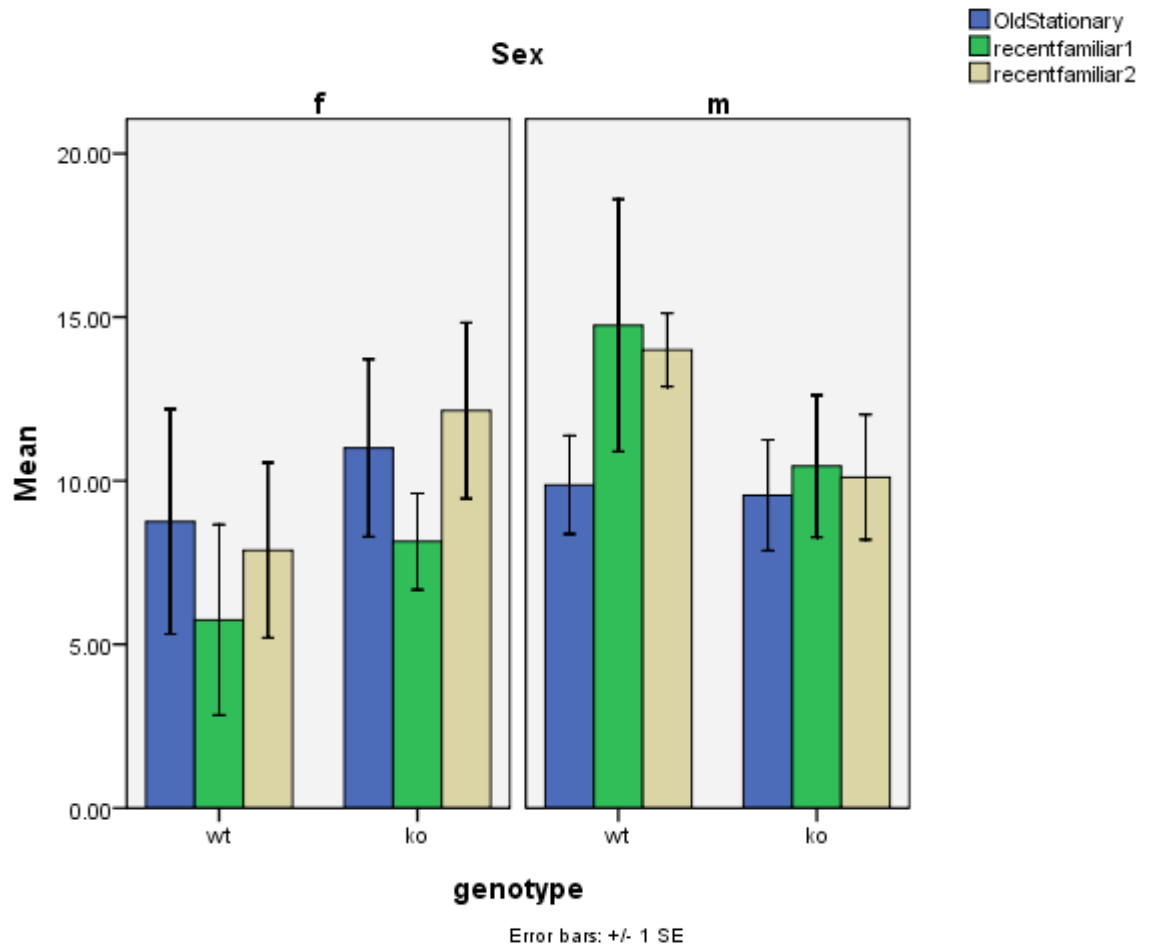
Figure 1g Graph showing raw means of exploration in D2R WT and KO of old stationary vs recent stationary objects 1 and recent stationary object 2 only. Both these recent objects were counterbalanced for place, as well as object type.

3.1 Appendix 5: Original episodic memory task, and its recent modification to segregate the influence of the spatial displacement of the object interacting with the temporal component of the episodic memory task in Chapter 8

The figure below shows the protocol we employed in mice as a measure of the episodic memory for what, where and when. As mentioned previously (Appendix 3) this protocol does not separate the temporal component from the spatial component, making it difficult to ascertain wHether object exploration is due to the memory for an old familiar object at a particular point in time, or simply a consequence of this object being displaced. See figure 1h. A recent modification of this protocol addresses this problem of spatio-temporal influence on objects, by displacing one of the recent objects in the test phase in addition to the old familiar objects. Now, a stationary old familiar object and a stationary recent familiar object are present in addition to a displaced old familiar and a displaced recent familiar object, thus facilitating a preference for objects solely on the basis of their occurrence at a particular point in time in terms of their recency and solely, due to the displacement , depending on wHether the old familiar object was recognised as being displaced or the recent familiar object was recognized as being displaced irrespective of its recency See figure 1i.
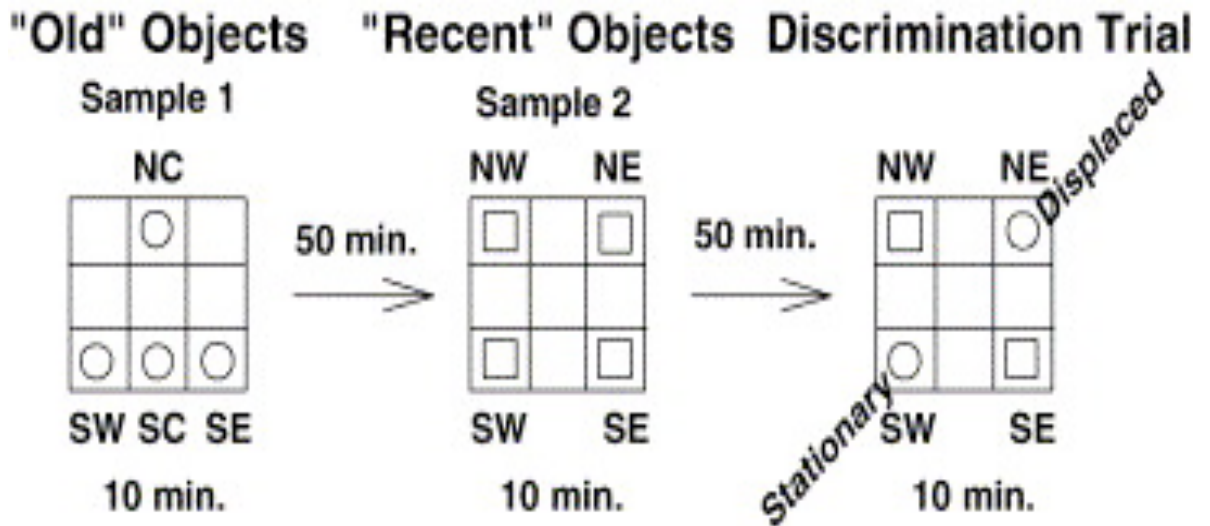
Figure 1h Schematic drawing of the what, where and when object exploration task, that was used in our experiment. The mice received three 10-min trials with a 50-min inter-trial interval. On sample trial 1, mice recive 4 novel objects were presented arranged in a triangle spatial configuration. On sample trial 2, another 4 novel objects were presented in a different spatial arrangement. During the test trial, two "old familiar" and two "recent familiar" objects known from the sample trials were presented as depicted. Circles and squares represent equal objects presented initially in the first and second sample trial respectively. Object locations: NW = north-west, NC = north-center, NE = north-east, SW = south-west, SC = south-center, SE = south-east.
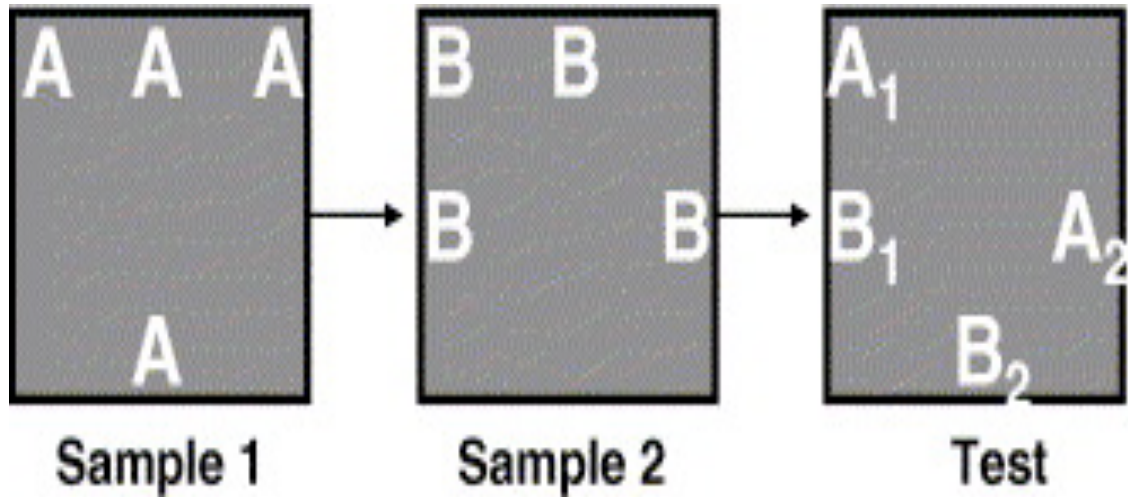
Figure 1i Schematic drawing of the experimental design shows modified object arrangement for the what, where and when task as previously mentioned. Rats received three 5 min trials with a 50 min inter-trial interval. During the test trial, two "old familiar" and two "recent familiar" objects known from the sample trials were presented at familiar and novel locations relative to the respective sample trials. A_1, "old familiar-stationary"; A_2, "old familiar-displaced"; B_1, "recent familiar-stationary"; and B_2, "recent familiar-displaced." (Kart-Teke, De Souza Silva et al., 2006)