Establishing a novel cognitive task in rats of relevance to schizophrenia

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Abstract

The most debilitating symptoms for patients with neuropsychiatric disorders, such as schizophrenia, are often cognitive deficits; yet they remain largely untreated by current medications. In preclinical animal models, the techniques used to measure cognitive deficits need to be improved to enhance our ability to screen novel drug targets and gain a better understanding of the neurobiological correlates of cognitive dysfunction. Therefore, the aim of this thesis was to develop and test a new high throughput cognitive task in rodents. I have designed a novel Signal Detection Task (SDT) for this purpose.

The first aim in developing the SDT was to compare alternative tasks (such as the 5-choice serial reaction time task) and address limitations including the extensive training time required and the lack of control over body position during stimulus presentation. Experiments were then selected to assess the face, construct and predictive validity of the SDT for measuring attentional deficits relevant to schizophrenia. Specifically, I determined if the effects of genetic, environmental, neurobiological and pharmacological manipulations could be detected in rats using this task. The SDT was conducted in rat operant chambers with a series of task variants to probe different components of performance, such as increasing detection difficulty and distraction. Briefly, the studies conducted compared strains (genetics), housing conditions (environment), the impact of a prefrontal cortical lesion (neurobiology) and the effects of amphetamine (pharmacology) on task performance. My findings indicate a relatively short training period was required for rats to perform the SDT with a high level of accuracy compared to other tasks; task acquisition was shown to be dependent on interactions between genetics and environment; prefrontal cortical lesions did not alter baseline performance but impaired attention during distraction and low dose amphetamine significantly improved accuracy. I demonstrated for the first time that the procognitive effects of amphetamine were dependent on baseline attentional performance. In addition, I found that individual variation in baseline performance was related to dopamine metabolism in the striatum.

The research outlined in this thesis presents a novel tool for researchers exploring the cognitive phenotype of animal models relevant to neuropsychiatric disorders and for exploring the neurobiology of attention, including mechanisms of action for procognitive medication.
Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Burne THJ, Alexander S, Turner KM, Eyles DW, McGrath JJ (2014) Developmentally vitamin D deficient rats are more sensitive to the behavioural effects of acute Δ⁹-tetrahydrocannabinol. Behav Pharmacol 25:236-244.


Conference abstracts

International


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Contributions by others to the thesis

Associate Professor Thomas Burne was my principle supervisor and provided support in designing and analysing each experiment. He has contributed to all publications, primarily as the senior author. Professor Darryl Eyles and Professor John McGrath also provided feedback on thesis drafts. Nick Valmas created the graphic images in Chapters 2, 3 and 6. Luis Sebastian Contreras Huerta scored the head angle and distance in the example used in Chapter 3. James Peak provided research support to conduct the studies in Chapter 3 and 6. Michelle Vega Sanchez scored ethological behaviours in Chapter 4. Suzy Alexander provided animal husbandry support, assisted with behaviour studies and perfusion procedures in Chapter 5. Jane Ellis provided histology support for slicing and staining brains in Chapter 5. Ava Solao conducted lesion scoring in Chapter 5 as part of an undergraduate research project. The contribution of co-authors to publications has been acknowledged in the previous section.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.
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cognition, schizophrenia, attention, neuropsychiatric disorders, behaviour, animal model, amphetamine, ADHD

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ANZSRC code: 170101, Biological psychology (Neuropsychology, Psychopharmacology, Physiological Psychology), 15%

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**Fields of Research (FoR) Classification**

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<th>Definition</th>
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<tr>
<td>5C-CPT</td>
<td>5-choice continuous performance task</td>
</tr>
<tr>
<td>5CSRTT</td>
<td>5-choice serial reaction time task</td>
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<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>Serotonin</td>
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<tr>
<td>6-OHDA</td>
<td>6-Hydroxydopamine</td>
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<tr>
<td>ADHD</td>
<td>Attention deficit hyperactivity disorder</td>
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<td>Amph</td>
<td>Amphetamine</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ASR</td>
<td>Acoustic startle reflex</td>
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<td>ASST</td>
<td>Attentional set-shifting task</td>
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<tr>
<td>Bdnf</td>
<td>Brain-derived neurotrophic factor</td>
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<tr>
<td>CATIE</td>
<td>Clinical Antipsychotic Trials of Intervention Effectiveness</td>
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<tr>
<td>Chrna7</td>
<td>Cholinergic receptor, nicotinic, alpha 7</td>
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<tr>
<td>CNTRICS</td>
<td>Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<td>CPT</td>
<td>Continuous performance task</td>
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<td>CPU</td>
<td>Caudate putamen</td>
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<td>d'</td>
<td>D-prime</td>
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<td>dB</td>
<td>Decibel</td>
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<td>DA</td>
<td>Dopamine</td>
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<td>DAT</td>
<td>Dopamine transporter</td>
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<td>DE</td>
<td>Deoxyepinephrine</td>
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<td>DMTP</td>
<td>Delayed match to position</td>
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<td>DOPAC</td>
<td>Dihydroxyphenyl acetic acid</td>
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<tr>
<td>dSAT</td>
<td>Sustained attention task distractor version</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>EE</td>
<td>Environmental enrichment</td>
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<tr>
<td>EPM</td>
<td>Elevated plus maze</td>
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<td>GDNF</td>
<td>Glial cell line-derived neurotrophic factor</td>
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<tr>
<td>Grin2b</td>
<td>Glutamate receptor, ionotropic,N-Methyl D-Aspartate 2B</td>
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<tr>
<td>GxE</td>
<td>Gene x Environment</td>
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<tr>
<td>HB</td>
<td>Hole board</td>
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<td>HE</td>
<td>Head entry</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>HVA</td>
<td>Homovanillic acid</td>
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<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
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<td>ITI</td>
<td>Inter-trial interval</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>LDT</td>
<td>Light dark test</td>
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<td>LE</td>
<td>Long Evans</td>
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<tr>
<td>LED</td>
<td>Light emitting diode</td>
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<td>LH</td>
<td>Limited hold</td>
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<td>MATRICS</td>
<td>Measurement and Treatment Research to Improve Cognition in SHI</td>
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<tr>
<td>MAO</td>
<td>Monoamine oxidase</td>
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<tr>
<td>MIA</td>
<td>Maternal immune activation</td>
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<td>mPFC</td>
<td>Medial prefrontal cortex</td>
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<tr>
<td>NA</td>
<td>Noradrenalin</td>
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<td>NGF</td>
<td>Nerve growth factor</td>
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<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<tr>
<td>NIH</td>
<td>National Institute of Health</td>
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<tr>
<td>NP</td>
<td>Nose poke</td>
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<tr>
<td>Nr3c1</td>
<td>Nuclear receptor subfamily 3 group C member 1</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCP</td>
<td>Phencyclidine</td>
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<tr>
<td>p(Hit)</td>
<td>Probability of a hit</td>
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<tr>
<td>p(FA)</td>
<td>Probability of false Alarm</td>
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<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>Polyl:C</td>
<td>Polyriboinosinic-polyribocytidilic acid</td>
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<td>PPI</td>
<td>Pre-pulse inhibition</td>
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<tr>
<td>PSA</td>
<td>Protected stretch attend</td>
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<tr>
<td>QBI</td>
<td>Queensland Brain Institute</td>
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<tr>
<td>RAM</td>
<td>Radial arm maze</td>
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<tr>
<td>RI</td>
<td>Responsivity index</td>
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<td>SAT</td>
<td>Sustained attention task</td>
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<tr>
<td>SD</td>
<td>Sprague Dawley</td>
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<td>SDT</td>
<td>Signal detection task</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SI</td>
<td>Sensitivity index</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>Tnfa</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>WCST</td>
<td>Wisconsin card sorting task</td>
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Chapter 1  General Introduction
1.1 Introduction

Cognitive deficits are considered core features of schizophrenia yet they remain largely untreated (Keefe et al., 2007; Keefe and Fenton, 2007). This is despite evidence showing they are one of the strongest predictors of patient outcomes and a surge of research in this field over the last decade (Green et al., 2000). One solution for overcoming the ‘translational bottleneck’ hindering the development of new medications may be the refinement of preclinical animal tasks used for measuring cognitive outcomes (Hyman and Fenton, 2003; Hyman, 2014; Young and Geyer, 2015). If animal models were more successful in detecting procognitive drug targets and for improving our basic understanding of cognitive deficits, this should lead to greater success in subsequent clinical trials. The research presented in this thesis focuses on the development and validation of a task for measuring attention in animal models with relevance to schizophrenia.

1.2 Schizophrenia

Schizophrenia is a disabling group of brain disorders affecting approximately 1% of the population worldwide (McGrath and Susser, 2009; Tandon et al., 2009; Javitt, 2010). It is characterised by positive, negative and cognitive symptoms. Positive symptoms include experiences such as hallucinations and delusions. Negative symptoms involve the lack of normal feelings or behaviours such as reduced speech, flattened affect and reduced motivation (Tandon et al., 2009). Cognitive symptoms include impairments across a range of domains and are central to the topic of this thesis.

The onset of schizophrenia typically occurs during adolescence and early adulthood, however this is often preceded by a prodromal phase. During the prodromal phase, psychotic symptoms do not reach diagnostic criteria and yet cognitive deficits may already be evident (Tandon et al., 2009). Schizophrenia is a leading cause of disability and suicide rates are 12 times higher in people with this diagnosis (Rezvani et al., 2002). Substance abuse, including the use of nicotine, alcohol, cannabis and cocaine, are common in schizophrenia patients (Green et al., 2004a). These factors combined with the increased rates of somatic diseases such as heart disease and diabetes, lead to increased morbidity and mortality in patients with schizophrenia (Caldwell and Gottesman, 1992; Rezvani et al., 2002; Saha et al., 2007; Koychev et al., 2011). No diagnostic biomarker has been detected thus far and symptomology can vary greatly between patients (Tandon et al.,
In the 1950’s the first antipsychotic, chlorpromazine, was discovered and this led to the development of many other D2 antagonist-based medications (Agid et al., 2008). Antipsychotic medications often lead to unacceptable side effects (e.g. tremor, tardive dyskinesia) and show greatest efficacy in reducing positive symptoms with very little improvement in negative or cognitive symptoms (Marder and Fenton, 2004; Agid et al., 2008; Carpenter and Koenig, 2008). In the 1970’s, the introduction of clozapine appeared to have superior efficacy for those with treatment resistance, which was followed by the introduction of a second generation of ‘atypical’ antipsychotics (Kapur and Remington, 2001). These antipsychotic medications have a different side effect profile (e.g. obesity) and they were initially thought to be more effective in the treatment of cognitive symptoms, however results of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study suggested this is not necessarily the case (Keefe et al., 2007). Despite the lack of progress that has been made in recent years, improving the treatment of cognitive impairments remains a priority for the field (Abbott, 2010; Hyman, 2014; Young and Geyer, 2015).

1.3 Cognitive deficits

Cognitive deficits often appear early in the development of schizophrenia and performance remains relatively stable when tested across different phases of disease progression (Rund, 1998; Nithianantharajah and Hannan, 2006). Patients have impairments across a number of cognitive domains, such as memory, attention, executive function, speed of processing and verbal memory (Marder and Fenton, 2004; Nuechterlein et al., 2004; Goff et al., 2011). This array of deficits may reflect specific areas of difficulty or more global cognitive dysfunction (Lesh et al., 2011). Poor cognitive performance has also been reported in un-affected relatives, which suggests that shared familial (e.g. genes, shared environment) factors contribute to impaired cognition in affected families (Harris et al., 1996; Cannon et al., 2000; Snitz et al., 2006). Despite cognitive dysfunction playing a prominent role in schizophrenia, they are not included in the DSM-V criteria (Widiger, 1994; Keefe and Fenton, 2007). Functional patient outcomes have repeatedly been shown to correlate more strongly with cognitive performance than psychotic symptoms, yet antipsychotic medications are only effective in treating positive symptoms (Green et al., 2000; van Praag et al., 2000; Kapur and Remington, 2001; Marder and Fenton, 2004; Keefe et al., 2007; Floresco and Jentsch, 2011; Simpson and Kelly, 2011). As positive symptoms represent only a portion of the disease state, it is not surprising that
Chapter 1. Introduction

Therapeutics have had limited success in improving the long-term prognosis for schizophrenic patients (Marder and Fenton, 2004).

With the lack of success in developing new medications, the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) initiative was developed to improve the transition from basic research to clinical outcomes and guide research investigating the treatment of cognitive deficits in schizophrenia (Green and Nuechterlein, 2004). They determined that cognitive deficits in schizophrenia were best measured across 7 domains: speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition (Nuechterlein et al., 2004). Based on these domains, a battery of the most promising clinical tasks was selected (Nuechterlein et al., 2008). Following this, the CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) initiative was formed to further these recommendations and address the need for translation between basic animal and human research (Carter et al., 2008). With the introduction of these recommendations, there has been an increasing focus on improving translation between rodent and human studies (Keeler and Robbins, 2011; Bussey et al., 2012b; Homberg, 2013; McKenna et al., 2013; Young et al., 2013a; Young and Geyer, 2015). The translation of clinically relevant tests may prove to be a highly valuable tool in the assessment of animal models and in the development of therapeutics (Barak and Weiner, 2011; Pratt et al., 2012). Human studies are often limited to observational data, while rodent-based studies allow researchers to ask more invasive questions using well-controlled experimental designs. However, schizophrenia is challenging to model since there are no diagnostic biomarkers and many of the symptoms (e.g. hallucinations, delusions) cannot be modelled in rodents. However, given many cognitive abilities can be assessed in rodents and the broader affliction of these deficits among high-risk individuals, cognitive testing may be one of the best ways to validate animal models of schizophrenia (Pratt et al., 2012).

1.4 Rodent models of Schizophrenia

Rodent models of schizophrenia have used pharmacological, neurodevelopmental or genetic manipulations, as well as interactions of genetic and environmental manipulations. Schizophrenia is a heritable disorder and there was hope that gene-targeted approaches would generate clear candidates. However, the last 10 years of research has painted a much more complicated picture. Genetic rodent models will be critical in understanding the
Chapter 1. Introduction

role of specific genetic modifications associated with schizophrenia, but it is clear no single gene is responsible for the development of this disorder (Kellendonk et al., 2009; Wray and Visscher, 2010; Pratt et al., 2012).

Many animal models of schizophrenia have been developed based on epidemiological evidence to assess risk factors thought to be associated with the disorder, and also to improve our understanding of the altered neurobiology (van den Buuse et al., 2005; Arguello and Gogos, 2006) (see database by Koenig et al. at http://schizophreniaforum.org/res/models/default.asp for further details). Key models in rats include developmental perturbations such as maternal infection (e.g. maternal immune activation (MIA) with PolyI:C (polyriboinosinic-polyribocytidilic acid)), developmental manipulations (e.g. developmental vitamin D deficiency) and anatomical alterations (e.g. neonatal ventral hippocampal lesioning) (Jones et al., 2011).

Pharmacological schedules using repeated administration of psychomimetics such as amphetamine, phencyclidine (PCP) and MK-801 have also been widely used (Lillrank et al., 1995; Marcotte et al., 2001; Boksa and Luheshi, 2003; Lipska, 2003; Rung et al., 2005; Amitai et al., 2007; Meyer and Feldon, 2010). These animal models attempt to reflect features of interest to clinical schizophrenia, including sensitivity to drug administration, and behavioural changes in tasks designed to measure traits such as attention, memory and sensorimotor gating (Pratt et al., 2012).

Unfortunately animal models have not been as useful as anticipated for developing and testing new antipsychotic medication. The use of drugs that appeared promising in preclinical studies have resulted in many failed clinical trials (Sarter et al., 1992b, a; Barak and Weiner, 2011). This major shortcoming added to the momentum for the formation of MATRICS and CNTRICS (Buchanan et al., 2005; Carter et al., 2008; Piontkewitz et al., 2012). Testing in rodent models has proven central in the development and screening of therapeutics for a range of diseases. Without adequate tools for the assessment of cognitive deficits relevant to schizophrenia, the development of procognitive medications will be limited.

Attention and vigilance is one of the cognitive domains identified by MATRICS with potential for measurement in humans and rodents (Nuechterlein et al., 2004). One of the human tasks selected for further development include variants of the Continuous Performance Task (CPT) (Carter et al., 2012). The CPT is a sustained attention task requiring participants to monitor a stream of stimuli and respond to targets. The robust
changes that have been detected using CPTs have lead to the suggestion that CPT deficits could be considered an endophenotype of schizophrenia, fitting the Gottesman and Gould (2003) criteria (Gottesman and Gould, 2003; Gur et al., 2007). The CPT protocol has been widely used with results robustly demonstrating deficits in people with schizophrenia and first-degree relatives (Snitz et al., 2006; Delawalla et al., 2008; MacDonald, 2008; Richard et al., 2013). Deficits have been shown to be stable from before first-episode of psychosis, during medication and remission, and performance errors do not correlate with psychotic symptoms in non-schizophrenia patients experiencing psychosis (Snitz et al., 2006; Richard et al., 2013). Together these findings suggest that deficits on CPT are at the foundations of this disorder. Deficits also correlate with the economic cost of the disorder in terms of loss of productivity and carer expenses, demonstrating the link between cognitive deficits and functional outcomes for patients (Ko et al., 2003).

1.5 Rodent tasks

1.5.1 Current tasks

The closest equivalent tasks to human CPT testing in rodents are the 5-choice serial reaction time task (5CSRTT), the 5-choice continuous performance task (5C-CPT), the sustained attention task (SAT) and more recently the touchscreen CPT task (McGaughy and Sarter, 1995a; Bari et al., 2008; Young et al., 2009b; Kim et al., 2015). The 5CSRTT, 5C-CPT and SAT were recommended by CNTRICS for further development (Lustig et al., 2013). To date, results from the touchscreen CPT have only been reported in a single study in mice (Kim et al., 2015) and although further studies in rats were indicated in a review (Hvoslef-Eide et al., 2015), they have not yet been published. This task may be a promising new avenue for research in the future, however the lack of data limit comparisons being made with the other more widely used tasks at this point in time. The 5CSRTT and 5C-CPT require rodents to attend to a spatial array of 5 holes and make a nose poke response when one hole illuminates. Each trial starts with either a fixed or variable inter-trial interval and if the rodent makes an impulsive nose poke response during this time, there is a time out period imposed. They also experience a time out for selecting the incorrect hole, but they receive an appetitive reward for selecting the correct hole. Omissions occur when the rodent does not respond within a limited hold period following stimulus presentation. The 5C-CPT differs by including a subset of trials where all 5 holes illuminate to indicate the rodent should withhold from making a response and therefore
Chapter 1. Introduction

includes a measure of response inhibition. The SAT differs in that the rodent is required to attend to a single stimulus and respond on one of two levers to indicate the presence or absence of a light presentation. This protocol is commonly presented with a distractor manipulation (dSAT), where a flashing houselight is presented during a block of trials. Each task has unique advantages and disadvantages. For example, the 5CSRTT has been more widely used and validated compared to the 5C-CPT and SAT. However, the 5C-CPT and SAT can easily be analysed using signal detection theory indices that are commonly used in human CPT studies. Although the SAT results in far fewer omission responses than the 5CSRTT and 5C-CPT, the application of this task to mice has been challenging (Martin et al., 2006; St Peters et al., 2011a). However, mice have been used in both the 5CSRTT and 5C-CPT (Young et al., 2009b; Harms et al., 2012; Sanchez-Roige et al., 2012). With a focus on translational testing, it is important to acknowledge that each of these protocols have similarities and differences when compared to human CPT testing.

1.5.2 Comparison with human CPT

There are a number of variants of the human CPT, for example the AX-CPT and identical pairs versions, which have been found to be sensitive to discriminating deficits in schizophrenia patients (Cornblatt and Keilp, 1994). Considering the substantial differences between rodent and human testing, I will consider a generic CPT-type task structure rather than aiming to replicate a specific CPT variant. Therefore, key features to be replicated are the rapid presentation of a single stimulus where a decision about signal and non-signal events determines the subject’s response. In addition, the subject should maintain attention towards the source of the stimuli and respond appropriately with minimal deviation away from the task. To achieve these behavioural goals in a rat, some features must in fact be deliberately different from the human CPT. As an example, in human CPT’s the use of externally controlled fast stimulus pace is potentially a very important element required to induce errors. However, in rats, a faster pace can be achieved by allowing self-initiation of trials with minimal delays imposed. Rats may have trouble maintaining task rhythm with a fixed, fast trial pace. However, by removing delays and allowing self-initiation of trials the rat can rapidly complete trials without losing track of the order of events. Differences in protocol design may in fact lead to more similar behaviour when species-specific requirements have been considered.

1.5.3 Body position and movement
Chapter 1. Introduction

A major difference between human CPT and rodent operant testing is the body position of the subject. Human subjects can be instructed to sit, facing a computer screen and maintain attention to detect a target stimulus. In contrast, a rodent may move freely around the chamber and perform alternative behaviours, such as grooming and even sleeping, during testing. The researcher cannot know whether the rodent was looking at the stimulus when it was presented or control the rat’s proximity to the stimulus. This may lead to incorrect responses due to poor visual angle rather than a lapse in attention. Indirectly, perhaps moving around the chamber is a form of inattention. However, it is not a brief lapse in vigilance and appears due to a loss of task-oriented goals. These alternative behaviours may even be considered ‘rest breaks’. This problem is exacerbated in the 5CSRTT, 5C-CPT and SAT tasks by delivery of the reward on the chamber wall opposite to the stimulus presentation, encouraging lapping behaviour in rodents across the chamber between trials (McGaughy and Sarter, 1995a; Bari et al., 2008; Young et al., 2009b). In addition, these three rodent tasks typically use relatively long inter-trial intervals and combined with imposed delays for reward collection and time out periods, the trial-to-trial interval is much longer than human CPT testing. Therefore in this study, I aimed to reduce excessive ambulation around the chamber and minimising delays between trials to decrease incongruous behaviours.

1.5.4 Omissions

Omissions can occur in rodents because they missed the stimulus presentation, but also because they were performing a competing behaviour such as grooming. Omission rates typically increase with drug administration and are virtually impossible to distinguish from a withheld response on the 5C-CPT. Although there are relatively few omissions on the dSAT, omissions can occur on up to 20% of trials on the 5CSRTT. Omission errors on human CPT’s can be very informative as an indicator of a lapse in vigilance, however because an omission in rodent testing can occur for a plethora of other reasons, the interpretation and cross-species comparison of this measure is complicated. In addition, the requirement for responding on every trial will be used in the rodent paradigm. Many, but not all, human CPT’s instruct the subject to respond to targets and inhibit responding to non-targets. Given a lack of response in rodents cannot always be attributed to a deliberate choice to withhold responding, a forced choice design may be more beneficial in rodents. Even though response inhibition is an important aspect of cognitive assessment, it was not the focus of this task. Therefore, one goal was to reduce the rate of omissions.
1.5.5 Stimulus properties

Next the type of stimulus must be considered. In human CPT’s, visual stimuli is most common, however there are auditory versions as well (Earle-Boyer et al., 1991; Riccio et al., 2002). Rodents do not have the visual capacity to perform human tasks using similar visual stimuli (Prusky et al., 2002). Rodents do not appear to identify colours, shapes and images in the same way as humans, although cognitive processing of images may be more advanced in rodents than generally assumed if sufficient training is provided (Jacobs et al., 2001; Zoccolan et al., 2009). Other sensory stimuli, such as olfactory cues, may be more informative or more easily acquired in rodents (Slotnick, 2001). However, a visual stimulus is advantageous in terms of control, both spatially and temporally, as compared to the presentation of olfactory stimuli. When testing many rodents simultaneously in the same room, a visual stimulus is unlikely to cause interference between animals compared with an auditory signal. Even with the use of sound attenuating chambers, the motoric sounds of a pellet dispenser can still be clearly heard throughout the room. Therefore, visual stimuli were used in the studies in this thesis. Species-specific differences in visual abilities must be considered when selecting appropriate stimuli. Due to the vast differences between rodents and human visual systems and the cognitive encoding of visual information, the presentation of stimuli will need to be carefully considered. At this point it should be noted that determining the correct choice based on the presentation of a single stimulus is likely to require different processes to the comparison and selection of two stimuli that are simultaneously presented. The use of an internalised rule, pairing a stimulus with the appropriate response in the human CPT is not replicated in the 5CSRTT where the location guides the response. In contrast, the 5C-CPT includes a rule about responding or inhibiting a response depending on the stimulus presented. And the SAT does require the rat to learn the association between different stimulus properties and the correct response, like the human CPT. Responding to the location of a stimulus is quite different to recognising features of a stimulus to respond correctly. Furthermore, identifying a stimulus without the opportunity to directly compare stimuli features may also require different cognitive processes. As construct validity is critical to translational testing and the aim was to maintain similar cognitive processes, stimuli were presented individually and rats were trained to make an association between the stimuli and response location.
1.5.6 Training

Other differences between human and rodent testing include the training technique and time to perform the task. Firstly, rodents require weeks or months of training to complete studies using tasks such as the 5CSRTT, whereas human subjects receive instructions prior to testing and complete the task within a single session. The instructions provided to human subjects are carefully selected and humans generally have ample life experience to perform CPT-type tasks without difficulty. However, studying cognition in humans brings a different set of challenges in terms of compliance, misunderstanding or different values/goals. On the other hand, rodents usually have no experience in learning tasks and are likely to have minimal handling or exposure to different environments prior to testing. So despite obvious differences in the ability to follow instructions, the prior relevant experience of human and rodent subjects are also substantially different. Needless to say, the extensive time required to train rodents is rate limiting and impedes high throughput preclinical drug studies. A further aim of this study was to reduce training time in rats, while maintaining task validity.

1.5.7 Motivation

A second major difference between human and rodent testing is the form of reward. To motivate rodents to perform operant tasks, appetitive rewards are provided after every correct trial and animals are normally further motivated using a food-restricted dietary schedule. On the other hand, human subjects may be motivated by money, course credit or psychological assessment. They may or may not receive feedback on a trial-by-trial basis and expectations may play a role in how much effort they expend. These differences cannot be easily overcome. Rodents require feedback after each trial to learn, particularly during task acquisition. Ultimately, if the subject is trying to respond correctly then the purpose of the motivator is accomplished. Therefore, a reward that leads to a high level of accuracy and trial completion will be used. Based on all the differences outlined above, a series of goals were defined for the development of a novel rodent attention task. To achieve these goals, species-specific behaviours were considered and protocol parameters and equipment were carefully chosen.

1.5.8 Attention

The main point in developing comparable tasks is to maintain the cognitive construct being measured, even after changing the stimulus input, the type of response and the training
protocol to suit species-specific abilities. The construct of attention can be further divided into many sub-types, including vigilance or sustained attention, divided attention and selective attention (Chudasama and Robbins, 2004). As the human CPT may be best described as a measure of vigilance or sustained attention, this was also a focus in the development of a rodent paradigm. A sustained attention task required the subject to maintain attention to a rather monotonous task in order to detect and respond to signals. Although the task may be best categorised as a measure of attention, there are a number of other outcomes that are generated by the CPT.

As with the human CPT, the rodent 5CSRTT and 5C-CPT measure attention, impulsivity, perseveration, response times and vigilance; with the 5C-CPT also measuring response inhibition. Human analogues of the rodent 5CSRTT have also been developed, including the CANTAB 5-choice task (Barnett et al., 2010), the spatial attentional resource allocation task (SARAT) (Hahn et al., 2012) and 4-CSRTT for humans (Worbe et al., 2014). The 5CSRTT, 5C-CPT and the dSAT were identified by CNTRICS as having face, predictive and construct validity when compared to the human CPT (Young et al., 2009a; Lustig et al., 2013). More recently the translational value of the 5C-CPT was demonstrated by comparing drug-induced deficits in mice to the performance of schizophrenia patients on the 5C-CPT (Young et al., 2013b). However, there are still a number of differences between the rodent and human versions of CPT including the spatial nature of stimuli and the speed of stimuli presentation. A critical goal for rodent tasks is being able to predict and detect drug targets for therapeutic use. Unfortunately, despite widespread use of task such as the 5CSRTT and major advances in understanding the neurobiology required for task performance, there has been limited success in discovering clinically effective novel drug targets (Robbins, 2002; Abbott, 2010; Hyman, 2014; Young and Geyer, 2015). Drugs that are known to have procognitive effects in humans, such as low doses of amphetamine (Wolraich et al., 2005), have not consistently demonstrated enhancement of performance in healthy, adult rodents on 5CSRTT (Bizarro and Stolerman, 2003). Therefore, further development of these (5CSRTT, 5C-CPT, dSAT) and novel tasks for rodent have been suggested, along with a series of recommendations to guide researchers (Pratt et al., 2012; Lustig et al., 2013).

1.6 Validation of rodent tasks

The translational validity of rodent tasks has been determined by a number of factors, including the involvement of homologous brain regions, response to psychoactive drugs,
similar response to manipulations of the task and response to factors known to alter performance (Young et al., 2009a; Lustig et al., 2013). The value of a task is often determined by assessing face, construct and predictive validity and here these ideas will be applied to the comparison of human and rodent tasks. Face validity indicates the similarity between tasks in appearance or design. Construct validity provides an indication of whether the tasks are measuring the same underlying function. Finally, predictive validity requires the results of the rodent task to be indicative of the response in humans, particularly in terms of drug effects (Homberg, 2013). In the case of 5CSRTT, construct validity has been demonstrated as the prefrontal cortex (PFC) and thalamus are involved in task performance (Muir et al., 1996; Baunez and Robbins, 1999; Passetti et al., 2003); reflecting the importance of these brain regions in human CPT performance (Salgado-Pineda et al., 2003; Young et al., 2009a). The 5CSRTT has also been shown to detect the attentional impairments that occur with ageing and sleep deprivation, which are known to alter attention in humans (Grottick et al., 2003; Cordova et al., 2006). Although low doses of amphetamine have been shown to reverse cognitive deficits in aged rats (Grottick and Higgins, 2002; Bizarro and Stolerman, 2003), reports of the positive effects of nicotine demonstrate the predictive validity in the 5CSRTT (Young et al., 2004). Predictive validity is further demonstrated by manipulations of the rodent task based on factors that alter performance on CPT in humans; for example reducing the stimulus duration (Grottick and Higgins, 2002). The translational value of the novel task developed in this thesis was determined using a number of experiments addressing the issues of face, construct and predictive validity.

1.7 Aims and outline

The overall aim of this project was to design and construct a novel rodent task analogous to human CPT for the purpose of identifying cognitive deficits in rodents relevant to the cognitive features of schizophrenia and for the assessment of procognitive treatments. Impairments on the CPT have consistently been associated with schizophrenia, leading to endorsements from leading consortia to pursue task development for wider use (Carter et al., 2012). This thesis presents the development of a modified signal detection task (SDT), which incorporates key aspects of the human CPT, which to date have not been mirrored in the rodent tasks (Chapter 2). After developing the SDT, a series of experiments were used to validate the task. Firstly, I compared performance outcomes on the SDT with a traditional rodent task for measuring attention, the 5CSRTT (Chapter 3). I then explored
different variables that were predicted to influence cognitive performance in rodents. For instance, it was predicted that genetic and environmental factors would influence a range of behavioural characteristics including cognition. This led to the next study where I determined the effect of different genetic strains and environmental housing conditions on a range of behavioural traits and SDT performance (Chapter 4). Following this study I aimed to establish neurobiological evidence for the SDT measuring processes relevant to attention and schizophrenia. Prior studies suggest that impairments of the PFC occur in schizophrenia and this region is known to be involved in executive control of behaviour in rats and humans (Dalley et al., 2004). Therefore, I assessed the effect of a PFC lesion on SDT performance to determine the role of the PFC on attentional performance (Chapter 5). One of the main purposes for task development is to improve the ability of preclinical tests to predict drug response in humans. Hence in Chapter 6, I investigated the effects of amphetamine, which is an indirect dopamine agonist known to alter (and in some cases improve) attentional performance in humans and rats. These results demonstrated the SDT could detect procognitive drug response. Consequently, this was followed by a replication study investigating the procognitive effects of amphetamine with an additional examination of neurochemical changes associated with SDT performance (Chapter 7). Together these studies provide evidence of face, construct and predictive validity of the SDT. While this task was designed within the context of deficits associated with schizophrenia, attentional impairments are also associated with a range of neurocognitive disorders. Therefore, this task may be used to assess deficits in models relevant to dementia, traumatic brain injury, depression or attention deficit hyperactivity disorder (ADHD). Overall, the results of this thesis provide a novel tool for researchers investigating disorders with cognitive impairments and also work towards the larger goal of improving treatment for patients with schizophrenia.
Chapter 2  Task Development: A novel Signal Detection Task
Chapter 2. Task development

2.1 Foreword

This chapter describes the development of the modified SDT with chamber design, protocol features and task modifications described. In addition, experiments were conducted to confirm (a) that visual stimuli were being used and (b) whether variations to the visual stimulus could be detected. Throughout the thesis some minor changes to the protocol have been made, for example signal durations vary, however the general flow of the paradigm remains the same. A thorough examination of unique task elements and contrasts with other rodent tasks has been discussed in the article in Chapter 3.
2.1.1 Abstract

The translational testing of cognitive deficits in animal models of schizophrenia has gained momentum with the growing appreciation of the role cognitive symptoms play on functional patient outcomes. Although there is a range of cognitive tasks that have been developed for use in rodents, few reflect the type of tasks clinically shown to differentiate patient and control groups. A novel SDT was developed to reflect aspects of the human continuous performance task. Task manipulations were then used to measure different aspects of cognitive performance. Rats were able to determine signal and non-signal trials with minimal training. Task variants using distracting stimuli and reversal of task contingencies were then utilised. Distraction within the same modality resulted in reduced accuracy, however cross-modal distraction using auditory stimuli reduced the speed of trial completion while accuracy remained high. Reversing the task led to extinction of the previous response and acquisition of the new stimulus-response pairing. A short training time was achieved using the SDT with rats able to perform the task after 20 training sessions. This task differed from other operant protocols in a number of ways; including faster trial rate, a near absence of omissions, trials were self-initiated with immediate stimulus presentation and the rat was in a central position on signal presentation. These features resulted in rats performing tasks when motivated and located appropriately within the chamber; reminiscent of the way standard human computer-based tasks are completed.
2.1.2 Introduction

Three rodent tasks have been identified as having face, construct and predictive validity for measuring the cognitive domain of attention/vigilance (Lustig et al., 2013). These are the 5CSRTT, 5C-CPT and dSAT. Each of these tasks has been said to reflect elements of the human CPT. However in comparing these tasks to the human CPT, a number of differences particularly in terms of face validity were identified. These differences have been discussed (Chapter 1) and therefore will not be repeated here. Based on these observations, species-specific differences in task performance and optimal task characteristics, a series of goals were established for the development of a novel task. These included:

- rapid presentation of a single stimulus with a response required for both trial types
- task acquisition should be relatively quick with minimal session duration
- the rat’s body position should be in front of the stimulus during presentation
- reducing unnecessary ambulation in the chamber and minimising task delays to decrease incongruous behaviours and decrease deviation away from the task
- reducing the rate of omissions
- carefully considering the visual stimuli to be used
- using a reward that leads to a high level of accuracy and trial completion

After the SDT was developed, performance was varied using a range of different manipulations. The purpose of these studies was to develop a method for the SDT, investigate factors involved in task performance and start to explore the use of variants that expand on the standard task.

2.1.3 Methods

2.1.3.1 Animals and housing

Male Sprague Dawley rats (N=16; ARC, WA) aged 8 weeks were pair-housed in cages with wire lids containing aspen chip bedding, nesting and a wood chew (Able Scientific, WA, USA). Male rats were selected as sex differences were not being examined in this study and males are more commonly used in similar experiments. There were housed at 21±2°C and 60% humidity on a 12-h light cycle (lights on at 0600 h). Each rat was regularly tail-marked for identification and weighed daily. At 10 weeks of age rats were food restricted to 90% for their free-feeding body weight and had *ad libitum* access to
water. All procedures were performed with approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

2.1.3.2 Apparatus

Training was conducted using Med Associates operant chambers for rats (Med Associates Inc., St. Albans, VT, USA). Each chamber was located within a sound attenuating box with a ventilation fan and overhead camera (CCD Mini CCIR, Samsung, Suwon, South Korea) for monitoring behaviour during training. The operant chambers were arranged specifically for the SDT as depicted in Figure 2.1A. All components were placed on a single wall with a houselight, custom-made signal display panel and a nose poke port located down the middle of the wall. On either side of the nose poke port there was a food magazine. Both the nose poke port and food magazines were equipped with a light and infra-red beam for head entry detection. The signal display panel was constructed using a filler panel with a grid of 3 x 3 light emitting diodes (5mm, green diffuse, 80MCD, Jaycar Electronics, NSW, Australia) that were plugged into output connections on the Med Associates SmartCtrl connection panel. For the distractor study a background noise generator was included on the back wall. Grain pellets (45mg, F0021 dustless precision pellet, Bioserv, Frenchtown, NJ, USA) were delivered to the food magazines to reward rats. All protocols were written using MedState Notation and Med-PC for Windows software (Med Associates Inc., St. Albans, VT, USA) was used for chamber operation and data collection.

2.1.3.3 Operant training

Firstly, rats were trained to collect pellets from a food magazine where each head entry resulted in another pellet being dispensed. After consuming 50 pellets on two consecutive days rats moved to the next level requiring a central nose poke to initiate trials. After nose poke detection, both magazines illuminated and head entry into a magazine lead to the delivery of a food pellet. A maximum of 100 pellets could be achieved with up to 50 pellets being delivered to each side. Collecting 80 pellets was considered sufficient to move to the next protocol. Two visual stimulus conditions (signal versus son-signal) were then presented after the central nose poke and if the correct receptacle was selected a reward was delivered (Figure 2.1B). The visual stimulus and response location contingency was counterbalanced across subjects in each study of this thesis. A list of outcome measures derived from the SDT is provided in Table 2.1.
Figure 2.1 Signal Detection Task.

(A) Graphic of chamber design. (B) Each trial runs through a procedure starting with the central nose poke, after which the stimuli was displayed and magazines become available for responding. If the correct magazine was chosen a pellet was delivered, however if the incorrect side was selected the trial ends with a time out. The next trial can be started when the central nose poke aperture illuminated.

1 Graphic image from Chapter 3 was published in Frontiers in Behavioral Neuroscience, 9:370, shared under Creative Commons Attribution Licence and originally created by Nick Valmas.
### Table 2.1 Outcome measures on SDT

<table>
<thead>
<tr>
<th>Measure</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials initiated</td>
<td>Number of trials initiated by centre nose poke</td>
</tr>
<tr>
<td>Session duration</td>
<td>Minutes taken to complete maximum trials (120)</td>
</tr>
<tr>
<td>% Omissions</td>
<td>Percentage of trials initiated but no magazine response within 4s</td>
</tr>
<tr>
<td>% Accuracy signal trials</td>
<td>Correct signal responses / (Correct signal + Incorrect signal responses)*100</td>
</tr>
<tr>
<td>% Accuracy non-signal trials</td>
<td>Correct non-signal responses / (Correct non-signal + Incorrect non-signal responses)*100</td>
</tr>
<tr>
<td>Latency to initiate trials</td>
<td>Time taken to initiate trial after central nose poke illuminates</td>
</tr>
<tr>
<td>Response latency</td>
<td>Time taken from stimulus cessation and magazine illumination to head entry detection</td>
</tr>
<tr>
<td>Premature HE during ITI</td>
<td>Number of head entries into the central nose poke prior to illumination during ITI</td>
</tr>
<tr>
<td>Premature HE during stimulus</td>
<td>Number of head entries into the magazine during stimulus presentation window</td>
</tr>
</tbody>
</table>

### 2.1.3.4 Signal Detection Theory

Other measures can be derived from the SDT using signal detection theory indices. The basis for signal detection theory analysis is that decision-making by the individual is determined by the statistical difference between the signal and noise. The advantages of using signal detection theory include the isolation of signal discriminability and response bias as opposed to using accuracy, within which these factors can vary independently. As the protocol has two choices and a response is recorded on every trial, four values can be calculated (Young et al., 2009b; Carandini and Churchland, 2013). A hit (correct signal trial), miss (incorrect signal trial), correct rejection (correct non-signal trial) or a false alarm (incorrect non-signal trial) can be made. These scores can be further analysed to determine an animal’s sensitivity to detecting the trial type with Sensitivity Index (SI) and a Responsivity Index (RI), which indicates if a rat was responding on one side more than the other. Calculations are as follows:

**Equation 2.1 Probability of a Hit**

\[
p(\text{Hit}) = \frac{\text{Hit}}{(\text{Hit} + \text{Miss})}
\]
Equation 2.2 Probability of a False Alarm
\[ p(FA) = \frac{\text{False alarm}}{(\text{False alarm} + \text{Correct rejection})} \]

Equation 2.3 Sensitivity Index
\[ SI = \frac{p(\text{Hit}) - p(FA)}{2[p(\text{Hit}) + p(FA)] - [p(\text{Hit}) + p(FA)]^2} \]

Equation 2.4 Responsivity Index
\[ RI = \frac{p(\text{Hit}) - p(FA) - 1}{1 - [p(FA) - p(\text{Hit})]^2} \]

The application here is slightly different to use in the human CPT in that the rat was required to respond, rather than inhibit responding, on non-signal trials. Therefore the interpretation also differs. RI has been defined as the likelihood to respond (effectively hits and false alarms vs. correct rejections and misses) and on an inhibitory task this would indicate the subject’s ability to withhold responding. However, in this study RI would be more indicative of a side bias. Although RI has been used on similar tasks, the different forms of responding (press/inhibit compared to left/right) must be considered when interpreting this measure.

Due to the near absence of omissions on the SDT, p(Hit) and p(FA) are equivalent to signal accuracy and the inverse of non-signal accuracy, respectively. Therefore, these measures replicate the results derived using % correct. SI and RI are derived from these values and represent a combined score, where SI indicates overall detectability and RI indicates the ratio of responding to the signal or non-signal side. These values are distinct from each other, but are both highly correlated with % correct on signal trials, % correct on non-signal trials, p(Hit) and p(FA). Thus, they provide useful information in some situations, such as when accuracy on both signal and non-signal trials is affected or when the decision-making criterion is altered. However, if only one parameter is manipulated these values become less informative, for example when using a manipulation of signal trials with reduced stimulus durations in a pseudorandom order interspersed with non-signal trials. The % correct on non-signal or p(FA) stays the same and % correct on signal or p(Hit) decreases as stimulus length was reduced. Using SI and RI in this instance does not provide additional information about performance as only one value was changing with the manipulation of interest.
Chapter 2. Task development

In considering the use of signal detection theory in this thesis, the application was deemed superfluous for the majority of chapters due to the manipulation of interest. In Chapter 3 signal detection theory could not be applied to the 5CSRTT and the key outcome was comparison of results between tasks. In Chapter 4, task acquisition rate was the primary outcome measured. In Chapter 5 data were analysed using both signal detection theory and % correct on signal and non-signal trials to demonstrate the application and similarity between the measures. In Chapter 6 and 7 the primary measures were reducing signal durations where signal detection theory measures only shift with the change in % correct on signal trials. Therefore, the majority of studies report % correct on signal and non-signal trials.

2.1.3.5 Manipulations

A range of manipulations was applied once the basic detection protocol was learnt. An occlusion manipulation was used in a restricted cohort (n=4) to confirm that the rats were only using the visual information and not potential tactical, auditory or olfactory cues from the illumination of the LEDs. The panel was covered with a lightproof shield but still operated using the same procedure as described previously. Responses were again rewarded based on the standard training schedule, despite the rat not being able to view the stimuli.

Based on the literature a number of stimulus changes were tested. Firstly, could rats discriminate between 3 vertical lights and 3 horizontal lights? Secondly, could patterns using different numbers of lights be discriminated? Thirdly, could rats discriminate between a red and green light? However, even after weeks of training, accuracy did not improve on any of these manipulations and therefore these data have not been presented. Although rats may be able to make these visual discriminations with extensive training, improvements were not observed within the time available for this study.

Four manipulations were used to further characterise performance under different experimental conditions. Firstly, the threshold for signal detection was determined by providing a range of illuminated LEDs from 0-9 and recording the proportion of responses to the signal and non-signal magazines (n=16). Trials were pseudo-randomly ordered from a list containing 8 non-signal (0 LEDs) trials, 8 signal (9 LEDs) trials or one of 8 intermediate stimuli (1-8 LEDs) trials. On the intermediate stimuli trials responses to both
left and right magazines were not rewarded to reduce reinforcement of either response type. Responses on signal and non-signal trials were rewarded as per standard training.

Then the effects of a visual and auditory distractor were used to determine if performance was differentially impaired by a distractor within the same or different modality in a subset of trained rats (n=4). The session was broken into 4 blocks of 30 trials, the first and last blocks containing standard trials while the second block included a visual distractor and the third block included an auditory distractor. The distractors pulsed on and off at 10Hz, using either the house light or a background noise generator, throughout the 30 trial block.

Next, a reversal of the schedule was performed to determine how many sessions were required for rats to flexibly switch their responses to the opposite contingency (n=16). For this manipulation rats that were trained to response left for signal and right for non-signal were switched to right for signal and left for non-signal and vice versa for the alternative contingency.

Finally, to determine if accuracy was dependent on motivation, a separate cohort of 20 male rats was trained on the SDT using 120 trials per session (60 signal and 60 non-signal). They were then individually pre-fed 200 reward pellets (9g food) 30min prior to testing. The amount of pellets they consumed prior to starting testing and the number of pellets attained, but not consumed, was also recorded.

2.1.3.6 Statistical analysis

Statistical analysis was performed using SPSS software package (ver.20, SPSS Inc. IL, USA). Where appropriate, repeated measures ANOVA, independent t-tests or paired t-tests were used. Bonferroni correction for multiple comparisons was used. Significance was set at $p<0.05$.

2.1.4 Results

2.1.4.1 Visual occlusion

To ensure the rats could not use other senses (for example auditory or olfactory cues) to determine if the visual stimuli were on or off, the panel was occluded but still operated for a test session (Figure 2.2A). Accuracy was significantly reduced (from 89% to 56% correct) when the panel was covered, indicating that rats were using visual cues to perform the task accurately ($t_{(3)}=13.68$, $p=0.001$).
2.1.4.2 Detection threshold

To determine the threshold for a response shift between signal and non-signal trials, a discrimination session was conducted where additional LEDs were presented but not rewarded (Figure 2.2B). While rats could clearly discriminate between 0 and 9 LEDs ($t_{(14)}=-9.41, p<0.001$), the illumination of a single LED resulted in performance at chance levels ($t_{(14)}=0.14, p=0.894$). Any more than 1 LED illumination resulted in a strong preference for signal responding (groups differed across 1-9 LEDs with a range of $t_{(14)}=2.82$ to 9.78, $p=0.014$ to <0.001). Therefore, it was decided that using all 9 lights on or off provided the best results and this was used for future studies.

![Figure 2.2 Performance measures on the signal detection task](image)

Figure 2.2 Performance measures on the signal detection task
(A) Performance indicated by correct detection of stimulus with the light panel either visible or occluded (n=4). (B) Discrimination curve showing the percentage of responses to the left magazine for rats in the group associating signal (closed) trials with a left response and the group associating non-signal (open) trials with a left response across increasing numbers of LEDs being presented (n=8/group). As all trials were completed and responses are mutually exclusive, the % responses to the right magazine for each group mirror these results and are not presented. Mean and SEM shown, *p<0.05.

2.1.4.3 Distraction

The results of the distractor manipulation were assessed using both % correct and signal detection theory indices. On both measures the distractor conditions differentially influenced performance. There was no main effect of Block on % correct for signal or non-signal trials. When the same data was analysed using signal detection theory indices it was found that p(Hit) and p(FA) also did not varying significantly by Block. SI was found to vary ($F_{(3,9)}=4.39, p=0.037$) with the visual distractor block being significantly lower than the auditory ($t_{(3)}=-3.87, p=0.030$) and end block ($t_{(3)}=-4.94, p=0.016$). However, RI did not differ
between Blocks (Figure 2.3). The auditory distractor did decrease trial rate compared to the normal trial blocks (Block 1 vs. 3 $t(3)=4.38$, $p=0.022$; Block 3 vs. 4 $t(3)=-9.86$, $p=0.002$; Figure 2.4).

Figure 2.3 Performance measures for distraction manipulation

(A) Signal accuracy and (B) Non-signal accuracy did not vary across blocks. (C) $p(\text{Hit})$ and (D) $p(\text{FA})$ also did not differ with distraction. (E) However, SI was reduced during the visual distractor compared to the auditory and end block but (F) RI did not differ across blocks. N=4, *$p<0.05$.
Chapter 2. Task development

2.1.4.4 Reversal learning

Rats were able to determine signal from non-signal trials with >80% accuracy. The protocol was then reversed so the correct response was now to enter the opposite magazine. Rats initially respond as per prior training, before gradually acquiring the new pairing (Figure 2.5).

Figure 2.4 Effect of distracting stimuli on trial rate
When compared for trial rates, the auditory distractor reduced trial pace more than the visual distractor (n=4). Mean and SEM shown, *p<0.05.

![Trial Rate during distraction](image)

**Figure 2.4 Effect of distracting stimuli on trial rate**
When compared for trial rates, the auditory distractor reduced trial pace more than the visual distractor (n=4). Mean and SEM shown, *p<0.05.

**2.1.4.4 Reversal learning**

Rats were able to determine signal from non-signal trials with >80% accuracy. The protocol was then reversed so the correct response was now to enter the opposite magazine. Rats initially respond as per prior training, before gradually acquiring the new pairing (Figure 2.5).

![Reversal Learning](image)

**Figure 2.5 Reversal learning**
Scatter plot showing individual values (as well as mean and SEM) for each session of reversal learning (n=6-16).
2.1.4.5 Pre-feeding

All rats consumed pellets prior to testing, with consumption ranging from 3.6-9.0g with 9.0g being equal to the entire 200 pellets supplied (mean was 7.8g). Paired t-test comparing measures from the day before and after pre-feeding found that the number of trials completed was significantly reduced ($t_{(19)}=-3.55$, $p=0.002$) and the number of omissions was significantly increased ($t_{(19)}=2.24$, $p=0.037$) after pre-feeding (Figure 2.6A,B). However, % correct was not significantly different ($t_{(19)}=-1.67$, ns; Figure 2.6 C). In addition, 10 out of the 20 rats did not consume all the reward pellets they received during task performance (ranging from 8-121 pellets left in magazines).
Prefeeding rats reward pellets before testing resulted in (A) fewer trials being completed and (B) a marginal, yet significant increase in omissions. (C) However, % correct remained high. *p<0.05.

**Figure 2.6 Pre-feeding manipulation**
2.1.5 Discussion

The aim of this study was to develop a novel SDT for measuring attention in rats. These preliminary experiments were conducted to explore aspects of the task design and derive pilot data for task manipulations, such as distraction and reversal learning. Firstly, I confirmed through the occlusion study that rats were using visual information to make decisions and not relying on other senses to determine when the display panel was illuminated. This may seem obvious, however this needed to be confirmed given the superiority of other senses, such as olfaction, in rodents. Having confirmed rats were using visual cues from the LEDs, the importance of the number and position of the LEDs were examined. Initial studies looking at different light patterns were not successful and this was not surprising given rats seem to use a detection strategy in this paradigm. When presented with varying stimuli, rats responded on the signal side with a strong bias after two or more LEDs were illuminated. Although rats may be able to learn to discriminate different patterns of visual stimuli, this would be likely to take significantly more training than was considered reasonable for this study. It has been shown that rats can discriminate very complex images, including photographs and morphed images (Bussey et al., 2008), although rats may use low level features rather than the whole image to discriminate. However, an important consideration for this study was that stimuli were presented in succession rather than simultaneously. Therefore, rather than being able to directly compare stimuli side-by-side and make a choice, the rat needed to maintain an internal rule about the stimulus-response pairing. It was also found that the threshold for switching from signal to non-signal responding occurs at a very low level of illumination with one LED leading to chance responding but two or more LEDs resulting in a strong preference for signal responding. This limits the use of variable LED patterns in terms of varying luminance levels, but clearly demonstrates the light from two LEDs was sufficient for detection. This may make more complex discriminations more difficult. Therefore, it was decided to use the light panel for detection (0 versus 9 lights) rather than display different arrangements of lights for the remainder of the thesis.

With the use of distractors, it was found that the auditory distractor did not reduce accuracy of responding, but did significantly slow trial completion rate. This is likely to occur because the rats were paying attention to the source of the noise rather than focussing on the task. In contrast, it was found that the visual distractor reduced performance to chance levels (50%) of accuracy on both signal and non-signal trials,
however this was only statistically detected using SI. Where signal detection theory was applied, SI was found to vary significantly between blocks whereas % correct for signal and non-signal, as well as p(Hit) and p(FA), were not different across blocks. As SI incorporates both these values, this may indicate a lack of power for detecting significance in these other measures. It would be expected that if accuracy on one trial type was driving the SI finding, then it would be significant on its own. However, if it is an additive effect, then either measure on its own may not reach significance. This is quite likely given the low number of animals used (n=4) in some experiments. These indices have been applied later in Chapter 5 where the distraction manipulation was used in study with greater sample numbers. It should be noted that these preliminary studies were conducted with small sample sizes and therefore may lack power to detect significant differences. However, the objectives for these studies were qualitative, rather than strictly quantitative. These results may indicate that the visual distractor is in fact preventing the rats from seeing the signal when it is presented. Rather than operating as a distractor that diverts attention away from the task, it may be visually interfering with their ability to complete the task.

Reversal learning was then examined and it was found that rats gradually acquired the new contingency over 2 weeks of testing. There was quite a lot of variation in the acquisition rate between individuals and this may be a useful manipulation for assessing behavioural flexibility in future studies. Finally, pre-feeding rats prior to testing examined the effect of reducing motivation on task performance. This experiment demonstrated that even when motivation was reduced, accuracy of responding was maintained. However, the number of trials completed was reduced and omissions increased as expected. This demonstrates that accuracy on the SDT provides a cognitive measure that is independent of motivational changes. Overall, these pilot studies were used to determine feasibility and protocol parameters that produced the desired outcome. From this work a modified signal detection task has been designed and task variations have been trialled. Having established training and testing procedures, the utility of the SDT can now be examined.
Chapter 3  Comparison of 5CSRTT and SDT
3.1 Foreword

The next step was to determine how the outcomes on the SDT compared to an alternative task. One of the most widely used and well-validated rodents tasks used to measure attention is the 5CSRTT. The tasks differ in a number of features, including predictability of stimulus onset and location, the number of response locations and stimulus properties (e.g. luminance). These differences are part of what makes the tasks unique and neither task was altered from the standard versions that have been previously published. Therefore the purpose of this study was to compare acquisition and performance on the 5CSRTT and SDT. Of particular interest was the time taken to train rats, the rate of omissions and level of accuracy on each task. A number of differences in task performance are highlighted including fast training time, reduced omission rate and the control of body position on the SDT. This article was published in *Frontiers in Behavioral Neuroscience*. 
3.2 Measuring attention in rodents: comparison of a modified signal detection task and the 5-choice serial reaction time task

Karly M. Turner, James Peak and Thomas H. J. Burne

3.2.1 Abstract

Neuropsychiatric research has utilised cognitive testing in rodents to improve our understanding of cognitive deficits and for preclinical drug development. However, more sophisticated cognitive tasks have not been as widely exploited due to low throughput and the extensive training time required. We developed a modified SDT based on the growing body of literature aimed at improving cognitive testing in rodents. This study compares performance on the modified SDT with a traditional test for measuring attention, the 5CSRTT. Adult male SD rats were trained on either the 5CSRTT or the SDT. Briefly, the 5CSRTT required rodents to pay attention to a spatial array of 5 apertures and respond with a nose poke when an aperture was illuminated. The SDT required the rat to attend to a light panel and respond either left or right to indicate the presence of a signal. In addition, modifications were made to the reward delivery, timing, control of body positioning and the self-initiation of trials. It was found that less training time was required for the SDT, with both sessions to criteria and daily session duration significantly reduced. Rats performed with a high level of accuracy (>87%) on both tasks, however omissions were far more frequent on the 5CSRTT. The signal duration was reduced on both tasks as a manipulation of task difficulty relevant to attention and a similar pattern of decreasing accuracy was observed on both tasks. These results demonstrate some of the advantages of the SDT over the traditional 5CSRTT as being higher throughput with reduced training time, fewer omission responses and their body position was controlled at stimulus onset. In addition, rats performing the SDT had comparable high levels of accuracy. These results highlight the differences and similarities between the 5CSRTT and a modified SDT as tools for assessing attention in preclinical animal models.

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3.2.2 Introduction

Cognitive symptoms are the strongest predictor of functional outcomes in patients with schizophrenia, yet current antipsychotic medications are no more effective in treating cognitive symptoms than those developed in the 1950's (Green et al., 2000; Keefe et al., 2007). To guide future clinical research in the development of more effective medications, the MATRICS panel was formed (Green and Nuechterlein, 2004). For each domain of cognition, tasks administered in human subjects were selected as part of a cognitive battery for assessing the efficacy of novel medications (Nuechterlein et al., 2008). Within the domain of attention/vigilance they selected versions of the CPT (Nuechterlein et al., 2008). Following these recommendations the CNTRICS panel devised a similar list of tasks for evaluating these cognitive domains in animals (Carter and Barch, 2007). These tasks were selected based on evidence of face, predictive and construct validity relative to the human CPT and each has been reverse-translated back into human tasks (Demeter et al., 2008; Young et al., 2009a; Young et al., 2013b; Worbe et al., 2014). A key issue that was raised throughout this process was the need for greater translational validity between rodent and human tasks (Hagan and Jones, 2005; Young et al., 2009a). The purpose of this study was to further develop a CPT-like task for the assessment of attention in rodents.

Firstly, elements of the human CPT and dissimilarities with current rodent protocols were carefully considered. The human CPT exists in many versions with deficits in schizophrenia patients widely reported (Earle-Boyer et al., 1991; Cornblatt and Keilp, 1994). These deficits have even been suggested to represent an endophenotype of schizophrenia as there is evidence of mild deficits in first-degree relatives, stability in patients from first-episode through to remission, and a lack of correlation between severity of psychotic symptoms and CPT deficits (Chen and Faraone, 2000; Snitz et al., 2006; Gur et al., 2007; Delawalla et al., 2008; Richard et al., 2013). A common feature of continuous performance tasks is the rapid presentation of stimuli where the subject is required to monitor, identify and respond to target stimuli. Key outcome measures are the accuracy of responding and the reaction times of participants, with both measures altered in schizophrenia patients. By translating important features of the human CPT into a rodent task, researchers can more invasively investigate how attentional deficits are related to neurobiological changes and test novel drug targets for treating cognitive symptoms in schizophrenia.
The corresponding rodent tasks selected for the domain of attention were the 5CSRTT, 5C-CPT and SAT (Lustig et al., 2013). The 5CSRTT has been widely used in rats and mice with extensive investigation of the underlying neurobiology and use of pharmacological agents to probe performance (Robbins, 2002). It has been shown to be highly sensitive to pharmacological agents and to manipulations used in animal models of schizophrenia (Chudasama and Robbins, 2004; Featherstone et al., 2007b; Fletcher et al., 2007; Paine and Carlezon, 2009). The 5CSRTT requires rodents to attend to an array of five apertures and make a nose poke response into an illuminated aperture. As an extension of this paradigm, the 5C-CPT incorporates a subset of trials requiring inhibition of responding when all five apertures illuminate to receive a reward. Therefore the 5C-CPT allows the assessment of response inhibition, which is an important component of executive functioning. A clear difference between human CPT and the rodent 5CSRTT is the use of a spatial array of stimuli and response locations. In contrast, a single, constant position is typically used for presenting stimuli and responding on the CPT for human subjects. While the rodent may correctly identify the response location via a spatial stimulus-response association in the 5CSRTT, the human CPT requires the maintenance of a rule to determine the correct response based on stimulus properties. Hence, the use of a rule is a valuable feature of the rodent SAT protocol when considering translational task components. The SAT requires the detection of a single, central stimulus followed by a response on the correct lever to receive a reward (McGaughy and Sarter, 1995a). On a subset of trials, a flashing light can also be presented to assess performance changes during dSAT. The SAT has incorporated the use of a rule about the properties of a single stimulus, however it has not been as widely used or validated as the 5CSRTT. In both these tasks a major issue that has not been addressed is the lack of control over the rodent’s body position during stimulus presentation.

In human studies the subject is often placed in a fixed position relative to the stimuli and maintains eye gaze in the direction of the stimulus stream. However, in rodent tasks, the animal can move anywhere within the operant chamber and may not have the stimuli within their visual field when it is presented. This leads to a number of differences in the interpretation of performance measures between human and rodent testing. Firstly, accuracy will depend on the body position of the rodent during stimuli presentation. However, body position cannot be determined without additional video recording and tracking analysis. Secondly, the lack of control over body position interferes with the interpretation of omission errors. In human studies an omission most likely occurs when
the subject misses a stimuli due to a lapse in vigilance, whereas in rodents studies an omission may occur for any number of reasons including grooming, sleeping or investigating the chamber. To demonstrate the importance of body position, we analysed video recordings from our previous 5CSRTT study in rats (Turner et al., 2013) and show that more omissions occurred when the rat was more distant and had their head turned during stimulus onset (see Figure 3.1).

![5CSRTT Body Position](image)

**Figure 3.1 5CSRTT Body Position**

On 5CSRTT the angle of the rat's head and distance from the stimulus are important for correct identification of the illuminated aperture. Correct responses typically occurred when the rat was very close to or looking straight at the 5-hole wall. However, rats were frequently on the other side of the chamber and looking away from the 5 holes prior to an omission (dotted line indicates half the width of the chamber). The plot shows individual responses from seven rats with correct, incorrect and omission responses.

Another point raised by CNTRICS for the optimisation of rodent testing included reducing the training time to encourage more widespread use (Lustig et al., 2013). Reducing the time required to train rodents on cognitive tasks would also promote preclinical screening of novel compounds, which has been limited due to the extensive investment required. Another issue that has been raised is the acknowledgment that no task provides a pure assessment of a single cognitive modality and therefore a number of behaviour measures should be considered when interpreting changes in performance. Therefore, it was suggested that tasks should endeavour to include dimensions where performance can be concurrently observed over a range of difficulties, such that deficits due to more general impairments can be isolated from cognitive deficits (Lustig et al., 2013). This would serve
as an internal control for changes in motivation, motoric effects of drugs and satiety as opposed to changes in attentional performance.

After considering the differences between rodent and human CPT testing and the recommendations made for optimising task qualities, a modified SDT was developed. We focussed on reducing training time, reducing omissions and controlling body position while maintaining construct validity for measuring attention. This fast-paced SDT was designed with consideration for the species-specific differences in task performance. This includes consideration of the stimuli and response devices used, but more importantly to improve task engagement and vigilance. The SDT was compared to the well-validated 5CSRTT to determine the advantages and disadvantages of each paradigm. It was hypothesised that acquisition of the SDT would be faster as the task has a simpler design with fewer outcomes that are punished, thereby limiting inappropriate responding and promoting rapid task acquisition. It was predicted that there would be fewer omissions on the SDT for a number of reasons. Firstly, trials are self-initiated without delay to signal or non-signal presentation and therefore the rat should be motivated to complete each trial. Secondly, the stimuli are immediately presented directly in front of the rat and responses require only minor movement from the initial start position. Thirdly, there is minimal delay between trials. Collectively these features promote engagement in the task rather than performance of alternative behaviours. Finally, it was predicted that accuracy would be comparable on both tasks during baseline testing and when challenged with more difficult stimuli.

3.2.3 Materials and Methods

3.2.3.1 Animals

Adult 12 week old male Sprague Dawley (ARC, WA) rats were housed in a room maintained at 21 ± 2°C and 60% humidity and on a 12-h light/dark cycle (lights on 0600 h). Male rats were selected as sex differences were not being examined in this study and males are more commonly used in similar experiments. They were pair-housed in polypropylene cages (41x28x24cm) with high-top wire lids, aspen chip bedding (Able Scientific, WA, USA), nesting and wood chew (Able Scientific, WA, USA), which was cleaned weekly after operant testing. Rats were micro-chipped (Microchips Australia Pty Ltd, Australia) and regularly tail marked to ensure accurate identification of individuals. Prior to training, rats were food restricted to 90% of their free-feeding body weight with free access to water. Throughout testing rats were weighed daily and food rations were
adjusted to maintain constant body weight. All procedures were performed with approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

3.2.3.2 Apparatus

Operant chambers were contained in sound attenuated boxes with ventilation fans (Med Associates Inc., St. Albans, VT, USA) and overhead cameras for monitoring behaviour (CCD Mini CCIR, Samsung, Suwon, South Korea). All chambers were 50x50x50cm and were assembled for either 5CSRTT or SDT training (Turner et al., 2013). For 5CSRTT there was a curved wall with five horizontal apertures each containing a light and head entry detector. On the opposing wall there was a house light and food magazine that was also equipped with a light and head entry detector. The arrangement for SDT training was on a single chamber wall with a house light, signal display panel and nose poke port in the middle and a food magazine on either side of the nose poke port. The central nose poke port and magazines each contained a light and head entry detector. The signal display panel consisted of a 3x3 grid of light emitting diodes (5mm, green diffuse, 80MCD, Jaycar Electronics, NSW, Australia). All rats were rewarded with 45mg grain pellets (F0021, dustless precision pellet, Bioserv, Frenchtown, NJ, USA) delivered to the food magazines. The protocol was designed using MedState Notation while operation and data acquisition was conducted using Med-PC for Windows software (Med Associates Inc., St. Albans, VT, USA).

3.2.3.3 5CSRTT Protocol

Training for the 5CSRTT (N=18) was conducted based on methods described previously (Bari et al., 2008; Turner et al., 2013) with a summary presented in Table 3.1. In the 5CSRTT protocol rats were required to attend to an array of 5 apertures and respond with a nose poke to the aperture that was briefly illuminated. Rats must withhold from responding during the inter-trial interval (ITI) where a premature response resulted in a time out (5s). One of the 5 apertures was then briefly illuminated and the rat must respond within the limited hold (LH) period (5s). Following selection of the correct aperture the rat received a food reward, however if an incorrect aperture was chosen there was a brief time out (5s). Unlike the methods described elsewhere, prior to training stage 1, this protocol required rats to be habituated to the chambers and collect reward pellets from the apertures and magazine. To automate this process, rats were first trained to collect
Chapter 3. Comparison of 5CSRTT and SDT

rewards from the magazine by placing 10 pellets in the magazine and every head entry resulted in the delivery of another pellet (Habituate). After achieving 100 pellets on 2 days, rats moved to level 0 where a response into any nose poke aperture results in a reward. These steps were used to automate the habituation procedure and ensure all rats had acquired the basic steps required for level 1. Rats were then trained progressively through levels 2-6 to collect rewards and respond to briefer stimuli until attaining level 7 with >80% accuracy and <20% omissions on a 1s stimulus duration. For details of each level see Table 3.1.

3.2.3.4 SDT protocol

An overview of the SDT protocol has been presented in Figure 3.2. For the SDT, rats were first trained to collect a food reward from the magazines. Each subsequent head entry resulted in another reward delivery until 50 rewards were collected from each magazine or 20min had elapsed (level 1). Next rats were trained to make a nose poke into the illuminated central port to activate reward delivery on head entry in the magazines (level 2). Finally, rats were trained to make a central nose poke, then the stimulus panel illuminated (signal trial) or remained off (non-signal trial) before both magazines illuminated and the rat could respond left or right (level 3, then level 4). A summary of the training step requirements has been listed in Table 3.2.

If the correct side was selected a reward was delivered; however if the incorrect side was selected a brief time out (5s) delayed the beginning of the next trial. Stimulus (signal or non-signal) and magazine (left or right) pairings were balanced across the cohort, but constant for an individual. By incorporating the central nose poke to start trials, the rat’s body position was confined to directly beneath the panel when the stimulus was presented. The location of the magazines on either side of the nose poke also reduces the amount of movement required to respond. During development of the task it was observed that if responses could be made immediately when stimuli were presented, more impulsive and inaccurate choices were made during training (data from pilot study not presented). As a result some animals did not learn the rule, preferring to respond quickly with 50% chance of success. Therefore the inclusion of a 1s stimulus presentation window when responses were not rewarded was critical to task acquisition. This also ensures that all animals are exposed to the same signal duration prior to responding, otherwise a faster response would reduce the amount of time the stimulus was presented. The session ended after 120
trials or 30min and rats were required to achieve >80% accuracy with an equal number of signal and non-signal trials presented pseudorandomly.
Figure 3.2 The Signal Detection Task
(A) Schematic of chamber arrangement including a house light, grid of lights for signal presentation and central nose poke with a magazine on each side. (B) Trials started with a brief inter-trial interval (ITI) before the central nose poke aperture illuminates and the rat makes a nose poke response to begin the trial. Immediately upon nose poke detection, the signal was presented (or absent for non-signal trials) for 1s. Following the signal presentation both the left and right magazines illuminated indicating the rat should make a choice. If the correct side was chosen a food reward was delivered, alternatively if the incorrect side was chosen there was a brief time out (5s). If no response was made after a 4s limited hold (LH) the trial ends with an omission scored. Nose pokes and head entries (HE) made at inappropriate times were recorded but not punished.
Chapter 3. Comparison of 5CSRTT and SDT

Table 3.1 Training requirements for the 5CSRTT

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<th>Level</th>
<th>Trials</th>
<th>Session min</th>
<th>Stim Dur s</th>
<th>Time Out s</th>
<th>LH s</th>
<th>Reward Dur s</th>
<th>ITI s</th>
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<td>30</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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Table 3.2 Training requirements for the SDT

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<tr>
<td>1 Collect pellets</td>
<td>100</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;=80 trials, 2 days</td>
</tr>
<tr>
<td>2 Nose poke</td>
<td>100</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0,2,4,6,8,10</td>
<td>&gt;=80 trials, 2 days</td>
</tr>
<tr>
<td>3 Signal Detection</td>
<td>120</td>
<td>30</td>
<td>Unlimited</td>
<td>-</td>
<td>-</td>
<td>1,2,3</td>
<td>&gt;=80% correct, 2 days</td>
</tr>
<tr>
<td>4 Signal Detection</td>
<td>120</td>
<td>30</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>1,2,3</td>
<td>&gt;=80% correct, 2 days</td>
</tr>
</tbody>
</table>
Chapter 3. Comparison of 5CSRTT and SDT

There are a number of modifications that have been made in the SDT compared to other signal detection tasks, such as the SAT. Changes to the chamber design include the location of the reward, the use of nose pokes instead of lever press responding, the use of a central nose poke aperture and the LED light panel. In terms of protocol design, one of the most influential changes was the positioning of the rat in the centre, underneath the light panel at stimulus onset. This ensured the rat was located in front of the stimulus when the signal was displayed. In addition, the ITI period occurs prior to the self-initiation nose poke, rather than after to encourage responding. Timed events and latencies are typically shorter than on other protocols to promote rapid trial pace (e.g. ITI). A mandatory pause (1s) was incorporated during stimulus presentation, however as all other delays are minimal a rat can rapidly complete 100 trials without stopping if they chose too. The continuous nature of task performance differs from other rodent tasks where delays often lead to alternative behaviours.

3.2.3.5 Signal duration manipulation

Following training, both tasks were adapted to include a variation in stimulus duration to increase attentional load. For both tasks there were 120 trials per session with 20 standard trials at the start and end of the session consisting of only 1s stimulus for 5CSRTT and 0 or 1s stimulus for SDT as per training. These trials could be used to assess the changes that occur across the length of the session. For the 5CSRTT the central block of 80 trials consisted of 0.5, 0.25, 0.12 or 0.06s signal duration trials. On the SDT, the central block of 80 trials consisted of 60 signal trials of 0.5, 0.25, 0.12 or 0.06s and 20 non-signal (0s) trials. These parameters were selected to derive similar measurements from each task although all analyses were conducted separately for 5CSRTT and SDT. Inter-trial interval (ITI) was fixed for both tasks to increase stimulus onset predictability in both tasks.

3.2.3.6 Behavioural measures

The primary outcome measure during training was the number of sessions required to reach criteria, which was >80% accuracy on 1s signal duration for both tasks. Once they reached this stage a range of measures were used to compare performance including % accuracy, % omissions, session duration, trial rate and response latency. Other measures could also be derived from each task but were not directly comparable due to differences in protocol requirements such as premature responses, latency to initiate trials and reward latency. For the signal duration manipulation, % accuracy at each signal duration was
calculated along with measures previously identified. The session was also split into three blocks including the start (first 20 standard trials), middle (80 reduced signal duration trials) and end (final 20 standard trials) blocks to investigate changes in performance that occur due to session length as compared to changes that occur due to altered stimulus duration.

3.2.3.7 Statistical Analysis

All data were analysed using SPSS software package (ver.20, SPSS Inc. IL, USA) and significance was set at $p<0.05$. Task acquisition and baseline performance measures were compared using independent $t$-tests. Comparison of performance measures across blocks in the signal duration manipulation were analysed by repeated measures ANOVA and followed with paired $t$-tests where appropriate. One rat was removed from signal duration analysis, as performance was unusually poor on the day of testing (<20% accuracy and 70% omissions on start block). Data are presented as mean ± S.E.M, *$p<0.05$.

3.2.4 Results

3.2.4.1 Task comparison

Performance was compared between rats trained on 5CSRTT and SDT with the number of sessions required for each training step presented in Table 3.3. On both tasks there were individual rats who took longer than average to reach criteria at certain steps, however to achieve an objective measure of training time on both tasks every animal was included and trained until they reached criteria.

The average number of sessions required to reach criteria with a 1s stimulus duration was significantly greater for 5CSRTT than for the SDT ($t_{(34)}=4.75$, $p<0.001$, Figure 3.3A). Trial rate was significantly greater for the SDT than the 5CSRTT ($t_{(34)}=-17.18$, $p<0.001$, Figure 3.3E) and consequently average session duration was significantly shorter on the SDT compared to the 5CSRTT ($t_{(20.72)}=9.17$, $p<0.001$, Figure 3.3D). Both groups of rats performed to a high level of accuracy (>87%, $t_{(34)}=-0.11$, ns, Figure 3.3B), however the 5CSRTT included significantly more omissions than the SDT ($t_{(17.05)}=11.36$, $p<0.001$, Figure 3.3C). Premature responses on the 5CSRTT were punished and therefore occurred infrequently (13.67±1.36) compared with premature responses on the SDT (155.28±9.75) where additional head entries were inconsequential. Non-signal trials only occur on the SDT, where accuracy was 71.9% ± 2.8, which was lower than for 1s signal trials (90.8% ± 2.3) possibly due to greater uncertainty when perceiving the absence of a signal.
## Chapter 3. Comparison of 5CSRTT and SDT

### Table 3.3 Session to criteria

<table>
<thead>
<tr>
<th>Training steps</th>
<th>5CSRTT</th>
<th>SDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sessions Mean ± SEM</td>
<td>Min</td>
</tr>
<tr>
<td>Habituate</td>
<td>3.06 ± 0.21</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>4.33 ± 0.46</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1.33 ± 0.16</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.06 ± 0.06</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1.22 ± 0.13</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3.61 ± 0.45</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>5.39 ± 0.76</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>5.94 ± 0.70</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>4.94 ± 0.86</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27.83 ± 1.65</td>
<td>19</td>
</tr>
</tbody>
</table>

Each training step for 5CSRTT (N=18) and SDT (N=18).
Chapter 3. Comparison of 5CSRTT and SDT

A Sessions to Criteria

B Accuracy

C Omissions

D Session Duration

E Trial Rate

F Signal Duration
Figure 3.3 Comparison of performance measures on 5CSRTT and SDT

(A) The number of sessions required to train rats to the final level of 5CSRTT was significantly greater than the number of sessions required to train rats on the SDT. (B) Accuracy was not different between the two protocols. (C) The number of omissions was greatly reduced on the SDT compared to the 5CSRTT. (D) The average session duration was significantly longer for rats to complete 5CSRTT (100 trials) than the time taken to complete the SDT (120 trials). (E) This was also reflected in the trial rate, where a significantly greater number of trials were completed per minute on the SDT compared to the 5CSRTT. (F) The reduced signal duration manipulation led to a decrease in accuracy on both the 5CSRTT and SDT. Compared to baseline testing with only 1s signal duration trials (Figure 3A), accuracy at 1s remained high on SDT (from 87.6% to 90.8%) but was reduced on 5CSRTT (from mean of 87.4% to 73.2%) possibly due to fatigue effects (Figure 4A). n=18/task, *p<0.05
3.2.4.2 Reduced stimulus duration

Accuracy was reduced with decreasing signal duration from very high accuracy to near chance responding on both protocols (see Figure 3.3F for SDT). The first and last blocks of 20 trials consisted of standard trials on both tasks. On the 5CSRTT it was found that accuracy was significantly different across blocks ($F_{(2,32)}=53.22, p<0.001$, Figure 3.4A) with a significant reduction from the start to end blocks ($t_{(16)}=4.23, p<0.001$), from start to middle ($t_{(16)}=12.16, p=0.001$) and from middle to end ($t_{(16)}=-5.35, p<0.001$); indicating reduced accuracy with the signal duration manipulation but also an overall decrease in performance over the session length indicative of fatigue. Omission rate varied across blocks ($F_{(2,32)}=5.85, p=0.07$, Figure 3.4C), with an increase from the start to middle block ($t_{(16)}=-4.15, p=0.001$), while an intermediate rate of omissions was found in the end block that did not differ from the start or middle blocks. Response latency ($F_{(2,34)}=0.78, ns$, Figure 3.4E) and reward latency ($F_{(2,32)}=3.09, ns$, Figure 3.4G) did not differ between blocks on the 5CSRTT. On the other hand, although there was a significant effect of block on accuracy on the SDT ($F_{(2,34)}=37.48, p<0.001$, Figure 3.4B) this was due to the reduced stimulus duration in the middle block and was not reduced from the start to end blocks ($t_{(17)}=1.35, ns$). There was only a single rat that recorded any omissions and therefore there was no effect of block on omission rate on the SDT ($F_{(2,34)}=1.00, ns$, Figure 3.4D). Also in contrast to the 5CSRTT, response latency on the SDT was reduced ($F_{(2,34)}=11.00, p<0.001$, Figure 3.4F) from start to the middle block ($t_{(17)}=5.12, p=0.001$) and middle to the end block ($t_{(17)}=-2.87, p=0.011$), indicating rats were responding faster across the session. In addition, centre latency was altered ($F_{(2,34)}=53.54, p<0.001$, Figure 3.4H) between start and middle blocks ($t_{(17)}=8.32, p<0.001$) and start and end blocks ($t_{(17)}=7.59, p<0.001$) but not middle and end blocks ($t_{(17)}=1.34, ns$). It was noted that centre latency was more variable in the start block on SDT and an individual plot of centre latency across trials has been provided as an example of the higher values commonly observed during the initial trials of a session (Figure 3.5A,B).
Chapter 3. Comparison of 5CSRTT and SDT

A 5CSRTT Accuracy

Start Middle End
Block

% Accuracy

B SDT Accuracy

Start Middle End
Block

% Accuracy

C 5CSRTT Omissions

Start Middle End
Block

% Omissions

D SDT Omissions

Start Middle End
Block

% Omissions

E 5CSRTT Response Latency

Start Middle End
Block

Av. Latency (s)

F SDT Response Latency

Start Middle End
Block

Av. Latency (s)

G 5CSRTT Reward Latency

Start Middle End
Block

Av. Latency (s)

H SDT Centre Latency

Start Middle End
Block

Av. Latency (s)
Figure 3.4 The reduced signal duration session
The session was broken into blocks for the first 20 standard trials (start), the reduced signal durations (middle) and the final 20 standard trials (end) on the 5CSRTT and SDT. Measures from both tasks are % accuracy (A, B, with dotted line indicating chance accuracy), % omissions (C, D), response latency (E, F) and reward latency for 5CSRTT (G) and centre latency for SDT (H). *p<0.05.
Chapter 3. Comparison of 5CSRTT and SDT

Figure 3.5 Variability in centre latency time on the SDT across session blocks
(A) A scatterplot of the mean centre latency values for each individual within each block. (B) A plot of an individual rat’s trial-by-trial values for centre latency across a session. Of interest is the occurrence of higher values in the initial trials of a session followed by more consistent, short latencies throughout the rest of the session.

3.2.5 Discussion

This study compared the performance of separate groups of rats on the modified SDT and the 5CSRTT under standard conditions and across reduced stimulus durations. Both tasks replicate features of the human CPT yet differ substantially in their protocol design. The 5CSRTT is a reaction time task with spatially separated response locations, whereas the SDT is a signal detection task where the presence or absence of a central signal indicates the correct response. In addition, a number of limitations were addressed in the development of the SDT, including reducing the time taken to train animals and limiting omissions, which may occur for different reasons in rodent tasks compared to human studies.

It was found that task acquisition to a comparable level of performance took 50% more sessions for the 5CSRTT compared to the SDT. Furthermore, the duration of each daily session on the SDT was nearly half the time taken for session completion on the 5CSRTT. Together, these findings suggest higher throughput studies would be possible with the SDT as more animals could be trained in less time. The investment of time required for operant testing is often seen as a drawback for researchers, but it is also a critical issue for preclinical testing (Young et al., 2009a). Therefore, tasks that can be implemented with faster outcomes would be beneficial, as long as they are still measuring the construct of...
interest. Both these tasks are targeted towards measuring the construct of attention and vigilance; hence accuracy of responding was a critical outcome on the rodent tasks. Importantly, accuracy was high (>85%) and did not differ between the two tasks. On the other hand, errors of omission are more difficult to interpret in rodent studies and therefore changes were made to the SDT protocol to reduce omission rate.

To encourage responding on every trial, rats were required to initiate trials and were then immediately presented with the stimuli. This ensured the rat was positioned directly in front of the stimuli when it was presented. By contrast, on the 5CSRTT rats may be anywhere within the chamber when the stimulus is presented and inaccurate responses may occur due to poor positioning, as indicated in Figure 1. In addition, all events in the SDT occurred on the same wall of the operant chamber to reduce the amount of ambulation required, promoting rapid and continuous task performance. Overall, these protocol differences resulted in negligible levels of omissions on the SDT (<0.1%) compared to the 5CSRTT (>10%). While omission rates are an important measure on human CPT’s, they are a more ambiguous outcome in rodent studies because they can occur for a number of reasons such as changes to sensory, motoric or motivational factors (Robbins, 2002). For example, rodents have been observed performing behaviours such as grooming, sleeping and exploring while in the operant chamber. These may be considered an indicator of distractibility, but do not seem comparable with a lapse in attention as recorded by an omission in human studies. Omissions also typically increase with drug exposure, irrespective of the pharmacological target (Robbins, 2002; Paine et al., 2007). Because the rat may not be engaged in the task, it is often difficult to simply interpret the lack of response in terms of attentional processing. Other measures such as magazine head entries, trials completed as well as response and reward latencies need to be considered before suggesting that increased omissions reflect reduced vigilance (Amitai and Markou, 2010). For these reasons, it is also difficult to measure response inhibition by including withhold responses in rodent paradigms without careful task design and interpretation.

In comparing the effects of the reduced stimulus duration block, it was found that accuracy dropped as predicted for both tasks. Performance decrements can occur for a number of reasons so other variables were carefully considered to determine the likely reason for reduced accuracy. We found that the number of omissions increased when accuracy decreased during the variable signal durations on the 5CSRTT, which was also found by Fletcher et al. (2007) and has been reported in mice (Sanchez-Roige et al., 2012).
Chapter 3. Comparison of 5CSRTT and SDT

the response latency and reward latency were unchanged in the 5CSRTT across the session indicating variable stimulus durations do not alter response speed, which is also in agreement with the literature (Fletcher et al., 2007). This indicates that the rats were not satiated or less motivated to respond across the 5CSRTT session.

By contrast, the reduced accuracy on the SDT reduced signal duration trials was not accompanied by an increase in omissions. However both response latency and centre latency were reduced during the variable stimulus duration block. Response latency was transiently reduced when stimulus duration varied. As stimuli are shorter than the standard 1s duration, response times may be faster due to rats moving to the chosen response side at signal offset. Centre nose poke latency was also reduced from the start block to the variable stimulus duration block and remained low for the final block of trials, indicating an effect of session rather than a transient shift due to changes in stimulus properties.

Because occasional large latency values were seen in the start block for individual rats (see Figure 4B), we suggest this reduction in centre latency time maybe due to habituation to the chamber during the first block of trials. Distractions, such as odours from the previous animal, and competing behaviours may reduce within a few trials as the rat becomes more focussed on performing the task. Despite the SDT trial rate being self-paced by the rat, the rate of stimuli presentation was roughly double that of the 5CSRTT (average inter-stimulus interval of 6.4s on SDT versus 12.7s on 5CSRTT) and was more similar to the fast rate used in the human CPT (commonly ranging between 0.5s - 2s) (Riccio et al., 2002). This indicates rats are initiating and responding on trials consistently and rapidly. At face level, this type of rapid and continuous responding reflects the monotonous pattern of responding required on the human CPT.

Compared to versions of the human CPT, there are still a number of missing features. There was no response inhibition or no-go component incorporated into this task, such as that in the rodent 5C-CPT and many human CPT's. However, some versions do require responses to both target and non-target stimuli, such as the CPT in the commercially available Cogtest battery. The focus of this study was on measuring attention and given the issues associated with correctly identifying an inhibited response in rodents, this component was not included. In human CPT studies a large range of visual stimuli (e.g. the alphabet) can be used simultaneously and easily identified by subjects, whereas this is not feasible in rodents. In addition, there are many versions of CPT to tax different processes, such as working memory and cognitive control. Given the heterogeneity in
human CPT design, incorporating core features like the continuous and rapid response to stimuli were the priorities in modifying the SDT. Additional modifications could be made in the future to measure other cognitive processes assessed by versions of the CPT.

Few studies have compared alternative paradigms to the 5CSRTT, however a recent study by Leite-Almeida et al. (2013) showed that impulsive responding on their novel Variable Delay-to-Signal task correlated with impulsivity during early stages of 5CSRTT training (although not when attentional load increased). It is important to note the 5CSRTT is very useful for measuring impulsive behaviour but this has not been a priority in developing the SDT. Although inappropriate head entries can be made on the SDT, they were not punished and therefore interpretation about this behaviour is quite different to premature responding on the 5CSRTT. In addition, preservative responding was easily measured with the 5CSRTT however this was not possible with the SDT as response and reward occur together. Therefore, if impulsive or compulsive behaviours are of interest, the 5CSRTT should be used. With the recent adaptation of many rodent tasks to use touchscreens, more tasks may be compared to the 5CSRTT using a battery approach (Hvoslef-Eide et al., 2015). Unfortunately, as the touchscreen chambers utilise an entire wall for stimulus display, rewards are delivered on the opposite side of the chamber to stimulus presentation. This limits the inclusion of modifications made in this study to control body position and promote fixation.

Another rodent task designed to measure attention is the sustained attention task (SAT or dSAT when a distractor is included) (McGaughy and Sarter, 1995a). This task is also a type of signal detection task, however there are a number of differences. In construction, these include the position of the reward magazine relative to the response panel and the use of levers in the SAT rather than nose poke receptacles in the SDT (McGaughy and Sarter, 1995a). By providing the reward on the same side of the chamber, ambulation was reduced allowing the rat to remain in front of the stimulus throughout training. This allows sessions to run at an increased task pace and promotes vigilance through stillness, as in human CPT testing. More recent SAT papers indicate the reward delivery system has been moved to the same wall as stimulus presentation (Demeter et al., 2008; Paolone et al., 2013); however the reward is provided in a central position rather than in the location of the correct response and there is not a separate port for trial initiation like in the SDT. Additionally, on the SDT the stimulus was presented when the rat makes a central nose poke directly underneath the light panel, controlling body position within the chamber. By
comparison, stimulus onset on the SAT occurs after a variable ITI during which time the rat can move anywhere within the chamber. The issue of body orientation has been acknowledged as ITI was reduced (from 12±3s to 9±3s) and stimulus duration was reduced (from 1s to 0.025-0.5s) in an attempt to “constrain their behaviour and presumably maintain persistent orientation towards the intelligence panel” (Demeter et al. (2008), p. 790). The self-initiation of trials also promotes trial completion and as an example omission rate on SAT has been reported around 2.5%, whereas on SDT it was 0.05% (Demeter et al., 2008). With the administration of pharmacological agents that typically increase omissions, self-paced trials on the SDT will allow the separation of inability to complete a trial from motivation or ability to start trials. The inclusion of levers has also limited the use of the SAT in mice, where a nose poke receptacle may be favourable (St Peters et al., 2011a). We have successful trained and tested pharmacological agents in two mouse strains using the SDT protocol with only minor changes, such as reward type, as the equipment used and protocol parameters were originally selected to accommodate both species (pilot study, unpublished). Other differences include the time schedule used. On the SDT, rapid trial rate was promoted through limited ITI’s of 2±1s versus 9±3s on the SAT. Substantial training is required for stable levels of performance on the SAT, with the suggestion that 4-8 months was required when training 5-6 days per week. This is around 80-200 sessions and significantly longer than the SDT training reported here, albeit stability criteria on each task have not been matched (Arnold et al., 2003). Therefore, there are a number of differences between these three tasks measuring attention that create unique forms of rodent performance and outcomes.

3.2.6 Conclusions

In summary, compared to the 5CSRTT, there were fewer training sessions and reduced session duration on the SDT, allowing higher throughput testing of animals. In preclinical settings this would reduce the time taken to test compounds, while in a research environment with limited operant chambers this would allow larger cohorts to be tested. Omissions can be difficult to interpret, particularly when they typically increase after drug administration, and hence modifications were made to reduce omissions on the SDT. We have also demonstrated that manipulating the signal duration leads to a comparable reduction in accuracy across both tasks. Importantly, we have controlled body position in relation to stimulus presentation and encouraged rapid trial progression on the SDT to
emulate features of the human CPT. This study highlights key differences and similarities between the traditional 5CSRTT and a SDT modified to meet modern demands.

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Conflict of interest statement: The authors declare no conflict of interest.
Chapter 4  Genetic and Environmental Conditions:
The Effects of Rat Strain and Housing
Chapter 4. Genetic and Environmental Conditions

4.1 Foreword

The SDT has now been developed and compared to an alternative task. The next goal was to determine if the task was sensitive to detecting factors relevant to cognitive performance. Genetic and environmental housing conditions are known to have a significant influence on behavioural outcomes in research animals. Furthermore, strain and housing conditions often differ between studies making generalisations difficult. Therefore, it was the purpose of this study to determine how performance was altered in different rat strains that were housed either under standard or mildly enriched housing. This work was also useful in determining the importance of strain and housing conditions for subsequent studies. This chapter contains a perspective article published in *Frontiers in Behavioral Neuroscience* discussing the importance of considering the background strain and housing conditions when assessing cognition in animal models of schizophrenia. The second manuscript is a research article published in *PLOS One* investigating behavioural and cognitive differences between two rat strains and housing environments. In this paper, the effects of strain and housing conditions were first characterised across a behavioural test battery prior to operant training. These experiments were designed to provide insight into the behavioural phenotype of each group and confirm the housing manipulation had altered behaviour. The results indicated both genetic and environment factors influence aspects of behavioural and cognitive performance. Furthermore, there was strain by housing interactions, where the effect of housing was only observed in one strain. In addition, some strains appear more useful for certain tests, for example due to cohort variability on pre-pulse inhibition or faster acquisition of the SDT. Given these strains differ in a range of behavioural traits, the differences observed on the SDT may be due to a number of factors, including cognitive abilities, visual abilities or motivational factors. Therefore, strain and housing conditions must be carefully considered in the interpretation of cognitive performance.
4.2 Interaction of genotype and environment: Effect of strain and housing conditions on cognitive behaviour in rodent models of schizophrenia

Karly M. Turner and Thomas H. J. Burne

4.2.1 Abstract

Schizophrenia is associated with many genetic and environmental risk factors and there is growing evidence that the interactions between genetic and environmental ‘hits’ are critical for disease onset. Animal models of schizophrenia have traditionally used specific strain and housing conditions to test potential risk factors. As the field moves towards testing gene (G) x environment (E) interactions the impact of these choices should be considered. Given the surge of research focused on cognitive deficits, we have examined studies of cognition in rodents from the perspective of GxE interactions, in which strain or housing manipulations have been varied. Behaviour is clearly altered by these factors, yet few animal models of schizophrenia have investigated cognitive deficits using different strain and housing conditions. It is important to recognise the large variation in behaviour observed when using different strain and housing combinations because GxE interactions may mask or exacerbate cognitive outcomes. Further consideration will improve our understanding of GxE interactions and the underlying neurobiology of cognitive impairments in neuropsychiatric disorders.

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Hyperlink: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3728474/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3728474/)
4.2.2 Introduction

Schizophrenia is a complex group of disorders in which genetic vulnerability may lead to greater sensitivity to adverse environmental conditions (Bayer et al., 1999; van Os et al., 2008; van Os et al., 2010; Tost and Meyer-Lindenberg, 2012). Psychiatric epidemiology has provided clues about biologically plausible combinations of genetic and environmental risk factors for the neuroscience field to examine (Caspi and Moffitt, 2006; Meyer and Feldon, 2010). For example, being raised in an urban environment has repeatedly been linked to an increase in psychotic symptoms, however this risk is amplified in individuals with a genetic predisposition to psychosis (van Os et al., 2004; Krabbendam and van Os, 2005; Spauwen et al., 2006; Weiser et al., 2007). Unravelling the neurobiological changes that lead to vulnerable or resilient phenotypes may provide important information about how gene (G) x environment (E) interactions occur and provide clues for the research community. Rodents have been used to model biologically plausible risk factors and we are beginning to appreciate the complexity of GxE interactions on outcomes relevant to schizophrenia. With the recent focus on measuring cognitive deficits in rodent models (Jentsch, 2003; Kellendonk et al., 2009; Young et al., 2009a; Keeler and Robbins, 2011; Bussey et al., 2012a) and the known influence of strain and housing conditions on cognitive measures (Chapillon et al., 2002; Harker and Whishaw, 2002; Wolff et al., 2002; Pena et al., 2009; Simpson and Kelly, 2011), it is important to consider whether schizophrenia-related outcomes are dependent on the strain or housing conditions used.

Currently there is a lack of animal models of schizophrenia investigating these GxE interactions on cognitive outcomes. For example, a PubMed search using the terms ‘strain’, ‘housing’, ‘schizophrenia’, ‘cognition’ and ‘animal model’ returned no results; substituting ‘strain’ for ‘gene’, and ‘housing’ for ‘environment’ or ‘enrichment’ only returned 7 research articles although none in which housing conditions were compared. Guidelines for cognitive testing in rodents have been established to improve the progression of novel drug treatments, and the use of animal models to examine GxE interactions on established cognitive tests are needed to bridge the translational gap. The next challenge is, therefore, to develop animal models to test the hypothesis that GxE interactions affect cognitive behaviour in animal models of schizophrenia. This article focuses on the consequences of strain and housing conditions on cognitive outcomes in rodent models of schizophrenia and how these factors may be useful in modelling GxE interactions.
4.2.3 Modelling the cognitive deficits in schizophrenia

Cognitive deficits are a core symptom group associated with schizophrenia and are the strongest predictor of functional patient outcomes (Green et al., 2000). While cognitive remediation techniques are beneficial, current drug treatments to improve cognitive deficits are largely ineffective and the failure to translate drug findings from animal models to clinical settings has impeded progress (Pratt et al., 2012). To guide future research an initiative of the NIMH was formed, MATRICS, to make suggestions for the development of cognitive testing in animal models of schizophrenia (Green et al., 2004b; Young et al., 2009a). Based on the core cognitive deficits found in patients with schizophrenia seven cognitive domains were identified including working memory and attention/vigilance (Green et al., 2004b). From these domains various clinical tests were selected by the follow-up group CNTRICS to be used in validating drug efficacy and to improve consistency between research groups (Carter and Barch, 2007). In order to bridge the translational gap, tests used in animal models have also been considered and selected for future use and development (Gilmour et al., 2013; Lustig et al., 2013). Domains such as verbal learning and memory cannot be translated to rodents, however processes such as attention, memory and executive control can be measured in a number of ways (Powell and Geyer, 2007). The tests selected for rodents that best reflect the cognitive constructs measured in patients include the 5CSRTT (Robbins, 2002) and dSAT for measuring attention (Lustig et al., 2013), the attentional set-shifting task (ASST) (Birrell and Brown, 2000) and reversal learning (Izquierdo and Jentsch, 2012) as measures of executive control and the radial arm maze (RAM) and delayed match to position (DMTP) task (Dudchenko, 2004), which provide the best assessment of working memory.

The CNTRICS panel reviewed the use of these tests in both rats and mice, however the selection of species and strain should be determined based on suitability for the experimental manipulation and the cognitive test being implemented (Young et al., 2009a). The use of non-human primates may also be warranted where processes need to be defined differently for humans and rodents, for example in tests of working memory (Castner et al., 2004). For example, GxE interactions were examined on cognition outcomes in an animal model of schizophrenia using catechol-o-methyl transferase (COMT) knockout mice and the 5CSRTT (Papaleo et al., 2012b). At baseline there was no effect of sex or genotype on cognitive performance, however by manipulating the inter-trial interval, measures of impulsivity were found to differ by sex and genotype. After a mild
stressor, males had impaired performance in terms of accuracy and impulsivity measures and this was particularly so for males with reduced (+/-) and absent (-/-) COMT. Other measures were found to differ based on sex and genotype only after reducing motivation. This study illustrates that phenotypes based on sex or genotype may not be readily apparent, however, differences were revealed after manipulating environmental conditions. These findings are in agreement with the suggestion that genes alone do not lead to schizophrenia, but they may predispose an individual to greater vulnerability following exposure to certain environmental insults or ‘hits’.

4.2.4 Environmental conditions and cognition

Epidemiological evidence for the role of environmental factors suggests that housing environment may be an important factor for modelling schizophrenia in rodents (McDonald and Murray, 2000). Housing conditions can have a significant influence on rodent behaviour and have been used to induce stress or anxiety and to alter cognitive development (van Praag et al., 2000; Nithianantharajah and Hannan, 2006; Burrows et al., 2011; Simpson and Kelly, 2011). Environmental enrichment has been incorporated to enhance sensory and motor experience through the inclusion of novel objects, expanded caging and larger social groups (van Praag et al., 2000). Environmental enrichment has been linked to a number of brain-related outcomes, such as increased brain weight, increased branching and synapse formation in the cortex, increased expression of brain-derived neurotrophic factor (BDNF), glial-cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) and increased acetylcholine levels (see review van Praag et al. (2000)). Many of these factors are likely to affect cognitive functioning, for example NGF and BDNF are both known to play a role in learning, while acetylcholine levels have been shown to correlate with attentional performance in rodents (St Peters et al., 2011b).

Housing conditions have been difficult to standardise across research groups, particularly when enrichment is used. Rather than viewing this noise as a nuisance, it could be seen as an opportunity to investigate how environmental conditions interact with proposed risk factors (Toth et al., 2011).

Rodents reared in more stimulating conditions often acquire tasks after fewer trials (Park et al., 1992), have reduced age-related deficits (Soffie et al., 1999; Harati et al., 2011) and recover from injury faster (Hicks et al., 2002) (see Pena et al. (2009)). This may indicate phenotypes are being rescued in enriched environments or that deficits only develop in a deprived environment. In some cases, such as animal models of depression, standard
housing may be largely contributing to the phenotype, possibly by reducing an animal’s compensatory ability to deal with additional challenges (Brenes et al., 2009). The brain may require stimulation beyond that provided in standard housing to develop sufficient connectivity and functionality to detect higher order cognitive deficits. Whether enrichment should be considered as a therapeutic intervention or the standard conditions required for developing a ‘normal’ brain continues to be debated (Wurbel, 2001).

4.2.5 Genetic background and cognitive performance

Mutant mouse models have been used to investigate other key candidate genes linked to schizophrenia (Chen et al., 2006). Despite the availability of tasks for cognitive testing in rodents, a recent review by Arguello and Gogos (2006) did not report any mutant mouse models in which the “top 30” genes linked to schizophrenia had been tested on an attentional paradigm. Considering the need to investigate cognitive symptoms in animal models, there is an obvious gap that needs to be addressed. The genetic risk for schizophrenia is likely to be the result of hundreds or even thousands of genes of small effect (Wray and Visscher, 2010). Systematically testing each individual mutation is unlikely to replicate the disorder, nor is this approach feasible. However, specific genetic mutants may be useful for identifying the origin of cognitive endophenotypes of schizophrenia (see review Kellendonk et al. (2009)). While using single gene mutants provides information about a particular gene of interest (Papaleo et al., 2012b), the polygenic nature of schizophrenia may be better modelled by comparing different strains.

Strain-dependent changes in behaviour have been observed on many cognitive tasks and in response to drugs; but these changes are also dependent on the manipulation applied (Andrews et al., 1995; Schmitt and Hiemke, 1998; Mirza and Bright, 2001; Harker and Whishaw, 2002; Wahlsten et al., 2003; Zamudio et al., 2005; Higgins et al., 2007). For example, a widely used task in animal models of schizophrenia, pre-pulse inhibition of the acoustic startle response (PPI), is a well validated test of sensorimotor gating but results are known to vary depending on the background strain (Rigdon, 1990; Glowa and Hansen, 1994; Varty and Higgins, 1994; Varty et al., 1999; Swerdlow et al., 2001; van den Buuse, 2003). Given the variability on this pre-attentive task, it is not surprising that strain differences have also been reported using more sophisticated cognitive tasks, such as the 5CSRTT (Didriksen and Christensen, 1993; Mirza and Bright, 2001; Higgins et al., 2007; Auclair et al., 2009). These studies also demonstrate the variability between studies using
the same strain, which may be due to variation in the protocol used or the source of the strain (Andrews, 1996; Karl et al., 2011).

Rat models of schizophrenia have been developed predominantly using two albino strains, however the reasons for these selections are not always obvious. Furthermore, studies of schizophrenia-related manipulations comparing rat strains are lacking. The neonatal ventral hippocampal lesion model was compared in the outbred SD and two inbred strains, Lewis and Fischer 344, which differed in stress responsivity (Lipska and Weinberger, 1995). For example, SD and Lewis rats show habituation of the HPA-axis response to a repeated restraint stress paradigm, whereas F344 rats do not habituate within or between stress-inducing sessions (Dhabhar et al., 1997). As predicted the hyper-responsive F344 strain showed greater behavioural vulnerability to the neonatal lesion, while the hypo-responsive Lewis rats showed greater resistance when both were compared to the SD strain. Thus, stress responsivity is a critical consideration both for models utilising stressful manipulations and for the interpretation of behavioural results from different strains (Faraday, 2002). Spontaneous and amphetamine-induced hyperlocomotion varied across development with strain, indicating genetic predisposition has a critical role in determining the phenotype derived from this neurodevelopmental model, although cognitive outcomes were not assessed in this study (Lipska and Weinberger, 1995).

4.2.6 GxE interactions and cognitive endophenotypes

The focus of GxE interaction studies in animal models of schizophrenia has taken advantage of the genetic tools available in mice, comparing mutant and control animals after adverse environmental exposures such as immune activation, stress or drug administration (Kannan et al., 2013). The influence of enriched housing conditions on rodent models of schizophrenia has been addressed by only a few studies (Karl et al., 2007; McOmish et al., 2008; Ishihama et al., 2010). However, the neurological and behavioural effects of environmental enrichment have been assessed in a range of other animal models including Huntington’s disease, Alzheimer’s disease, Parkinson’s disease, Epilepsy and drug addiction ((Bezard et al., 2003), see reviews Nithianantharajah and Hannan (2006); Laviola et al. (2008)). For example, the influence of environmental enrichment has been well demonstrated using the transgenic mouse model of Huntington’s disease (van Dellen et al., 2000). This neurodegenerative condition has a genetic cause, yet mice housed in enriched cages show delayed onset and progression of both the motor and cognitive deficits compared to standard housed controls (Hockly et al.,
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2002; Nithianantharajah and Hannan, 2006; Pang et al., 2006). Using animal models of schizophrenia, it will not only be important to address the detrimental effects of the environment, but also conditions that have a protective influence (Takuma et al., 2011; Pang and Hannan, 2013).

With the aim of developing biologically-relevant animal models of schizophrenia, studies using a GxE approach are rapidly emerging (Millstein et al., 2006; Millstein and Holmes, 2007; Oliver and Davies, 2009; Desbonnet et al., 2012; Hida et al., 2013; Petrovszki et al., 2013). Prenatal stress followed by acute stress during adulthood was used in three rat strains to examine how genetic background interacted with adverse environmental conditions to alter hippocampal gene expression (Neeley et al., 2011a). Five relevant genes (Nr3c1, Chrna7, Grin2b, Bdnf, Tnfα) were found to be altered by either strain or stress treatments, however changes were inconsistent across strains indicating a modulatory role of genotype. A second experiment comparing these strains using a stress protocol found that changes in Bdnf expression and associated pathways were also strain dependent (Neeley et al., 2011b). These studies demonstrate the importance of strain selection and genetic diversity in understanding GxE interactions.

In another recent study, rats were exposed to two commonly used risk factors, postweaning social isolation and chronic ketamine treatment, and selectively bred based on behavioural deficits relevant to schizophrenia to produce a vulnerable sub-strain (Petrovszki et al., 2013). After 15 generations, four groups were compared on three behavioural tests and the results were accumulated into an overall score. Rats with a standard genetic background raised under standard conditions were used as a control group. The environment-only group consisted of genetically-naïve rats that were then isolated and treated with ketamine. Rats from the selectively bred vulnerable sub-strain that were raised under standard conditions were used as the genetic-only group. And finally rats from the vulnerable sub-strain that also underwent social isolation and chronic ketamine treatment were used to investigate the GxE interaction. The GxE group scored the highest on schizophrenia-relevant deficits and the control group scored the lowest, indicating that both genetic and environmental insults were important. The behavioural tests used assess nociception, sensorimotor gating and recognition memory, which do not address the key cognitive domains identified by CNTRICS and therefore further work would be required to understand the influence of these manipulations on cognitive deficits.
relevant to schizophrenia. Nevertheless, this study does present a new way of investigating previously tested risk factors.

4.2.7 Future Recommendations

A recent review of mouse models of GxE interactions relevant to schizophrenia has discussed a comprehensive list of weaknesses to be addressed by future studies (Kannan et al., 2013). The authors suggested standardising strain and housing conditions to reduce variability between studies. However, genetic and environmental choices clearly alter outcomes relevant to schizophrenia and phenotypes may only be detected under specific strain or housing conditions. Furthermore, the way genetic and environmental conditions interact to protect or exacerbate phenotypes is of key importance in understanding the pathways that lead to schizophrenia.

Investigating genetic changes, such as mutant mouse models, may be easily replicated across laboratories, however environmental manipulations are more difficult to standardise. For example, wildtype mice show different behavioural phenotypes when tested under similar conditions but at different laboratories (Crabbe et al., 1999). More recently, heterozygous neuregulin mutant mice showed different behavioural phenotypes when tested in different laboratories, despite being on the same genetic background (Karl et al., 2011). Although these differences may be unavoidable, it is recommended the housing conditions of rodents be clearly stated in research methods. Unfortunately many articles do not list the forms of enrichment used (such as type of bedding, shelters, wood chews and tubes) however these should be indicated even if considered to represent ‘standard’ housing conditions. Recommending a standardised enrichment protocol would reduce variability between experiments, but would also limit the scope of enrichment studies (Wurbel, 2002). Protocol design should take into consideration the species-specific relevance of environmental changes, the timing and duration of exposure, the ethical implications and the reproducibility of the chosen design. Therefore, optimal enrichment conditions should be selected based on experimental aims.

Future studies could take a number of directions, including the use of GxG and ExE studies to identify the influence of genetic and environmental factors; as well as understanding the mechanisms that lead to increased vulnerability (Giovanoli et al., 2013). To more fully assess the effects of GxE interactions on cognitive endophenotypes, the field also needs to improve the range of the behavioural tasks available. The potential
therapeutic benefit of improved animal models may be limited by the sensitivity of the behavioural measures employed. Incorporating GxE clues from epidemiology into our animal models, and improving assessment techniques will advance our understanding of schizophrenia.

4.2.8 Conclusion

There is clear evidence to show that genetic and environmental conditions alter cognitive outcomes in rodents. However, the lack of studies comparing cognitive deficits in rodent models of schizophrenia using different strain and housing conditions is surprising. Schizophrenia develops from the complex interaction of GxE and we need to incorporate this complexity into animal models to understand the etiology of schizophrenia. While disorders, such as schizophrenia, cannot be fully encapsulated in a rodent model, the use of endophenotypes in carefully controlled experiments may allow us to understand some of the mechanisms behind GxE interactions. Current animal models are falling short of replicating the complex suite of risk factors implicated in schizophrenia and using a different strain or housing condition may provide an accessible stepping stone towards understanding altered brain development. Given the infancy of GxE interaction research in animal models of schizophrenia, manipulating these factors in existing as well as novel animal models will not only be informative in terms of GxE interactions, but will also allow researchers to determine a suitable foundation for further exploration. GxE interaction models will be particularly informative for understanding the role of vulnerable and resilient phenotypes in determining the influence of secondary ‘hits’ on cognitive outcomes in schizophrenia.

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4.3 Comprehensive behavioural analysis of Long Evans and Sprague-Dawley rats reveals differential effects of housing conditions on tests relevant to neuropsychiatric disorders

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4.3.1 Abstract

Genetic (G) and environmental (E) manipulations are known to alter behavioural outcomes in rodents, however many animal models of neuropsychiatric disorders only use a restricted selection of strain and housing conditions. The aim of this study was to examine GxE interactions comparing two outbred rat strains, which were housed in either standard or enriched cages. The strains selected were the albino SD rat, commonly used for animal models, and the other was the pigmented Long Evans (LE) rat, which is frequently used in cognitive studies. Rats were assessed using a comprehensive behavioural test battery and included well-established tests frequently employed to examine animal models of neuropsychiatric diseases, measuring aspects of anxiety, exploration, sensorimotor gating and cognition. Selective strain and housing effects were observed on a number of tests. These included increased locomotion and reduced pre-pulse inhibition in LE rats compared to SD rats; and rats housed in enriched cages had reduced anxiety-like behaviour compared to standard housed rats. LE rats required fewer sessions than SD rats to learnt operant tasks, including a signal detection task and reversal learning. Furthermore, LE rats housed in enriched cages acquired simple operant tasks faster than standard housed LE rats. Cognitive phenotypes in animal models of neuropsychiatric disorders would benefit from using strain and housing conditions where there is greater potential for both enhancement and deficits in performance.

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4.3.2 Introduction

Complex neuropsychiatric disorders, such as schizophrenia and autism, are affected by multiple genetic and environmental risk factors, possibly through the interaction between a vulnerable genotype (G) and an adverse environmental (E) ‘hit’ (Bayer et al., 1999; van Os et al., 2010; Tost and Meyer-Lindenberg, 2012). For example, cannabis use and having the catechol-o-methyl transferase (COMT) valine allele are separately implicated as schizophrenia risk factors (van Os et al., 2002; Chen et al., 2004) and the functional polymorphism in the COMT gene has been found to alter the effects of cannabis (Egan et al., 2001; Collins, 2004; Henquet et al., 2006). However, schizophrenia is associated with hundreds of genes of small effect rather than a few genes of large effect (Wray and Visscher, 2010). Genetic models of relevance to schizophrenia have investigated the functions of individual candidate genes (Chen et al., 2006; Kellendonk et al., 2006; Karl et al., 2007; O’Tuathaigh et al., 2007; McOmish et al., 2008; Papaleo et al., 2012a). While these models provide important information about the gene of interest, they cannot encapsulate the polygenic nature of this disorder. On the other hand, rodent strains were bred to produce consistent strain-dependent phenotypes that differ on a range of behavioural and physiological measures (Didriksen and Christensen, 1993; Andrews, 1996; Swerdlow et al., 2000; Faraday, 2002; Aubert et al., 2006). Comparing strains may provide an avenue to investigate how polygenic vulnerability interacts with environmental conditions to produce deficits relevant to neuropsychiatric disorders (Crawley et al., 1997). For example, a comparison of SD, Lewis and Fischer 344 rat strains demonstrated that F344 rats, which are more responsive to stress (Faraday, 2002) had the greatest vulnerability to a neonatal ventral hippocampal lesion (Lipska and Weinberger, 1995) which is used as a neurodevelopmental animal model of schizophrenia.

Both genetic and environmental risk factors have been investigated using animal models of neuropsychiatric disorders, however most of these manipulations have only been tested under standard housing conditions. Standard housing conditions vary between facilities and over time, however generally this is referring to a rather barren cage containing nesting material and minimal in dimensions. Environmental enrichment in rodents incorporates greater sensory, cognitive and motor stimulation (Nithianantharajah and Hannan, 2006) and may be considered therapeutic, or conversely standard housing may be seen as impoverished (van Praag et al., 2000; Hutchinson et al., 2005). Whether effects of enrichment are positive or negative may also depend on the animal model or...
disorder being investigated. Either way, enriching the housing conditions of laboratory rodents has been found to alter behaviour, stress hormone levels, neurogenesis, dendrite structure, and gene expression (see review Simpson and Kelly (2011). We need to consider how standard housing conditions are impacting on brain development, especially with increasing research focussed on GxE interactions (Burrows and Hannan, 2013). Despite evidence that environmental enrichment has reversed or retarded deficits in animal models of neurological disorders, few studies have assessed these effects in rodent models of schizophrenia (Laviola et al., 2008).

The validity of both genetic and environmental animal models has been assessed using tests that are relevant to the disorder being modelled. With many neuropsychiatric disorders diagnosed based on cognitive and behavioural symptoms, assessment of animal models also relies on measuring relevant behavioural characteristics (Moy et al., 2007). Using tests employed in behavioural screens, strain and housing conditions have been shown to affect multiple behavioural domains including locomotion, anxiety, pre-pulse inhibition and acquisition of operant tasks. However, few papers have directly evaluated strain and housing manipulations across a broad screen of behavioural tests in the same study, making comparisons between tasks difficult and often conflicting (Simpson and Kelly, 2011). Therefore, the aim of this experiment was to behaviourally phenotype pigmented LE, which are commonly used in cognitive experiments, and compare them to albino SD rats, which are frequently used to model neuropsychiatric disorders after they are reared in either standard or enriched housing conditions. Tests used in the behavioural test battery were selected to measure a broad range of behaviours that are frequently assessed in animal models of neuropsychiatric disorders. These tests highlight the behavioural alterations between strains and housing conditions and suggest animal models should consider using different strains for different purposes.

4.3.3 Materials and methods

4.3.3.1 Animals and housing

Nine-week old male SD (Asmu:SD) and LE (Asmu:LE) rats were both obtained at weaning (3 weeks of age) from Monash Animal Services (Melbourne, Australia) to ensure transport and housing prior to weaning were equivalent. Male rats were selected as sex differences were not being examined in this study and males are more commonly used in similar experiments. They were then housed in a room maintained at 21 ± 2°C and 60% humidity
and on a 12-h light/dark cycle (lights on 0600 h). Standard rat chow (Specialty Feeds, WA, Australia) and water were supplied ad libitum. On arrival rats were pair-housed in either standard or enriched cages (n=8/strain/housing) with groups balanced for initial body weight and pairs matched to avoid excessive dominance. Standard housing consisted of a polypropylene cage (41 x 28 x 24 cm) with a high top wire lid, aspen chip bedding (Able Scientific, WA, Australia), nesting, and wood chew (Able Scientific, WA, Australia). The alternative enriched housing condition used a larger sized polypropylene cage (54 x 36 x 30 cm) with a high top wire lid, bedding, nesting, wood chew, an enclosed shelter (15 x 15 x 12 cm) and running wheel (20.3 cm diameter, Super Pet Run-Around Wheel, IL, USA). Rats were weighed weekly and all testing was conducted during the light phase. Bicycle computers were used to record running wheel rotations (Bontrager Trip 2, Trek Bicycle Corporation, WI, USA). Rats were observed to play and jump on the wheels as well as running as a pair simultaneously as juveniles, which interfered with the accuracy of running distance. However, the values recorded confirmed the wheels were used particularly during the dark phase (data not presented). After completion of this study, rats were used for a follow-on experiment using the operant protocol. All procedures used were performed with the approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

4.3.3.2 Behavioural test battery

The effects of strain and housing conditions were characterised using a behavioural test battery that began at 9 weeks and concluded at 12 weeks of age. This was followed by a 3-week rest period and one week of food restriction before operant training started. Rats were weighed weekly and food restriction was delayed until adulthood when free-feeding weight stabilised to avoid changes in growth. The tests selected assessed a range of domains including anxiety-related behaviour, spatial memory, exploration, locomotion and sensorimotor gating. These tests have been widely used and standard protocols were adopted (Crawley, 1999; Karl et al., 2003; Burne et al., 2004). Tests were conducted during the light phase on separate days in the following order to reduce the influence of test order: elevated plus maze, hole board, light-dark emergence, open field, 8-arm radial maze, Y-maze, PPI of the acoustic startle response and finally operant task acquisition. Although the elevated plus maze may be more stressful than tests conducted afterwards, it is also very sensitive to order effects and hence was conducted first. An extra rest day was included after the elevated plus maze. All behaviours were recorded using automated
software or scored blind to treatment. Computerised tracking software, EthoVision ver.3.1 (Noldus, Netherlands) was used to analyse video recordings from a camera mounted above each testing arena. Ethologically relevant behaviours were scored using Observer ver.5.0 (Noldus, Netherlands). Rats were habituated to the testing room 30 minutes prior to testing and the apparatus was cleaned with 70% ethanol between each trial.

Elevated plus maze (EPM): The elevated plus maze was used to measure anxiety-related behaviours, but also provided measures of exploration and locomotion (Pellow et al., 1985). The plus-shaped platform was made of opaque grey plastic, elevated on a stand (70 cm) with two arms enclosed by walls (10 x 47 x 40 cm; 1 lux) and the other two arms open (10 x 47 cm; 8 lux). Rats were placed in the centre of the maze facing an open arm and allowed to explore the maze for 10 minutes. Measures included percentage of time spent on the open arms and distance travelled.

**Equation 4.1 EPM percentage time on open arms:**

\[
\% \text{ Open} = \left( \frac{\text{time spent open arms}}{(\text{time spent open arms} + \text{closed arms})} \right) \times 100
\]

These results were further investigated by scoring ethologically relevant behaviours such as rearing, grooming and risk assessment behaviours were also scored. Rearing was defined as standing with fore limbs lifted; grooming was scored when the animal was licking and cleaning their body; head dip was scored when the rats head was pointed downward over the edge of the platform; scanning was scored when the rats head was pointed outward and upward while the rat was stationary; and stretch attend posture was defined as stretching the head forward without stepping forward. If behaviours were split for zone, protected describes behaviours in the closed arms and centre zone, while open describes behaviours that occurred on the open arms.

Hole board: The hole board test was used to measure exploration and locomotion (File and Wardill, 1975). An opaque grey arena (60 x 60 x 40 cm; 8 lux in centre) with an elevated floor containing four holes (4 cm wide, 12.5 cm from each corner) was used. The rat was placed into a corner and explored the arena for 10 minutes. Measures included the distance travelled, time spent in the centre of the arena and the duration, frequency and latency to perform head dipping, grooming and rearing behaviour.
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Light dark emergence: Light dark emergence was used as an alternative measure of anxiety-related behaviours and exploration (Arrant et al., 2013). The dark compartment provides shelter and an increase in the time taken to leave the shelter to explore the open compartment is indicative of greater anxiety. A plastic arena (44 x 44 x 30 cm, 7 lux in open) was divided such that half the arena was open and the other half was completely enclosed with the exception of a central doorway (8 x 11 cm). To start the 10-minute trial the rat was positioned at the doorway and required to enter the dark section. The key measures were time to emerge from the dark chamber and the number of transitions between the light and dark sections.

Open field: The open field test was used to measure locomotion, however the use of different zones within the arena also provides information on anxiety-related behaviours (Prut and Belzung, 2003). Rats were placed in the centre of an open arena (60 x 60 x 40 cm; 5.5 lux) and tracked over 10 minutes. Measures included distance travelled, time spent in the centre and crossings into the centre.

8-Arm radial maze: The 8-arm radial maze has been used for a number of protocols to assess different aspects of cognition. A simplified protocol was used in this study to investigate exploratory behaviour in a novel arena (Olton and Samuelson, 1976; Nguyen et al., 2006). The opaque grey plastic maze consisted of 8 arms (60 x 10 x 20 cm, 3 lux) joined by a central octagon (25.5 cm wide; 8 lux). The rat was placed in the centre and the order and timing of arm visits was recorded over 10 minutes. The rat should visit each arm once until all arms have been explored. Revisiting an arm prior to exploring all 8 was considered an error. The number of errors and time taken to visit all the arms were recorded, as well as distance travelled.

Y-maze: Three arms of the 8-arm radial maze were used for this test and the other arms were blocked off at the centre. Two trials were conducted to investigate short-term recognition memory. During the first trial the rat was placed at the end of the ‘home’ arm and given access to one other arm, referred to as the ‘familiar’ arm for 10 minutes. After an inter-trial interval of one hour the rat was placed back in the home arm and has access to the familiar arm in addition to a previously occluded arm, the ‘novel’ arm for 5 minutes. The amount of time spent in each arm and the number of transitions was recorded. The rat should spend more time exploring the novel arm if it has remembered visiting the home and familiar arms in the previous trial. Within each pair the familiar and novel arm was
alternated between the left and right arms. The ends of these arms were decorated with either vertical or horizontal barred patterns to facilitate recognition.

Pre-Pulse Inhibition (PPI) of Acoustic Startle Response (ASR): PPI was used as a measure of sensorimotor gating, whereby a startle reflex is reduced if a weaker pre-pulse precedes the startling pulse. Responses were recorded in startle chambers by placing rats into clear Plexiglas cylinders on platforms connected to piezoelectric transducers, which were housed in sound attenuating chambers containing speakers and controlled using specialist software (SR-Lab, San Diego Instruments). The session consisted of pseudo-randomised presentation of the different trial types. The ASR was measured using a single 40 ms pulse at various intensities (70, 80, 90, 100, 110, 120 dB) and within-session habituation was measured as the change in startle response to a 110 dB pulse presented at the start, middle and end of the session. Pre-pulses at three different intensities (74, 78, 86 dB) were played at a variety of intervals (8, 16, 32, 64, 128, 256 ms) prior to the startle pulse (120 dB) to assess pre-pulse inhibition. Each trial type was presented five times and the median was used for further analysis. Percentage PPI was calculated as follows:

**Equation 4.2 % Pre-pulse Inhibition**

\[
\%_{PPI} = \left[ \frac{\text{startle amplitude ASR trial} - \text{startle amplitude for pre-pulse trial}}{\text{startle amplitude ASR trial}} \right] \times 100
\]

4.3.3.3 Operant training

Training was conducted in operant chambers housed in ventilated, sound attenuating boxes (50 x 50 x 50cm, Med Associates Inc., St. Albans, VT, USA). Rats were initially trained to collect a reward (45 mg, F0021, dustless precision pellet, Bioserv, Frenchtown, NJ, USA) from one of two receptacles equipped with head entry detectors that were located on the left and right side of the wall. Every head entry was rewarded with one pellet until 50 pellets were delivered from each receptacle or until the session ended after 20 mins. Once rats had attained >80 pellets on 2 days they were trained to nose poke a central aperture when it was illuminated to receive a reward, which was delivered to either the left or right receptacle. Finally, after learning to initiate trials by nose poking, the signal detection task was implemented. After initiating trials with a nose poke, a panel of 9 green LEDs were either illuminated (signal trial) or remained off (non-signal trial). After 1s both
the left and right magazines illuminated to indicate the rat should make a choice. Depending on the visual cue presented, a head entry into one side would lead to a pellet and the other had no consequence. The pairing of a trial type (signal or non-signal) and the correct magazine side (left or right) remained the same for each individual but was balanced across the group. Between trials there was a variable inter-trial interval (1, 2, 3s) and the session concluded after either 100 trials or 30min. The chamber was operated using MED-PC for Windows software and interfacing (Med Associates Inc., St. Albans, VT, USA).

4.3.3.4 Statistical analysis

Results were analysed using SPSS software (ver. 20, SPSS Inc., Chicago, Illinois). A power analysis determined that a group size of 8 was required to detect a difference with an effect size of 0.85 with 95% power and at the criterion of $p<0.05$. Key behavioural measures were assessed for skewness and kurtosis for confirm the assumption of normal distribution. The main effect of Strain (SD or LE) and Housing (standard or enriched) on key parameters for each test was subjected to an ANOVA or repeated measures ANOVA was applied where required. Significant interactions were then assessed using independent $t$-tests. Due to the large difference in variation, the effect of housing was assessed in each strain separately for PPI. Data is presented as mean ± standard error of the mean (SEM) and statistical significance was determined if $p<0.05$.

4.3.4 Results

The body weight of LE and SD rats housed in either enriched or standard housing was recorded from 3-16 weeks of age. Overall, there was a main effect of strain ($F_{(1,28)}=54.75$, $p<0.001$), but not housing ($F_{(1,28)}=0.24$, $p=0.625$; Figure 4.1). LE rats from standard and enriched housing tended to deviate more towards the end of the experiment, however this was not significant even at 16 weeks ($t_{(10.85)}=2.18$, $p=0.052$).
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4.3.4.1 Behavioural test battery

Due to the number of behavioural parameters reported, the main measures from the behavioural test battery are presented in Table 4.1, with ethological measures presented in Table 4.2.

Figure 4.1 Growth Curve

The body weight of Long Evans (LE, circles) and Sprague Dawley (SD, squares) rats from 3-16 weeks of age housed in either enriched (EE, open) or standard housing (SH, closed). Overall, there was a main effect of strain ($F_{(1,28)}=54.75, p<0.001$), but not housing ($F_{(1,28)}=0.24, p=0.625$). LE rats tended to separate more towards the end of the experiment, however this was not significant even at 16 weeks ($t_{(10.85)}=2.18, p=0.052$).

Elevated plus maze: There was a significant effect of both Strain ($F_{1,31}=9.45, p=.005$) and Housing ($F_{1,31}=17.24, p<0.001$) but no interaction ($F_{1,31}=0.31; p=.582$) on the percentage of time spent on the open arm of the EPM. LE rats spent significantly longer on the open arm than SD rats, and standard housed rats spent significantly longer on the open arms than those from enriched cages. There was no significant difference between Strains, however SD rats from enriched cages spent less time than those from standard housing in the centre of the maze ($t_{(14)}=-2.19, p=.046$). The total distance travelled on the EPM differed based on Strain, such that LE rats had greater locomotion than SD rats ($F_{(1,31)}=8.93, p=.006$). Within the ethological behaviours, it was found that enriched rats performed protected stretch-attend risk assessment behaviour earlier ($F_{1,31}=4.74, p=.039$) and more frequently ($F_{1,31}=6.58, p=.016$) than standard housed rats. There was no significant effect of Strain detected for these measures, however when analysed separately frequency of protected stretch-attend behaviour was only greater in enriched
LE compared to those in standard housing ($t_{(14)}=2.66$, $p=.019$). An analysis of grooming behaviour revealed main effects of Strain and Housing on latency ($F_{(1,31)}=4.31$, $p=.048$; $F_{(1,31)}=4.37$, $p=.046$) and duration ($F_{(1,31)}=12.45$, $p=.001$; $F_{(1,31)}=15.4$, $p=.001$) with a significant Strain x Housing interaction ($F_{(1,31)}=5.47$, $p=.027$) but no significant effect on frequency of grooming. These results indicated LE rats groom earlier and for longer than SD rats, and that rats from enriched housing groomed earlier and for longer than those from standard housing. A post-hoc $t$-test revealed LE rats housed in enrichment groomed for significantly longer than those from standard housing ($t_{(14)}=3.50$, $p=.004$).

Hole board: A main effect of Strain was found for distance travelled on the hole board with LE rats travelling significantly further than SD rats ($F_{(1,31)}=14.97$, $p=.001$) and spending more time in the centre zone ($F_{(1,31)}=7.21$, $p=.012$). The latency to head dip differed by Housing condition ($F_{(1,31)}=9.48$, $p=.005$) and there was a significant interaction of Strain x Housing ($F_{(1,31)}=6.10$, $p=.020$). SD rats reared in standard housing took significantly longer to head dip on the hole board compared to those housed in enriched cages ($t_{(14)}=-3.14$, $p=.007$). Frequency of head dipping ($F_{(1,31)}=7.15$, $p=.012$) and rearing ($F_{(1,31)}=5.45$, $p=.027$) was significantly greater in LE rats compared to SD rats, but there was no main effect of Housing.

Light dark emergence: The key measure of the light dark test was the latency to enter the open section and there was a significant effect of Housing in SD rats ($t_{(14)}=-2.85$, $p=.013$), without a main effect of Strain ($F_{(1,31)}=1.61$, $p=.215$). By contrast there was a main effect of Strain ($F_{(1,31)}=6.54$, $p=.016$) but not Housing ($F_{(1,31)}=0.64$, $p=.432$) on the percentage of time spent in the open. An effect of Strain ($F_{(1,31)}=23.79$, $p<0.001$) was again detected for distance travelled, indicating LE rats had increased locomotion compared to SD rats.

Open field: While distance travelled was found to differ between strains on a number of other tests, no significant effect of Strain ($F_{(1,31)}=3.53$, $p=.071$) was found on the open field test. However, there was a main effect of Housing ($F_{(1,31)}=9.09$, $p=.005$), in which enriched rats moved less than standard housed LE rats. While the total distance travelled after 10 minutes differed between strains, these groups did not differ after the 1$^{st}$ minute time bin, indicating altered habituation. There was a significant effect of Strain ($F_{(1,31)}=5.17$, $p=.031$) on number of crossings into the centre, but no significant difference between groups on the percentage of time spent in the centre.
8-Arm radial maze: Each rat visited all 8 arms of the maze. There was a main effect of Strain, but not Housing, on the time to enter all 8 arms ($F_{(1,30)}=23.22, p<0.001$), the total number of arms entered to visit all 8 arms ($F_{(1,30)}=14.75, p<0.001$) and the distance travelled ($F_{(1,30)}=7.63, p=0.010$). LE rats completed the 8-arm radial arm maze faster and with fewer errors than SD rats.

Y-maze: The percentage of time spent in the novel arm was greater than the familiar arm in each group, indicating rats were able to recognise the previously visited arm after a one-hour inter-trial interval. There was no significant effect of Strain or Housing on the percentage of time spent in the novel vs. familiar arm, the number of novel arm entries or the distance travelled.
### Table 4.1 Behavioural Test Battery

<table>
<thead>
<tr>
<th>Test and measure</th>
<th>Sprague Dawley</th>
<th>Long Evans</th>
<th>Strain ( (F_{1,31}) )</th>
<th>Housing ( (F_{1,31}) )</th>
<th>Interaction ( (F_{1,31}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated Plus Maze</td>
<td>Standard (n=8)</td>
<td>Enriched (n=8)</td>
<td>Standard (n=8)</td>
<td>Enriched (n=8)</td>
<td></td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>4057.2 ± 227.4</td>
<td>3863.5 ± 452.7</td>
<td>5555.0 ± 239.3</td>
<td>4494.1 ± 439.5</td>
<td>8.93** 3.10 1.48</td>
</tr>
<tr>
<td>Duration in Centre (s)</td>
<td>153.5 ± 15.8</td>
<td>112.2 ± 10.4</td>
<td>122.3 ± 13.9</td>
<td>125.2 ± 12.8</td>
<td>0.46 2.07 2.73</td>
</tr>
<tr>
<td>% Duration Open Arm</td>
<td>31.4 ± 5.6</td>
<td>10.3 ± 3.6</td>
<td>42.6 ± 4.4</td>
<td>26.6 ± 4.0</td>
<td>9.45** 17.24** 0.31</td>
</tr>
<tr>
<td>Hole board</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>3929.7 ± 186.2</td>
<td>3538.7 ± 261.9</td>
<td>4740.5 ± 150.8</td>
<td>4449.1 ± 268.1</td>
<td>14.97** 2.35 0.05</td>
</tr>
<tr>
<td>Time in centre (%)</td>
<td>2.1 ± 0.8</td>
<td>3.1 ± 0.9</td>
<td>5.7 ± 1.0</td>
<td>4.9 ± 1.3</td>
<td>7.21* 0.09 0.88</td>
</tr>
<tr>
<td>Latency Head Dip</td>
<td>60.5 ± 14.7</td>
<td>12.7 ± 4.1</td>
<td>29.2 ± 5.8</td>
<td>24.0 ± 5.7</td>
<td>1.35 9.48** 6.10*</td>
</tr>
<tr>
<td>Light Dark Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>1437.5 ± 94.7</td>
<td>1303.4 ± 185.2</td>
<td>2477.1 ± 251.7</td>
<td>2230.6 ± 236.5</td>
<td>23.79** 0.89 0.08</td>
</tr>
<tr>
<td>% Duration in Open</td>
<td>54.4 ± 2.7</td>
<td>45.5 ± 6.2</td>
<td>62.7 ± 5.2</td>
<td>63.4 ± 5.7</td>
<td>6.54* 0.64 0.88</td>
</tr>
<tr>
<td>Latency to Open (s)</td>
<td>20.3 ± 4.1</td>
<td>7.6 ± 1.7</td>
<td>12.7 ± 1.8</td>
<td>8.8 ± 1.5</td>
<td>1.61 10.93** 3.02</td>
</tr>
<tr>
<td>Open Field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>3485.0 ± 250.7</td>
<td>2832.4 ± 277.6</td>
<td>4189.0 ± 381.3</td>
<td>3169.9 ± 148.2</td>
<td>3.53 9.09** 0.44</td>
</tr>
<tr>
<td>Time in centre (%)</td>
<td>3.7 ± 1.1</td>
<td>3.6 ± 1.0</td>
<td>6.7 ± 1.7</td>
<td>4.9 ± 0.4</td>
<td>3.62 0.72 0.63</td>
</tr>
<tr>
<td>Entries to centre</td>
<td>11.0 ± 2.7</td>
<td>8.8 ± 1.7</td>
<td>17.9 ± 3.4</td>
<td>12.9 ± 1.2</td>
<td>5.17* 2.25 0.32</td>
</tr>
<tr>
<td>Y-Maze</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance Day 2 (cm)</td>
<td>2919.3 ± 90.2</td>
<td>2968.9 ± 174.3</td>
<td>3183.5 ± 210.5</td>
<td>2706.4 ± 125.7</td>
<td>0.00 1.85 2.81</td>
</tr>
<tr>
<td>Novel vs Fam Dur</td>
<td>63.1 ± 5.1</td>
<td>71.6 ± 3.1</td>
<td>59.2 ± 6.7</td>
<td>63.5 ± 6.2</td>
<td>1.22 1.37 0.14</td>
</tr>
<tr>
<td>Novel arm entries</td>
<td>6.4 ± 1.0</td>
<td>6.3 ± 0.3</td>
<td>7.9 ± 1.0</td>
<td>5.4 ± 0.6</td>
<td>0.16 2.75 2.25</td>
</tr>
<tr>
<td>Radial Arm Maze</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance (cm)</td>
<td>5770.4 ± 706.5</td>
<td>6424.6 ± 424.0</td>
<td>7709.3 ± 411.9</td>
<td>7692.0 ± 729.1</td>
<td>7.63* 0.30 0.33</td>
</tr>
<tr>
<td>Time to complete</td>
<td>226.7 ± 17.4</td>
<td>188.6 ± 19.0</td>
<td>137.0 ± 10.5</td>
<td>139.9 ± 8.5</td>
<td>23.22** 1.50 2.03</td>
</tr>
<tr>
<td>Entries to complete</td>
<td>12.0 ± 0.7</td>
<td>12.0 ± 0.8</td>
<td>9.6 ± 0.5</td>
<td>9.6 ± 0.5</td>
<td>14.75** 0.00 0.00</td>
</tr>
</tbody>
</table>
Key behavioural parameters from each test in the behaviour screen for Sprague Dawley and Long Evans rats housed in standard or enriched conditions. Data are presented as mean ± SEM and statistical results for strain comparison. Main effect of Strain and Housing was assessed by ANOVA and independent-samples t-tests were then performed if a significant Strain x Housing interaction was detected; *p<0.05, **p<0.01.
## Table 4.2 Ethological measures

<table>
<thead>
<tr>
<th>Test and measure</th>
<th>Sprague Dawley</th>
<th>Long Evans</th>
<th>Strain (F_{(1,31)})</th>
<th>Housing (F_{(1,31)})</th>
<th>Interaction (F_{(1,31)})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elevated Plus Maze</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to Protected Groom</td>
<td>338.5 ± 68.3</td>
<td>233.7 ± 53.1</td>
<td>234.4 ± 61.0</td>
<td>116.2 ± 24.3</td>
<td>4.31*</td>
</tr>
<tr>
<td>Duration Groom (s)</td>
<td>8.0 ± 2.4</td>
<td>30.5 ± 12.4</td>
<td>24.8 ± 7.4</td>
<td>113.6 ± 24.3</td>
<td>12.45**</td>
</tr>
<tr>
<td>Freq. Groom</td>
<td>3.3 ± 0.9</td>
<td>4.9 ± 1.1</td>
<td>5.1 ± 1.3</td>
<td>6.5 ± 1.0</td>
<td>2.69</td>
</tr>
<tr>
<td>Duration Protected Scanning (s)</td>
<td>61.8 ± 7.7</td>
<td>67.7 ± 9.9</td>
<td>27.5 ± 4.4</td>
<td>35.4 ± 3.2</td>
<td>23.78**</td>
</tr>
<tr>
<td>Freq. Open Head Dip</td>
<td>11.3 ± 3.0</td>
<td>3.0 ± 1.0</td>
<td>22.5 ± 2.9</td>
<td>16.9 ± 2.4</td>
<td>26.34**</td>
</tr>
<tr>
<td>Freq. PSA</td>
<td>4.3 ± 0.8</td>
<td>5.1 ± 0.8</td>
<td>2.0 ± 0.9</td>
<td>5.6 ± 1.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Latency PSA (s)</td>
<td>162.5 ± 35.2</td>
<td>69.4 ± 13.8</td>
<td>164.7 ± 41.9</td>
<td>128.0 ± 26.1</td>
<td>1.04</td>
</tr>
<tr>
<td><strong>Hole board</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to Groom (s)</td>
<td>229. ± 29.7</td>
<td>231.8 ± 57.0</td>
<td>204.9 ± 52.2</td>
<td>165.5 ± 56.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Duration Groom (s)</td>
<td>2.9 ± 0.6</td>
<td>7.0 ± 3.3</td>
<td>4.6 ± 0.8</td>
<td>7.8 ± 2.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Freq. Groom</td>
<td>3.9 ± 0.8</td>
<td>3.8 ± 1.0</td>
<td>5.0 ± 1.1</td>
<td>5.8 ± 0.8</td>
<td>2.76</td>
</tr>
<tr>
<td>Freq. Head Dip</td>
<td>23.0 ± 2.1</td>
<td>24.8 ± 3.7</td>
<td>34.4 ± 3.9</td>
<td>30.5 ± 2.8</td>
<td>7.15*</td>
</tr>
<tr>
<td>Freq. Rear</td>
<td>42.1 ± 4.0</td>
<td>41.9 ± 7.3</td>
<td>56.9 ± 3.5</td>
<td>54.6 ± 7.6</td>
<td>5.45*</td>
</tr>
</tbody>
</table>

Behavioural observations from EPM and Hole board tests showing the mean and SEM for each group. Data are presented as mean ± SEM and statistical results for strain comparison. Main effect of Strain and Housing was assessed by ANOVA and independent-samples t-tests were then performed where a significant Strain x Housing interaction was detected. *\(p<0.05\), **\(p<0.01\). PSA=Protected Stretch Attend posture.
Pre-Pulse Inhibition (PPI) of Acoustic Startle Response: All groups showed an increase in startle amplitude with increased pulse intensity, however using a repeated measures ANOVA no effect of Strain ($F_{(1,27)}=1.00$, $p=.326$) or Housing ($F_{(1,27)}=0.08$, $p=.774$) was detected (Figure 4.2A,B). Habituation was measured by comparing startle amplitude at the start and end of the session and while there was no difference between Strain or Housing at the start, at the end there was a main effect of Strain ($F_{(1,30)}=7.96$, $p=.009$) but not Housing ($F_{(1,30)}=0.84$, $p=.368$; Figure 4.2C,D). % PPI was pooled across pre-pulse intervals for each of the three intensities and analysed using a repeated measures ANOVA. A main effect of Strain ($F_{(1,30)}=4.68$, $p=.040$), but not Housing ($F_{(1,30)}=1.10$, $p=.304$) was found (Figure 4.2E,F). Within strains it was found that enriched SD rats when compared to standard housed SD rats had impaired PPI at 74dB ($t_{(14)}=-2.31$, $p=.038$). When pre-pulse interval was pooled across intensity there was also a reduction in PPI at 64ms in SD from enriched cages compared to those from standard housing ($t_{(8.27)}=-2.40$, $p=.042$; Figure 4.2G,H).
Figure 4.2 Pre-pulse inhibition

(A,B) Acoustic startle response in SD and LE rats demonstrating increasing startle amplitude with louder acoustic pulses. (C,D) Habituation of the startle response to 110db pulses presented at the start, middle and end of the session showing a clear within-session reduction in LE rats compared to SD rats, such that there was a main effect of strain at the end of the session ($F_{1,30}=7.96$, $p=.009$). (E,F) %PPI is presented as each intensity (74, 78 and 86dB) averaged across different six pre-pulse intervals. %PPI at 74dB was significantly reduced in SD rats housed in enrichment compared to standard housed rats ($t_{13}=-2.31$, $p=.038$). Variability of PPI within LE rats was noticeably greater than in SD rats and no significant effect of housing was found. (G,H) %PPI for each pre-pulse interval (averages across the three intensities) shows reduced PPI at 64ms in SD rats from enriched cages compared to standard housed SD rats ($t_{8.27}=-2.40$, $p=.042$) while LE rats from both housing conditions did not differ. Standard housing (open □), enriched housing (closed ■). Data presented as mean ± S.E.M.*$p<0.05$. 

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4.3.4.2 Operant training

After 3 days learning the initial protocol, where rats were required to respond with head entries to receive rewards, there was a significant interaction between Strain and Housing on the number of trials completed ($F_{(1,31)}=5.59, p=.025$); however there was no significant main effect of Strain or Housing. LE rats raised in enriched housing performed significantly more trials than those from standard housing conditions ($t_{(13)}=2.41, p=.032$; Figure 4.3A). Next rats learnt to nose poke a central aperture to receive a reward. On the first day of training there was a main effect of Strain ($F_{(1,31)}=26.3, p<0.001$) and Housing ($F_{(1,31)}=4.39, p=.045$), and a Strain x Housing interaction ($F_{(1,31)}=4.18, p=.050$). These results indicated that LE rats performed more successful trials than SD rats and that those from enriched housing performed better, which was most pronounced in LE rats ($t_{(13)}=2.41, p=.032$; Figure 4.3B). There was a main effect of Strain on the number of sessions required to learn the signal detection task ($F_{(1,31)}=6.47, p=.017$; Figure 4.3C) and the reversal ($F_{(1,31)}=21.21, p<0.001$; Figure 4.3D), finding in both cases that LE rats required less training than SD rats.
Chapter 4. Genetic and Environmental Conditions

Figure 4.3 Operant Training

(A) Fixed ratio (FR1) training in LE rats, showing the number of trials completed was greater in enriched rats compared to those from standard housing conditions after 3 days of training ($t_{(13)}=2.41$, $p = .032$). (B) On the first session of learning to nose poke to receive a food reward, LE rats completed more trials than SD rats ($F_{(1,31)}=26.3$, $p < 0.001$). Additionally, LE rats from enriched housing successfully performed more trials compared to those from standard housing ($t_{(13)}=2.41$, $p = .032$). (C) The number of sessions required to reach criteria on a signal detection task was greater in SD rats compared to LE ($F_{(1,31)}=6.47$, $p = .017$). (D) LE rats were able to acquire the reversed contingency on a signal detection task in fewer sessions than SD rats ($F_{(1,31)}=21.21$, $p < 0.001$). Standard housing (open □), enriched housing (closed ■). Data presented as mean ± S.E.M.*$p<0.05$.

4.3.5 Discussion

The genetic and environmental background of rodent models are known to influence behavioural phenotypes, however many animal models of schizophrenia use the same strain and standard housing conditions. In the current study we used two outbred rat strains, one used for modelling neuropsychiatric disorders (SD) and a strain more frequently used for cognitive testing (LE) and compared their behaviour when raised in standard or enriched cages. We found that strain and housing conditions influenced
behavioural measures across a number of behavioural domains relevant to animal models of neuropsychiatric disorders; including altered locomotion, increased anxiety, impaired PPI and altered learning (Duffy et al., 2008; Fone and Porkess, 2008; van den Buuse, 2010; Jones et al., 2011). Importantly, these genetic and environmental manipulations may help us to understand complex phenotypes relevant to human neuropsychiatric disorders.

4.3.5.1 Strain

Overall LE rats were more active, had greater exploration, reduced anxiety, reduced PPI and improved cognitive performance compared to SD rats. Sensorimotor gating was measured using PPI and LE rats showed greater habituated to the startle pulse than SD rats, however the increased variation observed in LE rats made PPI comparisons difficult. LE rats generally showed facilitation, whereas SD rats showed inhibition of the startle reflex. Facilitation can occur if the pre-pulse interval is too short or too long resulting in summation of the startle response. However, the same protocol has been used previously by our group using SD rats from a different supplier with similar results to the SD rats tested in this study (Kesby et al., 2012). Strain and supplier differences in PPI have been identified by a number of studies (e.g. Varty and Higgins (1994); Swerdlow et al. (2000); Aubert et al. (2006)) however the facilitation observed in LE rats is likely to be a characteristic of the strain under these testing conditions.

When compared using an operant task it was found that SD rats required more training sessions than LE rats to learn to self initiate trials, to learn a signal detection task and to acquire reversal of task contingencies. Strain differences in the acquisition of operant behaviours have been observed previously in studies comparing lever press acquisition in LE, SD and Wistar strains (Andrews et al., 1995). All strains acquired the task, however albino strains took longer than LE rats to learn this behaviour. It is interesting to note the widespread use of pigmented strains for cognitive tasks, while most animal models of schizophrenia are developed in albino strains. Comparing strains that differ in cognitive performance within existing animal model preparations may provide a useful tool for modelling and understanding cognitive deficits relevant to schizophrenia.

4.3.5.2 Housing

While some effects were strain-specific, it was found that minimal enrichment lead to a phenotype characterised by reduced anxiety-related behaviour, locomotion and PPI, and
enriched rats completed more trials successfully during operant training. On the EPM enriched rats of both strains spent less time on the open arms than those from standard housing, which is indicative of greater anxiety. This is contrary to many studies showing increased open arm usage in rats from environmentally enriched cages (Pena et al., 2009), however reduced open arm time after enrichment has been seen in mice (Pietropaolo et al., 2006) and after access to wheel running in rats (Burghardt et al., 2004). Latency to emergence on the light dark test was also used to measure anxiety and SD rats raised in the enriched environment emerged earlier than those raised in standard housing, a result found by others using Wistar rats and indicating enriched rats were less anxious (Harris et al., 2009). Additionally, SD rats from enriched cages performed head dips on the hole board test earlier than those from standard housing. Previously, enrichment in SD rats has been associated with greater exploration and reduced corticosterone levels on the hole board test (Pena et al., 2009). Together these results indicate enriched housing results in a less anxious phenotype. While this initially appears to conflict with the results from the EPM, the inclusion of a shelter in the enriched cages may have led to differential use of the protective zones.

Further analysis of ethologically relevant behaviours revealed that on the EPM, stretch-attend behaviour from the protected zone was performed earlier and more frequently in enriched rats. The stretch-attend posture is adopted when a rodent explores a novel or potentially dangerous space (Rodgers and Dalvi, 1997), indicating enriched rats used a protective posture to investigate the open arm rather than walking out. Rats from enriched housing also began grooming earlier and for longer than standard housed rats. Furthermore, although the duration of grooming was longer, the number of bouts was not the same, indicating that enriched rats were performing longer uninterrupted bouts of grooming. Increased grooming can be triggered in both high and low stress situations, however broken or rapid bouts of grooming are associated with increased stress in rats and mice (Kalueff and Tuohimaa, 2005). These results suggest that rats from enriched housing are less anxious than standard housed rats. Reduced latency to head dip on the hole board and earlier emergence on the light dark test provide further evidence for reduced anxiety-related behaviours in rats from enriched cages.

Enriched LE rats had reduced locomotion on the open field test, which is in agreement with a previous study in mice comparing standard and enriched conditions using either a freely spinning or locked wheel. This study isolated reduced locomotion in the open field
test to enriched caging with the addition of a functional running wheel (Pietropaolo et al., 2006). The inclusion of enrichment toys, but without a running wheel, has also been associated with faster habituation and reduced locomotion in the open field test in SD rats when compared to socially housed controls (Varty et al., 2000).

Enrichment within SD rats was associated with a PPI deficit at the lowest pre-pulse intensity and this pattern was also observed at higher intensities and when split for pre-pulse interval. Some studies report no effect of enrichment (Varty et al., 2000), however other studies have reported reduced PPI at low pre-pulse intensities after environmental enrichment (Pena et al., 2009), and this deficit appears to be modulated by exercise in mice (Pietropaolo et al., 2006). PPI is one of the most widely used tests for validating animal models of schizophrenia due to the translatability of this behaviour across many species and the well established deficit found in patients (Swerdlow et al., 1999). It was not expected that the simple environmental changes used in this study would disrupt PPI, but the results highlight the sensitivity of this test to environmental manipulations.

The acquisition of simple operant protocols was faster in LE rats raised in enriched cages compared to those from standard housing conditions. A study investigating performance on the Morris water maze found that enriched rats outperformed those from standard housing (Harris et al., 2009). However, this result could be explained by the difference in thigmotaxis, indicating enriched rats were less anxious rather than having improved cognition. This is an important consideration, as changes in stress or anxiety will interfere with performance measures on behavioural tasks. Rats in the current study had been handled extensively prior to operant training, however greater anxiety may have contributed to the impaired learning observed in standard housed LE rats.

Environmental enrichment altered the behavioural phenotype of both strains, confirming that including a running wheel and shelter were sufficient to alter adult behaviour. This design does not result in the large changes in complexity or novelty used in other EE studies, but has increased the opportunity for voluntary exercise. While SD rats grew significantly larger than LE rats, voluntary running did not alter body weight. This study used an enrichment protocol that was readily reproducible and required minimal equipment or labour. This makes improved housing more appealing, less expensive and reduces the variable experiences that would occur in a more dynamic environment. Investigating the compounding effects of GxE manipulations has become increasingly important for understanding neuropsychiatric conditions (Caspi and Moffitt, 2006; van Os
et al., 2008). Changing the background strain or housing environment of established animal models of schizophrenia may reveal important changes in behavioural outcomes and a better understanding of how GxE interactions impact on altered phenotypes (Turner and Burne, 2013).

While the order of testing has been shown to alter some behavioural outcomes (Blokland et al., 2012), the consistency of the results across tests suggests the differences observed can be reliably assessed using a behavioural test battery. As testing on one paradigm can influence performance on later tests, the order of testing was determined based on the requirement for novelty-based responding and to reduce the effects of stress. Running a battery in a different order may change the outcomes, however a key benefit of using test batteries is that responses to a number of different challenges can be compared and the most robust phenotypic features can be determined. Three tests of cognition were used in this study with no difference in performance on the Y-maze, the RAM found overall effects of strain, and the operant tasks found the same main effect of strain but also that LE rats with enrichment acquired tasks faster. These results suggest that LE rats can outperform SD rats on various measures of cognition, but also that cognitive performance in LE was sensitive to environmental manipulations. With an increasing interest in cognitive phenotypes, strains that not only display deficits but that are also capable of enhancement should be used. The lack of strain variation used in rat models of neuropsychiatric modelling may need to be revised to answer questions about cognitive functioning and for the development of procognitive treatments.

By comparing two strains commonly used for either neuropsychiatric animal models or cognitive studies, this study has highlighted that while SD rats have a less variable PPI response, they were outperformed by LE rats on cognitive tasks. Furthermore, the effects of enrichment on cognition were only apparent in LE rats. Using a strain that acquires cognitive tasks faster, makes fewer errors and shows greater sensitivity to environmental manipulations on cognitive measures may be beneficial when cognitive phenotypes are being explored.

Future studies should investigate whether changing strain or housing conditions alters the outcomes derived from manipulations relevant to some of the well-established animal models of neuropsychiatric disorders. Given the importance of understanding the GxE interaction in disorders, such as schizophrenia, it seems imperative that further consideration is given to the restricted range of strain and housing conditions being tested.
Chapter 4. Genetic and Environmental Conditions

Altering strain and housing conditions may provide important clues to help us understand how GxE interactions ultimately lead to changes in behavioural phenotypes relevant to human disorders.

**Acknowledgments:** The authors thank Michelle Sanchez Vega for scoring the ethological behaviours.
Chapter 5  Effects of Prefrontal Cortical Lesions on SDT Performance
5.1.1 Abstract

Due to the extensive literature highlighting the importance of the PFC for executive functioning in rodents and humans, this study aimed to characterise the deficits induced by a PFC lesion. Rats were well trained on the SDT before bilateral lesioning of the medial PFC (mPFC). Performance on the standard task was not expected to differ as rats had acquired the task prior to lesioning. Therefore, two manipulations were used to vary task requirements. The first manipulation used distracting stimuli to increase attentional load and the second changed the response contingency from using visual stimuli to applying a spatial rule. As expected it was found that the mPFC lesion did not alter performance at baseline, however during auditory distraction lesioned rats made more errors on non-signal trials than sham treated animals. Accuracy on signal trials during auditory distraction was not reduced, however detecting a signal may be easier than detecting the absence of a signal. There were no mPFC lesion-related deficits detected on either the switch to the spatial rule or the switch back to the visual rule. There was a trend level reduction in accuracy towards the end of the session in the lesion group and this may indicate that these animals are affected by fatigue across the extended session to a greater extent than the sham treated group. Overall, these results indicated that performance at baseline was not dependent on mPFC input, however deficits were detected under conditions where task conditions were altered. This demonstrates the importance of manipulations that challenge rodents as they may become very proficient and respond habitually on the standard task. Additional task demands may be required to engage the neural networks used for complex cognitive functions.
5.1.2 Introduction

One of the key hurdles in translating the results from rodent studies to humans has been determining whether behavioural functions occur by comparable neural pathways in rodents and humans (Pratt et al., 2012). This comparison has been important for determining the validity of cognitive tasks, but also for linking structural and functional abnormalities relevant to neuropsychiatric disorders, such as schizophrenia. Cognitive deficits occur across a range of different functions so it is not surprising that changes in the PFC have been associated with schizophrenia both structurally and functionally (Perlstein et al., 2003; Bonilha et al., 2008; Lewis and Gonzalez-Burgos, 2008). It is generally agreed that the PFC is important for ‘higher order’ functioning (Floresco et al., 2009). Ultimately however the rodent brain is far less evolved than the human brain, particularly in cortical areas.

Whether the rodent PFC is homologous to the highly evolved primate PFC has been extensively debated (Jones, 2002; Uylings et al., 2003; Holmes and Wellman, 2009). However, the relatively simple and restricted circuitry of the rodent brain provides a more tangible system for understanding cognitive processes shared by rodents and humans. This may be particularly important for understanding essential cognition functions, such as attention, memory and behavioural flexibility, which are altered in many disorders (Chudasama and Robbins, 2006; Kesner and Churchwell, 2011). The role of the PFC in cognitive performance has been explored using a number of tasks in rodents, providing valuable information about cortical functioning (Chudasama and Robbins, 2006; Young et al., 2009a).

The rodent PFC operates as part of a much larger network including projections to/from the thalamus, striatum, hippocampus, amygdala and subcortical areas. The PFC itself consists of a number of sub-regions including the prelimbic (PrL), infralimbic (IL), anterior cingulate (ACC) and orbitofrontal cortices (OFC). Each sub-region has been associated with different cognitive functions, for example the ACC has been associated with rule learning, while the PrL region has been associated with attention and response selection, and the IL region may be more important in fear-related learning (Uylings et al., 2003). Studies often manipulate two or three subregions simultaneously when exploring PFC function and collectively the PrL and IL cortices can be described as the mPFC.
In rodents, one function proposed for the mPFC is higher-order rule learning, while the OFC may be more important for lower-order rules. This was based on evidence that switching between a spatial and visual cue on the water maze or moving from matched to non-match versions of delayed match to position task were impaired after mPFC lesions (but not OFC). Conversely, reversal learning was hampered after OFC damage, but not after mPFC lesioning. Therefore, it would be predicted that changes in task contingency that involve abstract rule learning would require the mPFC, while simpler changes to stimulus valence would rely on the OFC. This is also in agreement with the idea that the mPFC is important for paying attention to the important aspects of stimuli during learning (see review Dalley et al. (2004)). The neurobiology behind performance on an alternate attentional task, the 5CSRTT, has been extensively studied.

It has been shown that mPFC lesions do not alter performance at baseline but impair performance on manipulations such as reducing the signal duration and after the inclusion of an auditory distractor (Robbins, 2002). Using the attentional set-shifting task, it was shown that mPFC lesioning prior to testing did not alter discrimination learning, reversal learning or ability to perform an intra-dimensional shift. However, lesioned animals required more sessions to acquire the extra-dimensional shift (Birrell and Brown, 2000). These studies indicate that the mPFC is recruited when greater attention is required for successful task performance.

We have designed a modified SDT and assessed performance in two outbred rats strains (SD and LE) housed under standard and enriched housing conditions (see 4.3). Rats had previously been trained and tested under baseline conditions on this task. Given the extensive literature on the role of the PFC on cognitive functioning in rodents and humans, the aim of this experiment was to determine how performance on the SDT was altered after lesioning the mPFC. It was expected that performance would generally remain intact, but that deficits, such as reduced accuracy, would be detected on more difficult task manipulations. Given rats in this experiment were well trained, performance at baseline was not expected to require PFC functioning. However when behavioural responses needed to adapt or when suppression of irrelevant behaviours was required, then we expected the PFC to be involved. Therefore, two additional manipulations were used in this study, firstly the inclusion of distracting stimuli and then secondly the task rules were changed. In addition, data from this study was examined using both standard measures and signal detection theory indices.
5.1.3 Methods

5.1.3.1 Animals and Housing

Male Sprague-Dawley (Asmu:SD) and Long Evans (Asmu:LE) rats were obtained (Monash Animal Services, Australia) at 3 weeks of age and housed in a room maintained at 21 ± 2°C and 60% humidity and on a 12-h light/dark cycle (lights on 0600 h). Male rats were selected as sex differences were not being examined in this study and males are more commonly used in similar experiments. Standard rat chow (Specialty Feeds, WA, Australia) and water were supplied ad libitum. Rats were housed in either standard or enriched cages in same-strain pairs (n=8). Standard housing consisted of a standard sized polypropylene cage (41 x 28 x 24 cm) with a high top wire lid, aspen chip bedding (Able Scientific, WA, USA), nesting, and wood chew (Able Scientific, WA, USA). The alternative enriched housing condition used a larger sized polypropylene cage (54 x 36 x 30 cm) with a high top wire lid, bedding, nesting, wood chew, an enclosed shelter (15 x 15 x 12 cm) and running wheel (20.3 cm diameter). Rats were weighed weekly and had performed previous behavioural testing (see 4.3). All testing was conducted during the light phase. All procedures used were performed with the approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

5.1.3.2 Apparatus

Training was conducted in operant chambers housed in ventilated, sound attenuating boxes (50 x 50 x 50cm, Med Associates Inc., St. Albans, VT, USA). Rats were initially trained to collect a reward (45 mg, F0021, dustless precision pellet, Bioserv, Frenchtown, NJ, USA) from one of two receptacles equipped with head entry detectors that were located on the left and right side of the wall. The chamber was operated using MED-PC for Windows software and interfacing (Med Associates Inc., St. Albans, VT, USA).

5.1.3.3 Protocol

Key features of the SDT and protocol design have been described previously in Chapter 2 and Chapter 3 (Turner et al., 2015). Manipulations were included in this study to measure task acquisition, rule switching, reversal learning and distraction of attention. Outcome measures included % correct on signal and non-signal trials, trial rate, head entries into the magazines either before the central nose poke or during the stimulus presentation.
5.1.3.4 Signal detection theory

Signal detection theory indices were calculated for comparison with the results generated from % correct signal and non-signal. Four values were derived and calculations are as described in Chapter 2. SI provided a measure of how distinct signal and non-signal trials were in terms of detectability. RI provided a measure of whether more responses were made on the signal or non-signal side and was indicative of biased responding.

5.1.3.5 Surgery

This experiment investigated deficits that would be detected across both strain and housing conditions. There were no significant main effects or interactions detected for strain or housing condition at baseline or after distractor. Therefore throughout this study data was pooled for strain and housing conditions and split for lesion and sham treatment groups. Within each strain and housing group (n=8) rats were split into lesion (n=5) or sham (n=3) and individuals were allocated to treatment groups based on pre-surgery cognitive performance. A greater number of rats were selected for the lesion group with the expectation individuals would be excluded for incorrect lesion placement. This led to a total of 20 lesioned and 12 sham treated rats. Rats were anaesthetised in an induction chamber using 4% isoflurane in medical oxygen before being placed in a stereotaxic frame (Koft Instruments, CA, USA). The eyes were protected with ointment (Polyvisc, Alcon Laboratories, TX, USA) and body temperature was maintained using a heating pad with rectal probe feedback (Harvard Bioscience, MA, USA). The incision site was shaved and a subcutaneous injection of bupivacaine and lidocaine was made to provide analgesia. A scalp incision was made and a small hole was drilled once the injection sites were identified. Injections were made using a 33G needle attached to a 5ul Hamilton syringe at the following co-ordinates relative to Bregma: AP+0.32mm, ML+/-0.07mm, DV-0.33mm from dura (Paxinos and Watson, 2005). Rats were injected bilaterally with 0.55µL of 8mg/ml ibotenic acid (American Radiolabeled Chemicals Inc (ARC), MO, USA) for the lesion group or 1M phosphate buffered saline (PBS, Lonza, MD, USA) for the sham group into the medial prefrontal cortex (mPFC) over 4min. The needle remained in place for a further 3min before being slowly removed. Bone wax was used to fill the cranial hole and the incision was sutured before post surgery analgesic and antibiotics were administered. Rats were housed individually overnight following surgery, but were then returned to their home cage and testing recommenced after a minimum of 10 days of recovery.
5.1.3.6 Histology

After testing was completed, rats were anaesthetised with an overdose of sodium pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were then removed and post-fixed in 4% PFA overnight at 4°C. Brains were blocked in agar and sectioned using a vibratome at 100µm thickness before being mounted and stained with cresyl violet. A scorer who was blind to treatment used the Paxinos and Watson atlas (Paxinos and Watson, 2005) to determine lesion placement and extent.

5.1.3.7 Drugs

Bupivacaine hydrochloride (0.5% in saline, Sigma-Aldrich, St. Louis, MO, USA) and lidocaine (1% in 0.1M HCl, St. Louis, MO, USA) solution was made 50:50 v/v and 0.8ml/kg was given subcutaneously prior to scalp incision. 5mg/kg of Baytril (Enrofloxacin 1µl/10g, Bayer Corporation, Germany) and 2.5mg/kg of Torbugesic (Butorphanol tartrate 2.5µl/10g) was given subcutaneously for analgesia immediately after surgery and Baytril was administered again two days post surgery.

5.1.3.8 Post Surgery Cognitive Testing

Ten days after recovering from surgery, rats were food restricted and returned to testing on the SDT. Rats were to perform a minimum of three days of baseline testing and achieve >70% correct before being tested on the distractor manipulation. During the distractor session, the first and last blocks of 30 trials were conducted as per normal, however during trials 31-60 a visual distractor was presented and during trials 61-90 an auditory distractor was presented (total of 120 trials in session). Both distractors operated at 1hz frequency throughout the block with the visual distractor being the house light and the auditory distractor was a background noise generator (80dB). Rats also performed a within-session rule-switching task to assess behavioural flexibility. Instead of following the rule imposed by the visual cues, rats were now only rewarded for responding to one receptacle side. Rats rapidly learnt the spatial rule and after 120 trials the rule switched back to using the visual cues for the remaining 120 trials (total of 240 trials per session). There were no cues to indicate the rule had changed in either case.
Chapter 5. PFC Lesion and SDT

5.1.3.9 Statistical analysis

Results were analysed using SPSS software (ver. 20, SPSS Inc., Chicago, Illinois). The main effect of Lesion on key parameters was subjected to independent $t$-tests, ANOVA or repeated measures ANOVA was applied where required. If a significant interaction was detected, post-hoc analysis using independent $t$-tests were performed. Data has been presented as mean ± SEM and statistical significance was determined if $p<0.05$.

5.1.4 Results

5.1.4.1 Histology

Histological assessment resulted in the exclusion of two lesioned and three sham animals from analysis, resulting in a final sample size of n=18 lesion and n=9 sham rats. Criteria was set based on previous literature (Birrell and Brown, 2000), with inclusion in the lesion group requiring significant damage to the prelimbic cortex with additional damage in the infralimbic cortex and may include damage in the anterior cingulate cortex. Representative sham and lesion sections are presented in Figure 5.1A and B, with a schematic of the mPFC outlined based on the rat brain atlas in Figure 5.1C (Paxinos and Watson, 2005).

![Histological verification of lesion placement](image)

**Figure 5.1 Histological verification of lesion placement**

Monochromatic microscopic images of rat brain slices stained with cresyl violet to identify apoptosis and gliosis. (A) The left image is a hemisphere from a mPFC lesioned animal with the lesion outlined by arrows. (B) The right image is from a rat after sham surgery. All rats received bilateral injections. (C) Corresponding image from rat brain atlas edited to highlight the position of the mPFC, composed of the prelimbic and infralimbic cortices (Paxinos and Watson, 2005).

5.1.4.2 Performance after mPFC lesion

Overall, there was no effect of lesioning on baseline SDT performance as predicted (Figure 5.2). With the introduction of distractor, there was an overall effect of block, such
that performance was significantly reduced during the visual distractor on a number of measures (% correct Signal $F_{(3,72)}=33.15$, $p<0.001$; % correct Non-Signal $F_{(3,72)}=13.41$, $p<0.001$; p(Hit) $F_{(3,72)}=33.15$, $p<0.001$; p(FA) $F_{(3,72)}=18.14$, $p<0.001$; SI $F_{(3,72)}=42.90$, $p<0.001$) however not on RI ($F_{(3,72)}=0.96$, $p=0.42$).

The a priori prediction was that mPFC-lesioned rats would perform worse than sham-treated rats during distraction. Independent group $t$-tests found that lesion and sham groups did not differ during visual distraction on any measure. However, during auditory distraction lesioned rats made more non-signal errors and had a greater p(FA) ($t_{(25)}=-2.19$, $p=0.038$; Figure 5.3B,D). But did not differ on % correct signal trials, p(Hit), SI or RI ($p=0.054$ to 0.866; Figure 5.3). The groups did not differ in the number of head entries made prior to the central nose poke or during stimulus presentation (Figure 5.2).

![Figure 5.2](image-url)

**Figure 5.2** Rats with PFC lesions were able to perform the SDT

(A) Measures of accuracy on signal and non-signal trials as well as (B) premature responding rates (measured as head entries before central nose poke and head entries during stimulus presentation) demonstrated that lesion and sham rats had comparable performance on the SDT.
Figure 5.3 Effects of distraction after mPFC-lesioning

The session started with a normal block of trials before the introduction of visual distractor, followed by an auditory distractor and concluded with a normal block of trials. There was no effect of mPFC lesioning on (A) signal trial accuracy, (C) p(Hit), (E) sensitivity index (SI) or (F) responsivity index (RI). However, for (B) more non-signal trial errors were made and (D) p(FA) was greater in lesioned rats during the auditory distractor. *p<0.05.
A repeated measures ANOVA considering three 10-trial bins during auditory distraction found a significant Bin x Lesion interaction, demonstrating a greater effect of lesioning during the first 10-trials after stimulus onset ($F_{(2,50)}=3.54, p=0.036$; Figure 5.4).

![Non-signal accuracy during Auditory Distraction](image)

**Figure 5.4 Non-signal accuracy in 10 trial bins during auditory distraction**
The difference in non-signal accuracy between sham and lesioned rats was greatest in the first 10 trials after the onset of auditory distraction.

After the initial switch to the spatial rule and the switch after 120 trials back to the visual rule there were no changes to performance accuracy. Both groups produced smooth acquisition curves showing a steady shift in response type as they acquired the new rule (Figure 5.5). It was noted that performance in the lesioned group did not stabilised across the session at the high level of accuracy seen in the sham group with a reduction in accuracy at a number of time points in the last quarter of the session. However, this observation was not supported by a repeated measures ANOVA (main effect of Lesion $F_{(1,20)}=3.67, p=0.07$; Bin x Lesion interaction, $F_{(6,20)}=1.45, p=0.21$).
Chapter 5. PFC Lesion and SDT

Figure 5.5 Switching rules in mPFC lesioned rats

(A) Rats acquired the side rule gradually over the first 120 trials before the rule switched back to using visual cues, indicated by vertical line. Performance on (B) signal trials dropped to less than 20% accuracy in the initial trials before improving to around 70%. Accuracy then dropped to chance levels when the switch to the previously learnt visual rule was imposed. (C) As the spatial rule required all rats to only respond to the side previously paired with non-signal trials, responding remained high on non-signal trials. Furthermore, when reward was contingent on visual cues, accuracy to non-signal trials remained high. There was no significant effect of mPFC lesion on contingency switching.
5.1.5 Discussion

The purpose of this study was to determine how performance on the novel SDT was altered by removal of mPFC function. The prelimbic cortex was lesioned with damage extending into the adjoining anterior cingulate and infralimbic cortices. Overall performance remained intact, however lesioned rats made more non-signal errors during auditory distraction.

It was predicted that performance would differ after mPFC lesion on manipulations that increased the difficulty of the task. The addition of distractors was used to make signal detection more challenging. A visual distractor was also used however performance was reduced to chance levels, suggesting the distractor was overwhelming and signal detection was not possible. It is likely the signal was not perceivable in the presence of a flashing house light. The luminance level of the house light could not be easily adjusted, however further investigation of visual distractors with varying luminance may yield better results. After the onset of an auditory distractor, lesioned rats made more non-signal errors than the sham-treated group. Errors on signal trials, as well as other measures of task performance, were not different between treatment groups demonstrating other aspects of task performance were unaffected. The non-signal trials may be more difficult to identify than the signal trials during distraction, as they require greater vigilance to determine the complete absence of stimuli as opposed to detecting its presence. Although not significant, it appears as though the sham rats have their greatest % accuracy in the auditory distractor block, especially in the first 10 trials, which may be due to rebound effect following the visual distractor. The lesioned rats also ‘rebound’ after the visual distractor, however not to the extent of the sham rats, and the lesioned rats continue to improve in subsequent auditory distractor trials. This may indicate lesioned rats have a delayed ability to adapt to the changing conditions. This experiment provides further evidence for the theory that the mPFC was required for selectively paying attention to relevant stimuli. The use of manipulations that alter performance may be critical for detecting deficits that do not present at baseline.

In comparison to other studies, it has been shown that intra-PFC infusion of the 5-HT2A/C antagonist, ketanserin, reduce premature responses on the 5CSRTT, but did not alter accuracy (Passetti et al., 2003). Similarly, Maddux and Holland (2011) found that lesions to either dorsal (PrL and IL) or ventral (dorsal peduncular cortex and tenia tecta) mPFC did not alter accuracy on the 5CSRTT. In contrast, dorsal PFC lesions did reduce accuracy on
the combined attention and memory (CAM) task, which incorporates components to test attention and memory (Chudasama et al., 2005). The effect of PFC lesions may also depend on post-surgery recovery as the initial 5CSRTT deficits in accuracy detected by Muir et al. (1996) began disappearing from 10 days post-operation. Although PFC lesions may impair other measures, such as premature responding or latency values, accuracy has not been consistently impaired on the baseline 5CSRTT. Therefore, further manipulations of task difficulty may be required.

Rats were well trained on the visual signal before the rule switch manipulation was used to assess behavioural flexibility. From the start of the session only responses to the non-signal side were rewarded, however visual signals were still displayed. All rats gradually acquired the new rule and after 120 trials the rule switched back to using the visual cues. This resulted in two strategy shifts, one at the start of the session and a second halfway through the session. Overall accuracy on the spatial rule reached 80% in both sham and lesioned rats with comparable acquisition curves. The decrement and recovery of performance mid-way through the session when the contingency shifted back to visual cues was also similar in both groups. Accuracy towards the end of the session appeared lower in lesioned rats, although this was not significant and caution must be used in considering this observation. Normal sessions ran for 120 trials, however to capture two acquisition curves in this manipulation the number of trials used was increased to 240 trials. This manipulation was used because reversing the contingency (switching response sides for signal and non-signals stimuli) was found in a previous experiment to take an average of 12 days to learn (see Chapter 2), whereas this shift was learnt in a single session. The extended version of the task may increase attentional fatigue and may be worth investigating further in future studies. It was expected that the change in rules would require the recruitment of the mPFC, however spatial rules are relatively easy for rodents to learn compared to using abstract cues. Switching between a spatial rule and a well learnt visual rule might have been too simple to observe deficits after mPFC lesioning.

For switching behaviour, it has been found that using the attentional set-shifting paradigm, rodents require the mPFC, but not the OFC, for making extradimensional shifts (Birrell and Brown, 2000). However, the mPFC lesion did not influence performance on intradimensional shift. The attentional set-shifting task is based on the human Wisconsin card-sorting task (WCST) and requires rodents to select the correct pot based on either the texture, digging medium or the odour of the pot (Birrell and Brown, 2000). Within this
task an intradimensional shift is a change in target stimuli within a dimension, for example from the texture of velvet to sandpaper. An extradimensional shift requires the rodent to focus on a target from a different dimension, for example from the texture velvet to the odour of cloves (Birrell and Brown, 2000). The authors suggest that this deficit is one of selective attention and while we did not observe a deficit on the switching task, this is in agreement with the finding that attention during distraction was impaired in the lesioned group. In addition, we did not use a range of stimuli within the same dimension to form an attentional set which may limit comparison between this manipulation on the SDT and the attentional set-shifting task.

Using an ibotenic acid lesion leads to death of cell bodies, however fibres transiting through the mPFC remain intact. This type of lesion would have prevented input being received and output being sent from the mPFC. This method was preferable to an electrolytic lesion, which would have damaged not only the cell bodies but also all tissue in the region. Lesion studies are not very specific but provide information for more targeted approaches. However they also need to be considered with caution, as cognitive performance is dependent on network function and not on a single brain region. The very specific effects of the lesion in this study indicate the network can cope without the mPFC, unless the task is more difficult. This study has only compared the effects of mPFC lesions in well-trained rats, however performing the lesion prior to acquiring the task may produce quite different results.

The results of the distraction manipulation were also analysed using signal detection theory indices, however in this case they did not provide any additional information in terms of differences between lesion and sham treated rats. These calculations could not be performed on the rule switch experimental data, as rats were required to respond to a single magazine. Therefore, although they provide a unique evaluation of SDT performance, the use of % correct for signal and non-signal trials has sufficiently captured the effects of the manipulations used in the current study and may be more widely applicable.

5.1.5.1 Conclusions

Using the SDT we have shown that lesions in the mPFC impaired accuracy only when an auditory distractor was implemented. However, lesioned rats were able to acquire a spatial rule and switch back to using visual cues without performance deficits. These rats were
highly trained and the switch to a relatively easy spatial task may have been too simple to require mPFC functioning. This experiment demonstrates the importance of using manipulations that increase task difficulty to observe performance decrements that are related to mPFC dysfunction. Future studies should incorporate manipulations that challenge performance when higher order cognitive functions are being investigated.
Chapter 6  Improvement of Attention with Amphetamine in Low and High Performing Rats
6.1 Foreword

After assessing the effects of genetic, environmental and neurobiological manipulations on SDT performance of rats, I wanted to explore the sensitivity of the task to detecting the pharmacological effects of amphetamine. In this study I used a range of doses to explore the beneficial and detrimental effects of amphetamine on cognitive performance. The study was conducted in male and female rats, although there were minimal differences between the sexes. The manuscript focuses on the inverted U-shaped response to amphetamine, which was given at four doses with the prediction that performance would be enhanced at low doses and disrupted by moderate doses. The results of this study demonstrate that low dose amphetamine improves attentional performance in rats on the SDT and that the magnitude of improvement was dependent on baseline performance. These results are important because the findings reflect the pattern of responding observed in human studies and provide evidence for the predictive validity of the SDT.
6.2 Improvement of attention with amphetamine in low and high performing rats

Karly M. Turner and Thomas H. J. Burne

6.2.1 Abstract

Rationale Attentional deficits occur in a range of neuropsychiatric disorders, such as schizophrenia and attention deficit hyperactivity disorder. Psychostimulants are one of the main treatments for attentional deficits, yet there are limited reports of procognitive effects of amphetamine in preclinical studies. Therefore task development may be needed to improve predictive validity when measuring attention in rodents.

Objectives This study aimed to use a modified SDT to determine if and at what doses amphetamine could improve attention in rats.

Methods Sprague-Dawley rats were trained on the SDT prior to amphetamine challenge (0.1, 0.25, 0.75 and 1.25mg/kg). This dose range was predicted to enhance and disrupt cognition with the effect differing between individuals depending on baseline performance.

Results Acute low dose amphetamine (0.1 and 0.25mg/kg) improved accuracy, while the highest dose (1.25mg/kg) significantly disrupted performance. The effects differed for low and high performing groups across these doses. The effect of amphetamine on accuracy was found to significantly correlate with baseline performance in rats.

Conclusions This study demonstrates that improvement in attentional performance with systemic amphetamine is dependent on baseline accuracy in rats. Indicative of the inverted U-shaped relationship between dopamine and cognition, there was a baseline-dependent shift in performance with increasing doses of amphetamine. The SDT may be a useful tool for investigating individual differences in attention and response to psychostimulants in rodents.

Key words: Attention, amphetamine, cognition, rat, behaviour

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Chapter 6. Amphetamine Dose-response Curve

6.2.2 Introduction

Cognitive deficits are a common feature of a range of neuropsychiatric disorders including ADHD and schizophrenia. Antipsychotic medications have been largely ineffective in treating cognitive deficits in patients with schizophrenia. Unfortunately, preclinical models have been largely disappointing in terms of developing medications with novel drug targets and may require further development to enhance predictive validity (Geyer et al., 2012; Young and Geyer, 2015). There is general consensus that further development of tasks used to measure cognitive performance in rodents is required if we are to understand the neurobiological mechanisms of cognitive dysfunction in complex disorders (Pratt et al., 2012; Lustig et al., 2013). Unfortunately, given the lack of medications that are effective in the treatment of cognitive deficits in disorders such as schizophrenia, there are limited drugs that can be used as a benchmark to assess the predictive validity of rodent tasks (Young and Geyer, 2015). However, low dose amphetamine has been widely used in the treatment of attentional deficits in ADHD (Fredriksen et al., 2013; Safer, 2015). In addition, a number of studies have demonstrated improved cognitive performance in schizophrenia patients when amphetamine was acutely given in combination with antipsychotic medication (Barch and Carter, 2005; Pietrzak et al., 2010). Despite the successful use of amphetamine in ADHD patients, few preclinical studies have demonstrated attentional improvements in rodents (Grilly, 2000; Bizarro et al., 2004; Andrzejewski et al., 2014). A task that can reliably detect procognitive effects on attention would allow researchers to explore the paradoxical effects of psychostimulant response and also screen novel compounds for improving attention. Therefore, this study tested the predictive validity of a modified SDT for measuring drug-induced improvements in attention using amphetamine.

Amphetamine is a non-selective indirect dopamine agonist widely used in the treatment of ADHD, but has also been used in military forces and is increasingly being used by college students (Smith and Farah, 2011). The growing interest in cognitive enhancing agents has led to controversial debates about the ethical use of psychostimulants in healthy individuals (Sahakian and Morein-Zamir, 2007; Greely et al., 2008; Ilieva et al., 2013; Allen and Strand, 2015). However in healthy control groups, the evidence suggests enhancement from stimulants may be dependent on baseline performance (Mattay et al., 2000; Allman et al., 2010; del Campo et al., 2013). Therefore, investigating the response to psychostimulants as a function of individual baseline performance should be incorporated into rodent studies, as a similar relationship should be expected (Levin et al.,
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2011). There are limited studies reporting attentional improvement in rats after systemic amphetamine (Grilly et al., 1998; Grilly, 2000; Grottick and Higgins, 2002; Andrzejewski et al., 2014). There are even fewer studies reporting baseline-dependent effects of amphetamine on cognition. This issue was highlighted by a recent paper that re-analysed data from prior publications and found that stimulant response was related to baseline impulsivity measures in >50% of studies (Bickel et al., 2016). It would be useful to determine if similar results hold for the effect of stimulants on attention in rodents. Although a positive influence on attention may not be readily available for review, determining whether the extent of disruption is baseline dependent would aid our understanding of the relationship between stimulants and attention.

Based on the relationship between dopamine and cognitive performance, it would be predicted that the relationship between stimulants and attention would follow an inverse U-shaped curve. This response pattern has been supported by a number of findings in humans demonstrating the complex interaction between dopamine function, cognitive performance and psychostimulant action (Mattay et al., 2000; Mattay et al., 2003; Arnsten, 2006; Cools and D'Esposito, 2011). While low doses have been used to treat attention deficits, higher doses of amphetamine are known to induce psychotic symptoms in humans and are widely used as a psychomimetic agent in animal models (Grilly and Loveland, 2001; Featherstone et al., 2007a; Nestler and Hyman, 2010). Therefore, this study selected doses ranging from 0.1mg/kg, where effects on cognition start to be reported, and up to 1.25mg/kg where mild locomotor effects are predicted to become evident (Grilly and Loveland, 2001). Therefore, the first aim of the current study was to use amphetamine at low doses (0.1mg/kg and 0.25mg/kg) expected to enhance cognitive performance and to assess whether this improvement was dependent on baseline performance. Secondly, we investigated the effects of moderate doses of amphetamine (0.75mg/kg and 1.25mg/kg), which were expected to disrupt performance without inducing severe motor disturbances.

6.2.3 Materials and Methods

6.2.3.1 Animals

Male and female Sprague Dawley (ARC, WA, Australia; n=20/sex) were housed in a room maintained at 21±2°C and 60% humidity with a 12-h light/dark cycle (lights on 0600 h) in polypropylene cages with wire lids, aspen chip bedding (Able Scientific, WA, USA), nesting
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and wood chew (Able Scientific). Rats were kept in same-sex sibling pairs with both male and female rats being used in this study following recommendations of the NIH (Clayton and Collins, 2014). At 15 weeks of age rats were food restricted to 90% free-feeding body weight with free access to water and began operant training. All procedures were performed with the approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

6.2.3.2 Apparatus

Training was conducted in rat operant chambers housed in ventilated, sound attenuating boxes (Med Associates Inc., St. Albans, VT, USA) and behaviour was monitored using an overhead mounted camera (CCD Mini CCIR, Samsung, Suwon, South Korea). All operant chamber components were mounted on a single wall and comprised of a central house light above a custom made stimulus panel (3x3 grid of light emitting diodes, 5mm, green diffuse, 80MCD, Jaycar Electronics, NSW, Australia). Below this was a central nose poke port fitted with a light and infrared beam to detect responses. Either side of the nose poke port was a food magazine also equipped with a light and infrared beam. These were attached to pellet dispenser to provide 45mg grain pellets (F0021, dustless precision pellet, Bioserv, Frenchtown, NJ, USA) as rewards. MedState Notation was used to design the protocol and Med-PC for Windows software (Med Associates Inc., St. Albans, VT, USA) was used for chamber operation and data acquisition.

6.2.3.3 Protocol

The modified SDT protocol has been described elsewhere (Turner et al., 2016). Briefly, rats were initially trained to collect a reward with every magazine head entry rewarded until 50 pellets were delivered from each magazine or for a maximum of 20min (level 1). Once rats had attained >80 pellets on two consecutive days they were trained to nose poke a central aperture when it was illuminated to activate either the left or right receptacle, which then delivered a reward upon head entry detection (level 2). Finally, after learning to initiate trials, the signal was introduced. After initiating trials with a nose poke, a LED panel was either illuminated (signal trial) or remained off (non-signal trial) and after 1s both magazines illuminated to indicate the rat should make a choice (level 3). A response limited hold period was then incorporated in level 4. A schematic of the chamber design and flow diagram of the protocol have been presented in Figure 6.1. Depending on the visual cue presented, a head entry into one side would lead to a reward, while the other
side was incorrect and ended the trial after a brief time out (5s). The pairing of a trial type (signal or non-signal) and the correct magazine side (left or right) remained the same for each individual but was counter balanced across the cohort. An equal number of signal and non-signal trials were presented in a pseudorandom order allowing a maximum of four consecutive trials of the same type. Between trials was a variable inter-trial interval (1, 2, 3s) and the session concluded after either 100 trials or 30min.

**Figure 6.1 Signal Detection Task protocol**

(A) Operant chamber wall containing house light, stimulus panel, central nose poke and a food magazine on either side. (B) Each trial started with the central nose poke, then the stimuli was displayed and the magazines become available for responding. If the correct magazine was chosen a pellet was delivered, however if the incorrect side was selected the trial ends with a time out. The next trial was started after the central nose poke aperture illuminated.

### 6.2.3.4 Task design features

This modified SDT contains a number of differences to other rodent tasks. These have been discussed at length elsewhere (Turner et al., 2016), and therefore will only be briefly mentioned here. Omissions were reduced to <1% trials by requiring trial initiation and presenting signal or non-signal stimulus immediately upon nose poke detection. Throughout the session there are minimal delays between trials as our previous experience indicated large time gaps between actions tends to lead to distraction and alternative behaviours (including sleeping and grooming). Constraining the rat’s body position during testing is difficult without restraint, yet in human testing head position and distance to stimulus presentation can be controlled through instruction. To gain better control over the rat’s position when the stimulus was presented, we immediately presented the stimulus after the central nose poke to initiate trials was detected. Given the stimulus
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panel was located directly above the central nose poke, stimulus onset always occurred when the rat was facing the stimulus panel. The rat could move freely at all times, however typically they only moved either left or right to respond into the appropriate receptacle and collect a reward. By locating the response receptacles, central nose poke and stimulus on the same wall, in conjunction with reduced time gaps, the SDT promotes very rapid task performance. From the literature, 5CSRTT was reported to take approximately 30min for 100 trials (Bari et al., 2008) (~18s/trial) and SAT requires approximately 35min for 100 trials (Andrzejewski et al., 2014) (~21s/trial), while in this study it took an average of 13min to complete 120 trials on the SDT (~6.5s/trial). This result alone promotes higher research throughput within a limited suite of chambers. These changes have also resulted in a task with short training time, brief session duration and a range of behavioural measures for interpretation of performance characteristics (see Table 6.1).

Table 6.1 Outcome measures derived from the SDT

<table>
<thead>
<tr>
<th>Measure</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trials initiated</td>
<td>Number of trials initiated by centre nose poke</td>
</tr>
<tr>
<td>Session duration</td>
<td>Minutes taken to complete maximum (120) trials</td>
</tr>
<tr>
<td>% Omissions</td>
<td>Percentage of trials initiated but no response to receptacles within 4s</td>
</tr>
<tr>
<td>% Accuracy signal trials</td>
<td>Correct signal responses / (Correct signal + Incorrect signal responses)*100</td>
</tr>
<tr>
<td>% Accuracy non-signal trials</td>
<td>Correct non-signal responses / (Correct non-signal + Incorrect non-signal responses)*100</td>
</tr>
<tr>
<td>Latency to initiate trials</td>
<td>Time taken to initiate trial after central nose poke illuminates to indicate trial can be started</td>
</tr>
<tr>
<td>Response latency</td>
<td>Time taken from stimulus cessation and receptacle illumination to head entry detection</td>
</tr>
<tr>
<td>Premature HE during ITI</td>
<td>Number of head entries into the central nose poke prior to illumination during ITI</td>
</tr>
<tr>
<td>Premature HE during stimulus</td>
<td>Number of head entries into the receptacle during stimulus presentation window</td>
</tr>
</tbody>
</table>
6.2.3.5 Task Manipulations

Reduction in stimulus duration has often been used to increase task difficulty in rodent studies of attention (McGaughy and Sarter, 1995a; Grottick and Higgins, 2002; Riccio et al., 2002; Bari et al., 2008). By decreasing accuracy across a range of signal durations, ceiling and floor performance effects can be avoided, which is particularly important for pharmacological investigations. A selection of reduced stimulus durations (0.01, 0.03, 0.12, 0.5 or 1s) was used to determine the effects of amphetamine on attention. All drug and saline test sessions consisted of 120 trials where the start block (trials 1-30) and end block (trials 91-120) presented standard non-signal (0s) and signal (1s) trials only, allowing the rats to start and end sessions with a high rate of reward delivery. The central block (trials 31-90) consisted of 20 non-signal trials and 40 signal trials with the stimulus duration varying across 0.01, 0.03, 0.12 or 0.5s (10 trials per duration). Performance measures included accuracy on signal trials and non-signal trials, percentage omissions, number of trials initiated, latency to start trials (centre latency), response latency, premature head entries into the central aperture during the ITI and premature head entries into either receptacle during the stimulus presentation (Table 1).

6.2.3.6 Pharmacology

All rats were then treated with vehicle (saline), followed by escalating doses of amphetamine (0.1, 0.25 mg/kg, 0.75 and 1.25mg/kg). Saline was also administered on test days between each dose and it was confirmed that performance had returned to baseline prior to the next drug administration. Escalating drug schedules were used for two reasons; firstly to ensure the effect of low dose amphetamine was not disrupted by prior drug exposure because this outcome was the priority for the study, and secondly because higher doses of amphetamine can result in a sensitised response to subsequent doses (Todtenkopf and Carlezon, 2006). The saline treatment day prior to the first dose of amphetamine was used for all comparison. d-amphetamine sulfate (Sigma-Aldrich, St. Louis, MO, USA) was diluted in 0.9% saline and given i.p. 1ml/kg and 20min prior to the session. Dosage and timing was selected based on previous studies (Grilly and Loveland, 2001; Klinkenberg and Blokland, 2010).

6.2.3.7 Statistical Analysis

From the literature on human studies, it was predicted that response to amphetamine would be dependent on individual baseline accuracy (Mattay et al., 2000; Allman et al.,
Therefore, analysis of performance sub-groups was conducted by median split into either low or high performing groups (n=20/group) based on signal trial performance (average accuracy on 0.12, 0.5, 1s) on the saline treatment day. The number of high and low performers of each sex was relatively even (n=9-11 in each performance group from n=20/sex). Performance on the SDT was initially compared using repeated-measures ANOVA incorporating Dose and Duration (0.12, 0.5, 1s) as within-subjects factors and Sex (male, female) and Performance group (low, high) as between-subjects factors. Signal durations of 0.01 and 0.03s were excluded as mean accuracy was near or below chance at baseline. There was no main effect of Sex or Sex x Dose interaction, results for signal duration analyses were subsequently pooled for Sex. Given opposing effects were expected for different doses of amphetamine, separate analyses were performed for low (0, 0.1, 0.25mg/kg) and moderate (0, 0.75, 1.25mg/kg) doses. However, due to some animals not completing enough trials after 1.25mg/kg amphetamine, the analysis of moderate doses was conducted for 0.75mg/kg and 1.25mg/kg separately. After the 1.25mg/kg dose a total of 17 out of 40 animals were removed from signal duration analysis due to a significant reduction in trials completed and insufficient data for each signal duration (see Table 6.1). The minimum number of trials initiated by an individual was 23, therefore all other performance measures, aside from signal duration accuracy, were analysed for all 40 animals. To further investigate individual differences in response to low doses of amphetamine, a difference score was calculated by subtracting accuracy after saline treatment from accuracy after amphetamine administration. The correlation between baseline performance after vehicle and the difference score after amphetamine was analysed. As a negative correlation was predicted, regression to the mean adjustment was made to the baseline score using the following formula (Finke et al., 2010):

\[
\text{Adjusted baseline, } x' = \text{ initial baseline score, } x + (1 - \text{retest reliability, } r_{xx})^* \\
(\text{mean of total sample, } \mu - \text{baseline score, } x)
\]

Retest reliability was calculated by correlating performance on saline treatment days prior to and at the end of the drug-testing schedule (Pearson correlation=0.593).

Significant effects were followed by post-hoc pairwise comparison with Bonferroni correction and individually assessed by paired t-test. Huynh-Feldt sphericity correction was
applied when required. Data have been presented as mean ± S.E.M, *\( p < 0.05 \). All data were analysed using SPSS software package (ver.20, SPSS Inc. IL, USA).

### 6.2.4 Results

To ensure performance had returned to baseline levels after each amphetamine dose, post-hoc analysis was conducted to compare signal or non-signal trial accuracy across the 9 days of testing using repeated-measures ANOVA with Bonferroni correction for multiple comparisons. It was found that neither accuracy measure was significantly different between any two saline treatment days. A main effect of Day was found however this was due to differences on amphetamine treatment days, which was explored in detail below.

#### 6.2.4.1 Low doses of Amphetamine

A 3x3 repeated measures ANOVA investigating the effects of low doses of amphetamine (0, 0.1, 0.25 mg/kg) on signal trial accuracy found a significant effect of Duration \((F_{(1.6,51.2)}=42.77, p<0.001)\), Dose \((F_{(2,66)}=3.90, p=0.025)\) and Performance group \((F_{(1,33)}=25.04, p<0.001)\). There was also a significant Dose x Performance group interaction \((F_{(2,66)}=3.76, p=0.028)\). There was no significant Dose x Duration interaction \((F_{(3.3,108)}=1.14, ns)\), indicating a consistent influence of these two factors. To further investigate the effects of low dose amphetamine, accuracy was averaged across 0.12, 0.5 and 1s signal durations. There was a significant improvement in accuracy after 0.1mg/kg \((t_{(36)}=-2.45, p=0.019)\) and 0.25mg/kg \((t_{(36)}=-2.22, p=0.033)\) amphetamine across the cohort compared to saline. However, when split for Performance group the low performing group improved significantly after both 0.1mg/kg \((t_{(16)}=-3.145, p=0.006)\) and 0.25mg/kg \((t_{(16)}=-3.72, p=0.002)\), while the high performance group did not improve after administration of either dose (0.1mg/kg, \(t_{(19)}=-0.49, ns\); 0.25, \(t_{(19)}=0.03, ns\); Figure 6.2).

Difference scores were then calculated by subtracting the averaged accuracy score after saline treatment from the average score after 0.1mg/kg or 0.25mg/kg amphetamine. There was a significant negative correlation (after regression to the mean adjustment) between performance after saline and the difference score calculated for 0.1mg/kg \((r=-0.380, p=0.017, N=39)\) and for 0.25mg/kg \((r=-0.496, p=0.002, N=37)\) amphetamine (Figure 6.3).

There were no significant changes found for accuracy on non-signal trials \((F_{(1.67,80)}=0.17, ns)\), premature responses during the stimulus presentation \((F_{(1.7,59.8)}=2.45, ns)\), number of
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trials initiated ($F_{(1.41,41.0)}=0.31$, ns), centre latency time ($F_{(1.52,54.9)}=3.05$, ns), omissions ($F_{(1.41,50.95)}=2.88$, ns) or response latency ($F_{(2,72)}=1.60$, ns); demonstrating most measures of general task performance were not altered by low doses of amphetamine. Performance groups were found to differ significantly on non-signal accuracy ($F_{(1.36)}=9.48$, $p=0.004$), however they did not differ on any other measure confirming the groups did not differ on other aspects of task performance. There was also a main effect of Sex on non-signal accuracy ($F_{(1.36)}=4.41$, $p=0.043$), omissions ($F_{(1.36)}=4.73$, $p=0.036$) and centre latency ($F_{(1.36)}=8.50$, $p=0.006$), with females having reduced non-signal accuracy, making more omissions (although still <0.5%) and initiating trials at a slower rate than males. There was a significant reduction in premature head entries during the ITI from 0.1mg/kg to 0.25mg/kg amphetamine ($F_{(1.9,69.4)}=4.04$, $p=0.023$; 0.1mg/kg versus 0.25mg/kg, $p=0.008$), however neither dose differed from saline (see Table 6.2).

Figure 6.2 Signal trial accuracy after low dose amphetamine.
Signal trial accuracy improved after both 0.1mg/kg and 0.25mg/kg amphetamine in the low performing group, but not in the high performing group (n=17-20/group). *$p<0.05$. 

<table>
<thead>
<tr>
<th>% Accuracy</th>
<th>Saline</th>
<th>0.1mg/kg</th>
<th>0.25mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
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<td></td>
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<tr>
<td>70</td>
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<td>80</td>
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<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
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</tbody>
</table>

Performance group
Figure 6.3 Correlations between baseline accuracy and the effect of amphetamine
There was a significant negative correlation between accuracy at baseline and the change in signal trial accuracy after (A) 0.1mg/kg ($r = -0.4$, $p = 0.017$) and (B) 0.25mg/kg ($r = -0.5$, $p = 0.002$) amphetamine.

6.2.4.2 Moderate doses of Amphetamine
Due to reduced trial completion and therefore differences in sample size for the signal duration trials, the 0.75mg/kg and 1.25mg/kg doses were compared to saline treatment in separate repeated measures ANOVAs. The 2x3 repeated measures ANOVA for 0.75mg/kg found a significant effect of Duration ($F_{(2,62)}=39.91$, $p<0.001$), Performance group ($F_{(1,31)}=9.64$, $p=0.004$) and a Dose x Performance group interaction ($F_{(1,31)}=7.77$, $p=0.009$). A paired t-test revealed the low performing group improved significantly after 0.75mg/kg ($t_{(15)}=-2.61$, $p=0.020$), however the high performing group did not ($t_{(22)}=1.40$, ns, Figure 5). The 2x3 repeated measures ANOVA for 1.25mg/kg found a significant effect of Duration ($F_{(2,38)}=35.28$, $p<0.001$). There was no significant main effect of Performance group ($F_{(1,19)}=2.73$, ns), however there was high variation due to the reduced sample size (n=11-12/performance group from N=40). The high performing group had a significant reduction in accuracy after 1.25mg/kg ($t_{(10)}=2.39$, $p=0.038$), although the low performing group did not ($t_{(11)}=0.85$, ns, Figure 6.4). There was no interaction between Dose and Duration for both doses and therefore accuracy was averaged across the three durations.
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Figure 6.4 Signal trial accuracy after moderate doses of amphetamine
Accuracy significantly improved in the low performing group after 0.75mg/kg and decreased after 1.25mg/kg in the high performing group (n=11-18/group). *\(p<0.05\) for dose accuracy compared to saline.

Difference scores were calculated as the change in performance after drug administration and correlated against baseline accuracy after saline. Pearson’s correlation found that baseline performance (after regression to the mean adjustment) was negatively correlated with the change in performance after 0.75mg/kg (\(r=-0.543, p=0.001, N=35\); Figure 6.5A). However, the association with 1.25mg/kg amphetamine (\(r=-0.409, p=0.053, N=23\)) failed to reach significance (Figure 6.5B).

Figure 6.5 Correlations between baseline accuracy and the effect of amphetamine
There was a significant negative correlation between accuracy at baseline and the change in signal trial accuracy after (A) 0.75mg/kg (\(r=-0.5, p=0.001\)) amphetamine. (B) However the association with baseline accuracy after 1.25mg/kg amphetamine did not reach significance (\(r=-0.4, p=0.053\)).
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Performance on non-signal trials was improved by 0.75mg/kg ($F_{(1,36)}=7.6, p=0.009$) but not by 1.25mg/kg ($F_{(1,36)}=0.68, ns$). There was also a main effect of Performance group after 0.75mg/kg ($F_{(1,36)}=8.24, p=0.007$), but not after 1.25mg/kg ($F_{(1,36)}=0.24, ns$). There was also a significant Dose x Performance group interaction ($F_{(1,36)}=4.33, p=0.045$) indicating the performance groups differed after saline but were equivalent after 1.25mg/kg amphetamine in non-signal accuracy. Both 0.75mg/kg and 1.25mg/kg amphetamine significantly impaired performance across many measures including increased omissions (0.75mg/kg, $F_{(1,36)}=13.50, p=0.001$; 1.25mg/kg, $F_{(1,36)}=51.74, p<0.001$), increased centre latency (0.75mg/kg, $F_{(1,36)}=9.66, p=0.004$; 1.25mg/kg, $F_{(1,36)}=35.28, p<0.001$), decreased the number of trials initiated (0.75mg/kg, $F_{(1,36)}=9.68, p=0.004$; 1.25mg/kg, $F_{(1,36)}=48.79, p<0.001$), increased response latency (0.75mg/kg, $F_{(1,36)}=4.74, p=0.036$; 1.25mg/kg, $F_{(1,36)}=37.59, p<0.001$), decreased premature responses during the ITI (0.75mg/kg, $F_{(1,36)}=18.64, p<0.001$; 1.25mg/kg, $F_{(1,36)}=59.01, p<0.001$), and during the stimulus after 1.25mg/kg only (0.75mg/kg, $F_{(1,36)}=0.60, ns$; 1.25mg/kg, $F_{(1,36)}=29.41, p<0.001$). Overall performance was disrupted after both doses, however this was generally dose-dependent with greater impairment at 1.25mg/kg than 0.75mg/kg (Table 6.2). After 1.25mg/kg the number of trials completed was reduced and omission rates were particularly high (25%), indicating rats were starting but then not completing the trial.

Overall females made more omissions ($F_{(1,36)}=4.47, p=0.042$) and a Dose x Sex interaction ($F_{(1,36)}=4.35, p=0.044$) indicated this difference occurred after 0.75mg/kg ($t_{(26.5)}=-2.14, p=0.041$) but not after saline ($t_{(19)}=-1.44, ns$). There was no effect of Sex on omissions after 1.25mg/kg. Male rats initiated more trials than females after 1.25mg/kg ($F_{(1,36)}=5.97, p=0.020$), but there was no effect of Sex after 0.75mg/kg amphetamine. There were no other main effects of interactions with Sex after moderate doses of amphetamine. Low and high performance groups did not differ on any task measure other than signal and non-signal accuracy, confirming the groups did not differ on other aspects of task performance.

Across all doses of amphetamine (0.1-1.25mg/kg), low and high performing groups responded in a pattern that reflects the inverted U-shaped relationship between dopamine and cognitive performance. Furthermore, the optimal level of performance differed between groups, producing a rightward shift in the curve for low compared to high performers (Figure 6.6A). These results were not due to ceiling or floor effects, as both groups performed worse at short durations and better at long durations compared to the brief durations used in these analyses (Figure 6.6B).
**Figure 6.6** The shift in performance curve with amphetamine differs for low and high performing groups

(A) The low performing group improves from saline at the lower doses, however the high performing group does not improve at low doses and had reduced accuracy at the highest dose. (B) Comparison of % correct responses for low and high performers after saline, demonstrating the brief durations (average 0.12, 0.5, 1s) used for analysis in this study were free from ceiling and floor effects as performance was worse on short (0.03s) durations and better on long (1s) durations in both groups. *p<0.05 for dose accuracy compared to saline.
## Table 6.2 Behavioural measures on the SDT after amphetamine

<table>
<thead>
<tr>
<th></th>
<th>% Omission</th>
<th>No. Trials Initiated</th>
<th>Centre Latency (s)</th>
<th>Response Latency (s)</th>
<th>Premature Responses during ITI</th>
<th>Premature Responses during Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>Saline</td>
<td>0.1±0.1</td>
<td>116.9±2.2</td>
<td>2.5±0.3</td>
<td>138.8±11.1</td>
<td>81.2±6.9</td>
</tr>
<tr>
<td><strong>Low doses</strong></td>
<td>0.1</td>
<td>0.1±0.1</td>
<td>116.5±2.5</td>
<td>2.5±0.4</td>
<td>149.4±10.7</td>
<td>89.3±7.3</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.4±0.2**</td>
<td>115.3±2.3</td>
<td>3.4±0.5</td>
<td>131.6±10.8</td>
<td>80.2±7.1</td>
</tr>
<tr>
<td><strong>Moderate doses</strong></td>
<td>0.75</td>
<td>9.0±2.4**</td>
<td>106.3±4.0**</td>
<td>8.4±2.1**</td>
<td>98.0±10.1**</td>
<td>86.7±7.9</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>25.8±3.6**</td>
<td>80.6±5.5**</td>
<td>18.5±2.7**</td>
<td>58.7±7.4**</td>
<td>49.2±6.8**</td>
</tr>
</tbody>
</table>

Compared to saline treatment with paired t-test if a main effect of Dose was detected by repeated measures ANOVA, *p<0.05, **p<0.01, N=40
6.2.5 Discussion

These results demonstrate the paradoxical and baseline-dependent effects of amphetamine on attention in rats. Low dose amphetamine (0.1mg/kg and 0.25mg/kg) enhanced accuracy, whereas the highest dose of amphetamine (1.25mg/kg) disrupted performance. Furthermore, it was found that cognitive improvement after amphetamine was dependent on baseline accuracy, which has not previously been reported in the literature for rats. Importantly, these dose-dependent and baseline-dependent results were in agreement with the inverted U-shaped response to psychostimulants observed in humans.

Low dose amphetamine was predicted to enhance performance, particularly on brief signal durations where accuracy was challenged and not restricted by ceiling effects. Both 0.1mg/kg and 0.25mg/kg amphetamine selectively improved accuracy on signal trials without altering other behavioural measures such as omissions, premature responses or response latency. When split based on individual baseline performance, the low performing group significantly improved in accuracy after low dose amphetamine, but the high performing group did not. Increasing the dose to 0.75mg/kg again led to an improvement only in the low performers. However, at the highest dose of 1.25mg/kg the high performing animals had a significant reduction in accuracy while the low performing group returned to baseline levels of performance. These results are consistent with the inverse U-shaped function of dopamine and cognitive performance (Cools and D'Esposito, 2011). When comparing the effects of increasing doses of amphetamine, there was a rightward-shift in the inverted U-shaped performance curve from low to high performing groups (Figure 6.6). Furthermore, there was a significant correlation between the degree of improvement in performance and baseline accuracy. These results reflect the baseline-dependent effects of psychostimulants observed in humans on attentional tasks (Koelega, 1993; Riccio et al., 2001). Demonstrating improved performance after low dose amphetamine supports the predictive validity of this task and demonstrates that systemic amphetamine can enhance attention in adult rats (Young et al., 2009a). There are relatively few studies demonstrating enhancement of attention with systemic low dose amphetamine in rats (Grilly, 2000; Grottick and Higgins, 2002; Chudasama et al., 2005; Andrzejewski et al., 2014). Therefore, given the widespread use of psychostimulant agents in attentional disorders (Wolraich et al., 2005) and the clinical importance of predictive...
validity in animal models (Hagan and Jones, 2005; Pratt et al., 2012), these results are promising and should be further explored.

On another signal detection task, it was found that 0.25mg/kg and 0.75mg/kg, but not 0.125mg/kg amphetamine, improved the number of correct responses in SD rats (Grilly, 2000). This was also found on a similar sustained attention task where 0.25mg/kg but not 0.1mg/kg improved detectability of stimulus by improving signal trial accuracy (Andrzejewski et al., 2014). These dose-response patterns are generally in agreement with the effects observed in the current study. However, neither study compared response to amphetamine with individual baseline performance levels. Other reports of improvement after amphetamine have shown the reversal of a deficit in treated animals, but no improvement in controls. For example, on a combined attention and memory task it was found that animals with a dorsal prefrontal cortical lesion experienced improvements in accuracy after systemic amphetamine (0.2mg/kg), however no improvement was found in sham animals (Chudasama et al., 2005). Using the 5CSRTT in Lister Hooded rats it was found that 0.1mg/kg amphetamine improved accuracy and reduced omissions in 2-year old, but not 1-year old rats (Grottick and Higgins, 2002). This was replicated in a separate study of 1-year old rats, suggesting procognitive effects could only be observed in aged rats (Bizarro and Stolerman, 2003). While the remediating effects may be important where the deficit is relevant to a specific disorder or system disturbance, amphetamine is known to influence human performance in healthy as well as patient populations. Therefore, similar actions should be expected in rodent studies using normal, healthy animals. It has also been shown that direct infusion of the D1 DA receptor agonist (SKF 38393) into the medial prefrontal cortex (Granon et al., 2000) and into the nucleus accumbens (Pezze et al., 2007) of Lister Hooded rats improved performance on the 5CSRTT. While these studies are important for investigating drug targets, they may not reflect the results of systemic administration in patients. Not surprisingly, parallel dosing and administration of cognitive enhancing agents has been highlighted as a critical step in improving the translation from preclinical to clinical testing (Lustig et al., 2013). Furthermore, understanding individual differences in response to psychostimulants will also be highly relevant for understanding clinical efficacy.

Few studies have correlated changes in drug response in individual animals with baseline performance despite the use of this measurement in human studies (del Campo et al., 2013; Cherkasova et al., 2014). This was highlighted by a recent study that reanalysed a
series of studies for individual baseline dependency of drug effects (Bickel et al., 2016). The authors focussed on clinical and preclinical studies using stimulants on tasks measuring impulsivity. Overall, they showed that drug effects were dependent on individual baseline impulsivity for the majority of studies, including 72% of the studies using amphetamine. Studies using other agents have found evidence for baseline-dependent improvement after splitting animals in sub-groups (Mohler et al., 2010; Tomlinson et al., 2014). Previously, it was shown that administration of a D4 antagonist in rats could improve working memory performance in below average, but not above average individuals (Zhang et al., 2004). Furthermore, the change in performance after drug administration was correlated with individual baseline accuracy. Individual heterogeneity in drug response may also be highly informative in terms of understanding pharmacological action, particularly given the inverse U-shaped relationship between basal dopamine function and cognitive performance. Future work should now investigate individual differences in neurobiological measures, such as neurotransmitter levels or receptor density, that correlate with baseline attentional performance and psychostimulant response.

Moderate doses (0.75 and 1.25mg/kg) of amphetamine dose-dependently increased omissions, reduced the number of trials initiated, increased response latency and significantly reduced accuracy in high performing animals after 1.25mg/kg amphetamine. These measures provide a good indication of the competing effects of amphetamine on cognition and general task performance at the highest dose. In agreement with these findings, doses from 0.5-1mg/kg have been shown to mildly increase locomotion whereas doses >1mg/kg induce more substantial hyperlocomotion (Grilly and Loveland, 2001). Changes in behavioural measures other than accuracy did not differ between the low and high performance groups (Appendix II), indicating cognitive performance and changes to general task performance may occur independently. It is important to note that ceiling and floor effects were minimised in this study by using accuracy at stimulus durations that produce sub-optimal performance, but were above chance levels of responding. Therefore, both low and high performing groups could perform better on longer stimulus durations and worse on shorter stimulus durations at all doses of amphetamine.

In conclusion, this study reports the first demonstration of baseline-dependent attentional improvement in rats after systemic amphetamine, reproducing a phenomena reported in the human literature. Few studies have reported an improvement in rodents after low dose
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amphetamine, despite its widespread use in ADHD treatment. This demonstration of baseline-dependent effects of amphetamine is reflective of the pattern observed in both ADHD and healthy controls, highlighting a novel tool for investigating individual differences in attentional performance and psychostimulant response in rodent models. Future studies should further investigate the neurobiological basis of the relationship between baseline performance and psychostimulant response, and explore the cognitive enhancing properties of other known and novel drugs for the treatment of attentional deficits.

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The authors declare no conflict of interest.

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Chapter 7  Attention is Improved by Amphetamine and is associated with Striatal Dopamine Levels
7.1 Foreword

The experiment in this chapter was designed to see if the amphetamine finding observed in Chapter 6 could be replicated. My aim was to improve the experimental design with an a priori hypothesis that baseline performance would correlate with the degree of improvement after low dose amphetamine. The study used only male rats, a greater sample size and a Latin square within-animal design to increase power and reduce variability. Not only were the results replicated but I was also able to investigate neurochemical changes related to task performance. I found that across reduced signal durations, the impaired accuracy observed low performing rats was reversed by 0.1mg/kg amphetamine. Furthermore, across the cohort the level of improvement correlated with baseline performance. I was able to show for the first time that baseline performance also correlated with levels of dopamine (DA) and the dopaminergic metabolite, homovanillic acid (HVA), in the striatum, but not the PFC. Given that amphetamine is an indirect dopamine agonist and the relationship between dopamine and cognition is expected to follow an inverse U-shaped function, these results indicate a critical role for the striatum in understanding attentional deficits and how they may be reversed with psychostimulant medication.
7.2 Acute amphetamine administration improves attention in rats with low baseline performance

Karly M. Turner, James Peak and Thomas H. J. Burne

7.2.1 Abstract

Background: Psychostimulants, such as amphetamine, are widely used to treat attentional deficits. However, few studies have demonstrated procognitive effects of amphetamine in rodents. In humans, response to dopaminergic medications is complex and task dependent with improvement often dependent on baseline performance. Here, our goal was to determine if poor attention in rats could be improved following low dose of amphetamine. We then examined the relationship between baseline performance, drug response and catecholamine levels in corticostriatal tissue.

Methods: Rats performed a SDT with varying signal durations before systemic administration of saline, 0.1 or 0.25mg/kg amphetamine (N=18). Neurochemical analysis of catecholamine levels was performed on the PFC and dorsal striatum (CPU).

Results: Reducing the signal duration impaired accuracy, providing a performance window in which accuracy could improve or worsen. Following 0.1mg/kg amphetamine, accuracy in poor performing individuals increased to that seen in high performing rats. Furthermore, baseline accuracy correlated negatively with the magnitude of improvement after amphetamine across all rats. CPU homovanillic acid (HVA) levels were increased in poor performers and were also negatively correlated with performance. No changes were found in the PFC.

Conclusions: These results indicated poor performance was associated with greater response to amphetamine and altered CPU DA metabolism. In humans, response to amphetamine is hypothesised to occur via an inverse U-shaped relationship between prefrontal DA and performance. However, these results suggest the balance between cortical and striatal DA levels may be fundamental to explaining individual differences in response to psychostimulants.
Significance Statement

This study examines the effect of amphetamine on attention in rodents. It was found that drug response was dependent on baseline performance, such that poor performing rats have the greatest improvement after amphetamine. We demonstrated for the first time that levels of a dopamine metabolite were also related to an individual’s baseline performance, providing an explanation for the variable action of amphetamine. These results replicate patterns observed in humans and indicate rats may be useful for improving our understanding of individual differences in attention and response to amphetamine.

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Chapter 7. Amphetamine and Dopamine

7.2.2 Introduction

Altered DA function in cortical and striatal brain regions have been implicated in a number of neuropsychiatric disorders, such as schizophrenia, Parkinson's disease and ADHD. Cognitive deficits are a prominent feature of these disorders and drugs acting on dopaminergic pathways are often used for treatment. Therefore, it is critical to understand the complex relationship between DA activity in the brain, cognitive functioning and the effect of dopaminergic drugs.

Psychostimulants are highly effective in the treatment of attentional deficits in ADHD, but are increasingly being used for performance enhancement in healthy individuals (Sahakian and Morein-Zamir, 2007; Greely et al., 2008; Ilieva et al., 2013). Irrespective of diagnosis, psychostimulants can improve performance in individuals with low baseline performance and an individual’s response has been linked to a range of neurological changes (Mattay et al., 2000; del Campo et al., 2013). Studies have focussed on the role of cortical and striatal brain regions, which are the likely site of neurobiological deficits and medication targets (Mattay et al., 2000; Crofts et al., 2001; Cools and D'Esposito, 2011; Klanker et al., 2013). However, response to DA agonists is paradoxical with positive and negative changes in cognitive performance dependent on dose and task; as well as individual characteristics such as baseline performance, genetics and state when tested (e.g. stress, tiredness) (Mattay et al., 2003). However, despite being the mainstream treatment for attentional deficits in humans, few studies have found that amphetamine improves attention in preclinical studies (Grilly et al., 1998; Grilly, 2000; Grottick and Higgins, 2002; Andrzejewski et al., 2014). This may be due to species-specific differences in drug metabolism or because the relationship between cognitive performance and DA function is very complex.

Human studies demonstrate the importance of considering individual differences in response to pharmacological treatment (Klanker et al., 2013). For example, improvement after amphetamine has been shown to be dependent on baseline performance, such that low performers had the greatest improvement (Mattay et al., 2000; Allman et al., 2010) and this has also been shown for methylphenidate (Finke et al., 2010; del Campo et al., 2013). However, rodent studies commonly compare groups of subjects, rather than taking advantage of the variability between individuals (Dellu-Hagedorn, 2005; Dalley et al., 2007). It remains to be seen if individual differences in baseline attention are related to amphetamine-induced enhancement in rats. To enhance individual variability, it should be
advantageous to use an outbred strain, such as SD rats. Individual variability in SD rats has been used to demonstrate the relationship between working memory performance and response to L-745,870, a selective D4 antagonist in rats (Zhang et al., 2004); as well as between working memory performance and locomotor response to amphetamine (Dellu-Hagedorn, 2005). These findings support the hypothesis that individual differences in baseline cognitive functioning and response to dopaminergic agents are linked.

The aim of this study was to determine if attentional performance in rats could be improved with low dose amphetamine, and to examine the relationship between task performance, drug response and catecholamine levels in corticostrital tissue. Firstly, it was hypothesised that low dose amphetamine would improve accuracy on the SDT in low performing rats. Secondly, it was expected that the improvement in performance would correlate negatively with baseline accuracy. Finally, this study examined differences in catecholamine levels within two task-relevant brain regions; the dorsal striatum and prefrontal cortex. Our results suggest that rodents can be used to investigate amphetamine-induced improvement in attention and striatal DA metabolism is associated with differences in attentional performance.

### 7.2.3 Methods and Materials

#### 7.2.3.1 Animals and housing

Adult 12 week old male Sprague Dawley (ARC, Australia) rats (N=18) were housed in pairs in a room maintained at 21±2°C and 60% humidity and on a 12-h light/dark cycle (lights on 0600h) in cages with a high top wire lid, aspen chip bedding, nesting and wood chew (Able Scientific, WA, USA). Male rats were selected as sex differences were not being examined in this study and males are more commonly used in similar experiments. Prior to training, rats were food restricted to 90% of their free-feeding body weight with free access to water. All procedures were performed with approval from The University of Queensland Animal Ethics Committee, under the guidelines of the National Health and Medical Research Council of Australia.

#### 7.2.3.2 Apparatus

Operant rat chambers were contained in ventilated, sound attenuated boxes and all responding occurred on a single wall. The wall contained a central house light, signal display panel and nose poke port, and a food magazine on either side of the nose poke
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port. Rats were rewarded with 45mg grain pellets (Bioserv, Frenchtown, NJ, USA) delivered to the food receptacle, which was equipped with a head entry detector. Prior to testing rats were habituated to the dimly lit testing room for 30min. The chamber was operated using MED-PC for Windows software and interfacing (Med Associates Inc., St. Albans, VT, USA).

7.2.3.3 Protocol

The training and testing conditions for the SDT used in this study has previously been described in detail, including a comparison with other rodent tasks (Turner et al., 2016). Briefly, training commenced with a fixed ratio schedule where every head entry into a food receptacle was rewarded until 50 pellets were delivered from each receptacle or after 20min. After receiving >80 pellets on 2 days, rats were trained to nose poke an illuminated central aperture. After learning to nose poke to initiate trials, the SDT was implemented. In this protocol the trial was started by a nose poke after which a panel of 9 green LEDs (5mm, 80mcd, Jaycar Electronics, NSW, Australia) were either illuminated on signal trials or remained off on non-signal trials. Following a 1s delay, both receptacles illuminated and the rat needed to make a choice between left or right. Each side was paired with either signal or non-signal visual presentation such that the rat would receive a reward for the correct choice or a brief time out for the incorrect choice. Each animal experienced the same pairing throughout training, with half the group assigned to each combination. A variable inter-trial interval occurred between reward collection and nose poke initiation (1, 2, 3s) and the session ended after 100 trials or 30min.

7.2.3.4 Signal Duration

By reducing the signal duration, the task difficulty was varied from near chance performance to very high accuracy (55-95% accuracy). Using this manipulation, performance could be assessed across a performance range where both ceiling and floor effects could be avoided. The stimulus durations used were 0.06, 0.12, 0.25, 0.5 and 1s with both magazines illuminating 1s after stimulus onset. The sessions consisted of 120 trials broken into three blocks. During the first 20 trials and last 20 trials of the session the standard signal (1s) and non-signal (0s) trials were presented equally. The reduced signal durations occurred in the central block of 80 trials with 20 non-signal (0s) and 15 trials for each of the reduced signal durations (0.06, 0.12, 0.25, 0.5; total 60 trials). Rats were familiar with responding equally to both receptacle sides and using this adjusted ratio
accommodates for the increased erroneous non-signal selection at very low signal durations (unpublished pilot study). Rats were trained on the signal duration manipulation for a minimum of 15 sessions to ensure performance had plateaued.

7.2.3.5 Pharmacology

All rats were treated with d-amphetamine (0.1mg/kg and 0.25 mg/kg) or saline according to a Latin-square design balanced for performance prior to drug. d-amphetamine (Sigma-Aldrich, St. Louis, MO, USA) was diluted in 0.9% saline and given i.p. at 1ml/kg, 20min prior to operant testing. Prior to pharmacology experiments, rats were habituated to the injection procedure over 13 consecutive days to reduce stress and variability in responding during the drug schedule.

7.2.3.6 Neurochemistry

After testing was completed rats were housed without testing for a minimum of 12 weeks before being euthanised with an overdose of Lethabarb (Virbac Pty. Ltd., Australia). Microdissection of brain regions was rapidly performed and sections were immediately frozen in liquid nitrogen (Heffner et al., 1980). Criteria for obtaining brain regions was based on Paxinos and Watson (2005) with the PFC consisting of the PrL and IL regions and the CPU consisting of the region labelled CPu. Neurotransmitter analysis was conducted by high performance liquid chromatography (HPLC) to measure DA, dihydroxyphenyl acetic acid (DOPAC), HVA and noradrenaline (NA) against the internal standard deoxyepinephrine (DE). Tissue was prepared for HPLC analysis by sonication in ice cold 0.1M perchloric acid containing 50ng/ml DE before centrifuging samples at 13000rpm for 5min at 4°C. After filtering (0.22µm, 4mm), 20ul of supernatant was loaded for a 10µl injection on the HPLC. An isocratic pump, degasser and autosampler (Model 1100, Agilent Technologies, Inc., CA) were connected to a Sunfire C18 4.6mm x 100mm x 5um column (Waters Corporation, MA) maintained at 30°C and followed by a Coulochem III electrochemical detector (ESA Laboratories, Inc., MA, USA). A guard cell (Model 5020) and analytic cell (Model 5014B) were operated at -150 and +300mV (ESA Laboratories, Inc., MA, USA). The mobile phase consisted of 75mM monosodium phosphate, 1.4mM octane sulfonic acid and 1mM EDTA adjusted to pH 4.13 with phosphoric acid, before adjusting to 12% acetonitrile. Flow rate was 1ml/min with a run time of 10 minutes. Analyte concentrations were determined by calculating peak area relative to internal standard and a standard curve using ChemStation software (Agilent Technologies, Inc., CA).
7.2.3.7 Statistical Analysis and Calculations

Previously, it was found that the procognitive effects of low dose amphetamine were dependent on baseline performance and therefore drug response was compared between low and high performance groups (Turner et al, unpublished). The number of animals used was determined by a power analysis using these previous results. A median split (at 80% accuracy) from baseline performance was used to allocate rats into low (n=8) and high (n=10) performance groups. Repeated measures ANOVA was used to compare groups across signal durations at baseline and after amphetamine. Following this performance groups were compared using independent t-tests and paired t-test were used to compare measures within groups. Bonferroni adjustment for multiple comparisons was used where appropriate.

A previous study has shown a negative correlation between baseline performance and improvement after low dose amphetamine (Mattay et al., 2000). Because this finding would be supported by regression to the mean effects, baseline accuracy values were subjected to a normalisation adjustment. The following formula was used (Finke et al., 2010):

Equation 7.1 Regression to the mean adjustment:

\[
\text{Adjusted baseline, } x' = \text{initial baseline score, } x + (1 - \text{retest reliability, } r_{xx}) \times (\text{mean of total sample, } \mu - \text{baseline score, } x)
\]

Reliability was calculated by correlating accuracy after vehicle treatment within the Latin square design and after vehicle treatment at the end of the drug schedule (Pearson’s correlation, r=0.817). To assess relative improvement in accuracy, a difference score was calculated by subtracting accuracy after vehicle from accuracy after amphetamine. All data were analysed using SPSS software package (ver.20, SPSS Inc. IL, USA). Significance was set at \( p<0.05 \) and all data are presented as mean ± S.E.M, *\( p<0.05 \).

7.2.4 Results

Accuracy in response to 0.5s and 1.0s stimuli was very high (90%) and appeared to plateau due to ceiling effects, but was reduced with brief signal durations (Figure 7.1A). There was a main effect of duration after vehicle treatment (\( F_{(3,64)}=18.59, p<0.001 \)) and a
significant difference in accuracy between low and high performers across all durations as expected ($F_{(1,16)}=42.51, p<0.001$, Figure 7.1B).

Figure 7.1 Manipulating accuracy by reducing signal duration
(A) Decreasing signal duration led to a reduction in accuracy on the SDT where 50% is chance accuracy (N=18). (B) There was a significant difference between low and high performance groups (n=8-10) across all signal durations, ***$p<0.001$.

7.2.4.1 Pharmacology

A repeated measures ANOVA with 3 drug levels (vehicle, 0.1, 0.25 mg/kg), 5 durations (0.06s, 0.12s, 0.25, 0.5, 1s) and a between subjects factor of performance group found a main effect of duration ($F_{(4,64)}=51.99, p<0.001$), performance group ($F_{(1,16)}=17.21, p=0.001$) and a drug x performance group interaction ($F_{(2,32)}=7.98, p=0.002$). As there was no interaction with duration, accuracy across the five signal durations was averaged for each individual. Using the average accuracy across durations, independent t-tests revealed that the difference between performance groups was present after vehicle ($t_{(16)}=-6.52, p<0.001$), absent after 0.1mg/kg ($t_{(16)}=-1.35, p=0.195$, Figure 7.2A) but not 0.25mg/kg amphetamine ($t_{(16)}=-3.08, p=0.007$, Figure 7.2A). Paired samples t-tests found that the low performing group significantly improved after 0.1mg/kg ($t_{(7)}=-3.07, p=0.018$) but not 0.25mg/kg amphetamine ($t_{(9)}=-1.56, p=0.163$). The average accuracy of the high performing was not significantly different after either dose. When compared using a difference score (accuracy after amphetamine – accuracy after vehicle) the groups varied
in response to 0.1mg/kg amphetamine ($t_{(16)}=3.67$, $p=0.002$, Figure 7.2B) and 0.25mg/kg amphetamine ($t_{(16)}=2.16$, $p=0.046$, Figure 7.2B).

**Figure 7.2 Performance accuracy after amphetamine**

(A) The reduced accuracy observed in the low performing group after vehicle was corrected by 0.1mg/kg amphetamine (Amph), but not by 0.25mg/kg amphetamine. (B) A difference score was calculated to compare accuracy after vehicle and amphetamine, demonstrating a significant difference between low and high performing rats in response to 0.1mg/kg amphetamine and 0.25mg/kg amphetamine ($n=8-10$), $p<0.05$.

Next the relationship between baseline performance level and response to amphetamine was analysed. A significant negative correlation was observed for 0.1mg/kg ($r=-0.764$, $p<0.001$) but not 0.25mg/kg amphetamine ($r=-0.336$, $p=0.173$). Because the relationship was negative, scores were adjusted for regression to the mean effects. The adjusted baseline score was also significantly correlated with the recalculated difference score after 0.1mg/kg ($r=-0.763$, $p<0.001$, $N=18$, Figure 7.3A) but not after 0.25mg/kg amphetamine.

There were no significant differences between groups or with the administration of amphetamine with respect to non-signal accuracy, response latency, latency to initiate trials, or the number of additional head entries made during the session. At the conclusion of the drug schedule, all rats were treated with vehicle and tested again. There was a significant difference in accuracy between performance groups ($t_{(16)}=-2.71$, $p=0.015$) and a strong correlation between testing days ($r=0.817$, $p<0.001$), demonstrating stability of the performance measure and the reversible effect of acute amphetamine.
Figure 7.3 Correlations with baseline performance and response to amphetamine
After adjustment for regression to the mean effects response to amphetamine was found to negatively correlate with baseline performance after (A) 0.1mg/kg amphetamine ($r = -0.763$, $p < 0.001$) but not (B) 0.25mg/kg amphetamine ($r = -0.336$, $p = 0.173$), (N=18).

7.2.4.2 Neurochemistry
Low performers had significantly higher striatal HVA levels than high performing rats ($t_{(16)} = 3.26$, $p = 0.005$, Figure 7.4F) with a subsequent increase in the ratio of HVA to 3MT ($t_{(16)} = 2.14$, $p = 0.048$). Furthermore, a significant negative correlation was found between baseline accuracy and striatal HVA levels ($r = -0.494$, $p = 0.037$, N=18, Figure 7.5A) and a negative non-significant relationship was found for striatal DA levels ($r = -0.455$, $p = 0.058$, N=18, Figure 7.5B). There were no other differences found between performance group measures in the PFC or CPU levels of noradrenaline, dopamine or metabolites (Figure 7.4).
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Figure 7.4 Catecholamine levels
(A, C, E, G) There were no detectable differences in the PFC. (B, D, F, H). However, low performing rats had significantly more homovanillic acid (HVA) in the CPU (n=9), *p<0.05. Dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), noradrenaline (NA).
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7.2.5 Discussion

The major findings from this study were that (1) attention was improved in low performing animals using low dose amphetamine; (2) baseline performance correlated with the magnitude of improvement after amphetamine; and (3) HVA levels in the striatum were higher in low performing rats with the concentration correlating with baseline performance. Only a few studies have demonstrated the effects of amphetamine on attention are dependent on baseline performance in rats. Furthermore, we found that individual differences in baseline attentional performance were related to DA metabolism in the striatum, but not the prefrontal cortex. These findings are significant as they demonstrate that individual differences in attention and response to amphetamine, as well as the dopaminergic mechanism underpinning this relationship, can be modelled in rats.

Studies investigating attentional processing in adult rats using the 5CSRTT have mostly demonstrated accuracy is unaffected by low doses of amphetamine (<1.0mg/kg) (Cole and Robbins, 1987; Bizarro and Stolerman, 2003; Baarendse and Vanderschuren, 2012) but see (Grottick and Higgins, 2002; Bizarro et al., 2004). Using signal detection tasks, amphetamine has been shown to impair performance (McGaughy and Sarter, 1995a; Paolone et al., 2013) or improve accuracy (Grilly, 2000). Previous studies using low dose amphetamine in rodents have produced mixed results, however our findings support the

Figure 7.5 Correlations with baseline performance and neurochemistry
Baseline performance accuracy was found to negatively correlate with striatal (CPU) levels of (A) homovanillic acid (HVA) ($r = -0.49$, $p = 0.037$) and (B) dopamine were not significantly correlated but showed a trend in the same direction (DA) ($r = -0.46$, $p = 0.058$), (N=18).
procognitive effects observed on signal detection tasks at reduced stimulus durations (Andrzejewski et al., 2014). Steps were taken in both the present study and Andrzejewski et al. (2014) to improve stability of performance and reduce injection-related stress. Both studies also used decreasing signal durations that minimised ceiling and floor effects. By manipulating task difficulty, accuracy could be used to assess drug effects on challenging trials where performance could be improved or further impaired. However, a unique consideration in our study was determining how individual responses differed with respect to their baseline performance.

The investigation of individual differences is imperative given that the effects of psychostimulants have been shown to depend on baseline performance in humans. Typically, poor performing individuals have the greatest improvement after psychostimulants (Mattay et al., 2000; Finke et al., 2010; Ilieva et al., 2013). Therefore, finding the same pattern in the current study supports the validity of using animal models to understand the mechanisms of psychostimulant action on attention. However, few studies have explored whether the effects of amphetamine are related to baseline performance accuracy in rodents. Paterson et al. (2011) examined the effect of amphetamine (0.1-1.0mg/kg) on 5CSRTT performance in rats selected for sub-optimal performance (<75% accuracy) but did not find accuracy was improved. Individual differences in response to methylphenidate were noted on a working memory task, whereby performance was optimised at different doses, but not explored further (Arnsten and Dudley, 2005). One of the few reports investigating individual differences in rodents found baseline working memory performance was correlated with response to L-745,870, a selective D4 antagonist (Zhang et al., 2004). Together with our data, these previous studies indicate there is sufficient variation in SD rats to measure individual differences in cognition and pharmacological response. The need for analysis of individual difference in stimulant response was recently highlighted by Bickel et al. (2016). They reanalysed data from preclinical and clinical studies investigating correlations between individual baseline impulsivity and psychostimulant response. They found a significant relationship in the majority of studies using amphetamine (72%). Although it remains to be seen if the same effects would be observed for attentional measures, the current study provides evidence for the baseline dependence of amphetamine action on attention in rodents. Many rodent studies thus far have not consider individual differences in response to drug and in addition to using a dose with a small effect size, this may in part explain the inconsistent results for low dose amphetamine.
We next examined catecholamine levels in brain regions relevant to task performance to explore the neurobiological differences between individuals. It was predicted that DA levels in the striatum and prefrontal cortex would be altered. Although there were no changes in the PFC, striatal levels of the final DA metabolite, HVA, were greater in the low performing individuals. This finding may be due to overall greater DA production or increased turnover. Given, there was a trend towards higher, rather than lower, DA levels in the striatum of poor performing animals, we would speculate that greater DA production might be increasing HVA levels. Although this hypothesis would require further testing as there was not a significant relationship between striatal dopamine and baseline performance. This finding is in agreement with studies demonstrating that in children with ADHD, greater behavioural response to amphetamine was correlated with higher levels of CSF HVA (Castellanos et al., 1996).

Although it was predicted from the inverted U-shaped relationship between DA and cognitive performance that individuals responding positively to amphetamine should have low DA levels at baseline, this is likely to depend on many factors including brain region of interest, DA D1/D2 receptor activation and task features (Floresco, 2013; Klanker et al., 2013). For example, it has been suggested that DA may have opposing roles in the striatum and prefrontal cortex in terms of modulating behavioural flexibility and stability (Cools and D'Esposito, 2011). The balance between these functions would then depend on the task. For example, greater cognitive flexibility may improve attentional set shifting performance but lead to distraction in a sustained attention task. It has been suggested that the balance between striatal and cortical dopaminergic activity regulates these cognitive functions, such that relatively high DA levels in the prefrontal cortex promotes stability whereas relatively high DA levels in the striatum promote flexibility. In healthy people, amphetamine-induced improvement on a switching task was not only dependent on baseline performance, but also on DA release in the caudate where greater enhancement of performance was associated with greater DA release (Samanez-Larkin et al., 2013). Samanez-Larkin et al. (2013) provide evidence in humans that higher DA levels in the striatum lead to increased behavioural flexibility. Furthermore, del Campo et al. (2013) found that irrespective of ADHD diagnosis, deficits in sustained attention were related to reduced D2/D3 binding potential in the caudate. In addition Clatworthy et al. (2009) have demonstrated a relationship between DA binding potential in the striatum, cognitive performance on different tasks and response to methylphenidate. These findings
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further implicate striatal DA in baseline cognitive performance and in relation to psychostimulant-induced improvement.

Corticostriatal regions are proposed to operate in a complimentary yet competitive fashion (Crofts et al., 2001). In marmosets it has been shown that a reduction in prefrontal catecholamine levels (via 6-hydroxydopamine (6-OHDA) lesioning) leads to increased DA release in the striatum (Roberts et al., 1994). Furthermore, it was shown that depleting DA in the frontal cortex impaired visual discrimination acquisition and increased distraction, whereas DA depletion in the striatum resulted in less distraction compared to controls (Crofts et al., 2001). These results support the hypothesis that DA in the frontal cortex drives cognitive stability and striatal DA increases flexibility/distractibility. These results can be extended to explain the outcomes of the current study where low performance was associated with higher DA levels in the striatum, which would promote flexibility at the expense of greater distractibility. In rats it was shown that low dose psychostimulants preferentially act in the PFC and that working memory was improved by the local administration of methylphenidate in the PFC but not the dorsal striatum despite both regions being essential for task performance (Berridge et al., 2006; Cools and D'Esposito, 2011; Spencer et al., 2012; Schmeichel and Berridge, 2013). Therefore, it could be expected that in this study low doses of amphetamine would act to a greater extent, although not exclusively, within the PFC to modulate performance. Therefore, we speculate that low performance may be remedied by amphetamine via increases in cortical DA level. This may shift the balance between striatal and cortical DA such that cognitive stability was restored and the rat performed better on a task requiring attention (Cools and D'Esposito, 2011). It should be noted that there were no associations between PFC DA levels and task performance. In addition, catecholamine levels were measured post mortem and the dynamic changes that occur with amphetamine administration and during task performance were not directly measured in this study. This hypothesis could be tested in future experiments using an in vivo recording technique, such as microdialysis, during drug and task administration.

The results of this study could be extended by also comparing performance on a task requiring behavioural flexibility. Low dose amphetamine has been shown to impair reversal learning in rats (Idris et al., 2005), and it would be powerful to show opposing task-related effects within the same individuals. In addition, future studies could infuse specific dopamine-altering drugs into the PFC or CPU to further test the hypotheses generated.
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from this study. This study only included male SD rats and it would be valuable to ascertain whether the same pattern of drug response holds true in female subjects (given the potential role of oestrogen in modulating dopamine functions) and other rat strains. When differences in drug response are observable in individuals from the same cohort, it would be expected that the use of different strains, ages or genders would also lead to some variation in drug response patterns.

Human studies have indicated an effect of amphetamine on reaction time, however in this experiment rats were not encouraged to respond as fast as possible. There was a 1s delay from stimulus onset until a response could be made to prevent ‘guessing’ behaviour in rats. Although reaction time can be an important measure in human tasks in terms of speed/accuracy trade off costs this task was optimised to train rats for accurate responding rather than speed of responding. There were no motoric effects seen at these low doses of amphetamine. These results are testament to the stability of performance generated prior to drug administration and the absence of non-cognitive side effects that are associated with higher doses of amphetamine.

7.2.6 Conclusions

Cognitive deficits related to dysfunction of corticostriatal catecholamine activity have been found in a variety of neuropsychiatric disorders including schizophrenia, ADHD, OCD and Parkinson’s disease. However, studies exploring the role of DA in cognitive functioning have produced paradoxical findings with both increasing and decreasing levels of DA improving or impairing performance on different tasks. Here we demonstrate on the SDT that poor attentional performance can be improved by low dose amphetamine in rats. Furthermore, the degree of amphetamine-induced improvement was associated with baseline performance levels across the cohort, which has not previously been reported in rodents. Finally, we were able to extend these findings to show that individual differences in baseline performance correlate with DA metabolism in the striatum, but not the PFC. From these results we propose that high baseline levels of striatal DA production may be counteracted by low dose amphetamine treatment, possibly via increased cortical DA levels. Low dose psychostimulants have been shown to act preferentially in cortical regions and an increase in cortical DA relative to striatal DA would promote stability of task representation and reduce distractibility. This study provides new evidence to suggest that the balance between cortical and striatal DA levels is central to understanding individual differences in attention and response to psychostimulant drugs. Future studies should
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utilise animal models to further understand how corticostriatal functions differ in individuals during task performance, after administration of psychostimulants and characterise the dynamic interaction between drug and task-related activation.

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Chapter 8  General Discussion
8.1 Introduction

Cognitive deficits are a core, but non-diagnostic feature of schizophrenia (Keefe and Fenton, 2007). The severity of cognitive symptoms is independent of psychotic symptoms; they are common among patients and occur in some first-degree relatives without a schizophrenia diagnosis (Green et al., 2004b; Keefe and Fenton, 2007). Treatment for cognitive deficits has largely been ineffective despite a surge in research and these symptoms being linked to functional outcomes in patients (Green et al., 2000; Hyman and Fenton, 2003). Furthermore, newer second-generation antipsychotics are no more efficacious than older first-generation treatments in the management of neurocognitive deficits in schizophrenia (Keefe et al., 2007). Around 10 years ago the MATRICS consortia established recommendations based on prior studies for researchers and the pharmaceutical industry to improve treatment research and drug development (Green and Nuechterlein, 2004; Marder and Fenton, 2004). Seven separate cognitive domains were identified as being disrupted in schizophrenia, however not all can be tested in preclinical animal studies. Those determined to be appropriate for animal studies were working memory, speed of processing and attention/vigilance (Nuechterlein et al., 2005). For the domain of attention/vigilance, CPT-like tasks were suggested as providing a translatable platform for preclinical testing (Nuechterlein et al., 2005). From this panel it was concluded that:

“A high priority was recommended for research to identify parallel animal and human cognitive paradigms that would allow the prediction of human pharmacological response from animal pharmacological response within the same domain.” page 873, (Nuechterlein et al., 2005).

To achieve this goal, preclinical paradigms need to be further developed to improve the translatability of findings (Young et al., 2009a; Hyman, 2014). Therefore, the aim of this thesis was to design and construct a novel task for measuring attention in rodent models. To improve translatability, features of the human CPT were incorporated. The overarching goal was to develop a task with face, construct and predictive validity for detecting attentional deficits in rodent models, particularly for detecting procognitive effects of drugs.

There are significant differences between rodent and human testing, however not all of these differences can be easily addressed. Rodents require training, whereas humans can be provided instructions. The provision of rewards and motivation to perform a task may
differ substantially between species. There are also significant differences in the visual abilities of humans and rodents. Although these factors (and many others) are important to consider, the primary goal was to measure attention. Paying attention in human studies typically requires a subject to sit in front of a screen to monitor and respond to stimuli. However, in a rodent operant chamber, the rat can freely move around and perform a variety of behaviours during stimulus presentation and fixation on the stimulus is lost.

My primary goal was to develop a task that measured attention in rats with parallels to human CPT testing. The task was designed based on elements of tasks already used by the field, particularly other signal detection tasks. However some key changes were made to improve translatability. The SDT was designed with standard commercially available operant chambers with all equipment located on a single wall. Previously rodents had been observed to engage in alternative behaviours such as grooming, exploring the chamber and even sleeping during operant testing. It was decided that rats should be freely moving within the chambers to reduce stress and allow measurement of response times without restriction on mobility. Thus to encourage engagement in the task, all equipment used in the SDT was mounted on a single wall. This was done to reduce ambulation and support the rat staying within the vicinity of the stimulus and response receptacles.

A custom-made stimulus panel was used to present stimuli. However after using many stimuli variations it was found that detection, rather than discrimination, could be achieved more consistently and acquired more rapidly by SD rats. In addition the reward delivery was also located on the same chamber wall to reduce the likelihood of the rodent turning away from the stimulus panel. This also promoted rapid trial pace, as minimal movement was required. This is in contrast to the traditional arrangement of the 5CSRTT and dSAT where the reward is delivered on the opposite wall to the stimulus and leads to lapping behaviour within the chamber (McGaughy and Sarter, 1995b; Arnold et al., 2003; Bari et al., 2008). Separating the response and reward locations can serve a purpose, for example to allow measurement of reward latency, however it appeared to interfere with continuous task performance, which was deemed a higher priority in this study. Thirdly, the use of the central nose poke was critical to task design and sets the SDT apart from other rodent tasks. The self-initiation of trials ensures the rat is motivated to complete the trial and aids the reduction of omissions. This is critical because omissions may be due to a number of reasons in rodents, such as grooming or exploring the chamber. Omissions
typically increase with drug administration and it can be difficult to determine whether the impairment is due poor motoric or cognitive functioning. However, the issue I wanted to address is the lack of attention/vigilance during omissions. If the rat is performing an alternative behaviour, it is not likely to be focussed on the task. Reduced omission rate in combination with a fast trial rate, provides an indication of continuous task engagement by rats on the SDT.

The self-initiating nose poke location was directly beneath the stimulus panel to ensure the rat is located directly in front of the stimulus during the presentation window. Without head fixing or physically restraining the rat, this feature of the SDT can control the body position of the rat at stimulus onset, which has not previously been used in rodent studies using 5CSRTT or dSAT. This was particularly important given the stimulus was visual and body position would alter the detectability and accuracy of decision-making. In comparison to the human CPT where chin rests, eye tracking and fixation points may be used to reduce variation in responding due to shifting position, this element was used to improve the translational validity of the SDT. Finally, the timing of events was reduced to promote rapid trial pace and reduce alternative behaviours, such as grooming and exploring. This also contributed to reducing daily testing duration and may have supported swift task acquisition.

When visually monitoring the rodent performance, it was observed that the rat was positioned in front of the stimulus when it was presented and rarely moved away from the active chamber wall. By minimising the need for ambulation across the chamber, as occurs in the 5CSRTT and dSAT, the rat can pay attention to the stimulus panel and rapidly perform trials in a continuous manner comparable to human CPT performance. This rapid responding appears to be much more similar to human computer-based testing. Rats would continue to respond until all trials were completed, often without breaking. This led to a self-directed inter-stimulus interval of around 6.4s (compared to 12.7s on 5CSRTT), where human CPT’s are fixed and between 0.5s-2s. This demonstrates that rats are capable of, and willing to, perform in a fast and continuous manner. The response and reward have been spatially combined, which prevents the recording of reward latency. However, human CPT subjects would be likely to experience ‘reward’ when they make their selection and as they are not normally required to physically collect a reward, there is no measure of reward latency. Overall these changes have resulted in a task that can be rapidly acquired, with virtually no omissions, a fast trial pace and high level of accuracy.
These changes may seem incremental, however the overall effect is rats performing trials very rapidly (hence the short session duration), with negligible numbers of omissions (<0.5%) and rats appear to be paying attention in a more continuous manner. Ultimately, the purpose of these changes is to improve the validity of the task for measuring attention in rodents. It was predicted that by making the task more similar to the way testing is conducted in humans, that the results from rodent studies should be more translatable. To test this idea, I performed a series of experiments using different manipulations that were known to alter attentional performance. Here they will be discussed in terms of how these results address the issues of task validity.

8.2 Validation of the SDT

Validation is frequently considered across three separate areas; face, construct and predictive validity (Young et al., 2009a; Young and Geyer, 2015). **Face validity** is determined by the degree to which the condition appears similar between rodents and humans. In terms of cognitive task development, this has been used to describe common features of task performance between species and is useful to consider during initial task development. **Construct validity** is arguably the most important as it determines the specificity of the task to measure the cognitive process it was proposed to measure. **Predictive validity** refers to the ability of the task to provide an outcome that would be predicted by prior knowledge or results. In this context, we would expect the same result in rodents and humans. Perhaps most critically, predictive validity determines the ability of a pharmacological agent to affect task performance thus potentially identifying medications that will be useful for treating cognitive deficits. Experiments addressing these forms of validation are discussed below.

**Face Validity: Does the SDT appear to measure attention in a similar way to the human CPT?**

Face validity was carefully considered throughout task design and development. There are already a number of rodent tasks that were designed to replicate features of the human CPT, including the 5CSRTT, 5C-CPT and SAT (McGaughy and Sarter, 1995b; Bari et al., 2008; Young et al., 2009b). There are also many variants of the human CPT; therefore features common to many variants were used for rodent task development. I decided to use a signal detection task as it has many parallel features in comparison with the human CPT. In the human CPT, a single stream of stimuli are presented and the subject must
remember a rule about the type of stimuli to respond appropriately. On a rodent signal
detection task, trials of present or absent stimuli are presented and the rat must also
remember a rule about how to respond correctly. However, in previous versions of the
rodent task, the rat must move from the stimulus to the opposing wall to collect a reward
after each correct trial. This diverts the rat’s attention away from the stimulus wall and can
result in distraction as observed by initiation of alternative behaviours. As a consequence,
the scoring of omissions in rodents is not the same as the interpretation of an omission in
human testing. Omissions in humans are likely to occur due to a lapse in concentration,
however in rodents it may occur because they were performing another action, such as
grooming or exploring the chamber. At face level changes such as controlling body
position and increasing trial rate, have resulted in a task where the rodent attends to
stimuli and responds rapidly with few breaks. There is limited ambulation required and
rodents rarely make omissions. Although these protocol changes may appear minor, they
have a significant overall effect on the rat’s behaviour, as evidenced by the results in
Chapter 3. Therefore, it was concluded that the SDT has improved face validity for
measuring attention in a similar manner to the human CPT.

At this point it is important to note that there was no inhibitory component incorporated in
the rodent SDT, despite inclusion in many versions of the human CPT. Inhibitory control is
an important component of cognitive deficits in many disorders and has been extensively
studied. Within this study, faster responding was prioritised over a measure of inhibition.
There are other rodent tasks that excel in measuring impulsive behaviours (including the
5CSRTT, stop signal reaction time task and delay discounting). Although impulsivity is
linked to attention, it is not strictly used as a measure of attention and therefore was not
incorporated into the SDT. It could be included, for example by delaying responding and
punishing premature head entries into the magazines; however this would significantly
slow trial rate and may increase task disengagement and omissions. In addition, response
times are not as informative in the rodent SDT as they are in the human CPT. When rats
were allowed to respond immediately with stimulus presentation, they were found to
impulsively guess. With a 50% chance of reward and the fast trial rate, many did not learn
to associate the stimuli and response side. By imposing a 1 second delay on responding,
they slow their responses and learn the contingency. Although they are not responding as
fast as possible, assessing the rat’s ability to make the correct decision was deemed more
important. In human testing, this response may be more comparable performance where
the instruction is to maintain accuracy rather than speed.
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Construct Validity: Does the SDT measure attention?

Determining a method for measuring attention and confirming the task is measuring attention is not as simple as it may seem. Generally, both the human CPT and rodent equivalent tasks are said to measure ‘sustained attention’, which may be observed by a reduction in accuracy over time. However, satiation levels also influence motivation when using food rewards in rodents and lead to a reduction in accuracy over time. The task also relies on both perceptual and attentional capacity of the rat. The concepts are tightly interconnected and it is experimentally difficult to measure either ability without influence of the other. Therefore, experiments will aim to test stimuli within the perceptual limits of the rat, but use manipulations that challenge attentional demands. Rather than aiming to measure a specific type of attention, the goal was to measure the same form of attention as the human CPT. Hence, the assessment of construct validity relied on manipulations with a known effect on attentional performance in humans and rodents. For example, performance was impaired by reducing the signal duration or by including distracting stimuli. Both these manipulations have been used in rodent and human studies (Riccio et al., 2002; Bari et al., 2008; Demeter et al., 2013). In addition, performance on the SDT was directly compared to performance on the 5CSRTT (Chapter 3), one of the most well validated tasks for measuring attention in rodents (Robbins, 2002). Furthermore, the importance of the PFC in maintaining attention during distraction was demonstrated (Chapter 5). Together these findings indicate that the construct of attention can be measured and manipulated using the SDT.

Predictive Validity: Does performance on the SDT reflect the outcomes demonstrated in humans on measures of attention?

This question was addressed by investigating the influence of genetics and environmental factors and through pharmacology experiments. Firstly, it is known that genetic and environmental factors influence a range of cognitive outcomes, as well as being involved in the development of numerous neuropsychiatric disorders. While the complex interplay of genetic and environmental factors experienced by humans is not easily replicated in rodents, it is well known that different strains and housing conditions alter behavioural phenotypes. Therefore, it was predicted that the SDT would detect differences between strain and housing conditions (Chapter 4). This study found that genetic and environmental factors have an influence on cognitive performance that can be measured with the SDT.
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A key feature of predictive validity for tasks used in preclinical animal models is the ability to predict drug response in humans. However, there is a lack of benchmark drugs to determine task sensitivity because there are no effective medications for the treatment of cognitive deficits in schizophrenia. To determine if the SDT could detect procognitive drug effects, I used low dose amphetamine as it is widely used in the treatment of attentional deficits in ADHD (Chapter 6). This study found that low-dose amphetamine significantly improved performance in rats, indicating the potential of the SDT for detecting procognitive effects. Furthermore, efficacy was baseline-dependent as has been found in human studies. These outcomes were replicated in a second study where the findings were extended to reveal a relationship between striatal DA metabolism and baseline performance (Chapter 7). These results are highly relevant to the inverted U-shaped relationship between cognition and DA that has been hypothesised from human and non-human primate studies. Overall, these results support the predictive validity of the SDT, indicating this task may be sensitive to detecting the procognitive effects of medications.

Task development

From a practical perspective there were also a number of goals set in terms of improving feasibility for researchers using operant testing paradigms in rodent models. The time required for training animals repeatedly for weeks or months makes these studies expensive and unfeasible for many researchers. Therefore, I wanted to develop a task that could be acquired relatively quickly and required minimal time per session to encourage higher throughput. This goal was achieved and is demonstrated by the rapid training and testing times presented in Chapter 3 when the SDT was compared to a traditional task for measuring attention, the 5CSRTT. In comparison to another rodent signal detection task, the SAT, the SDT also required considerably less training time. The SAT is reported to require 4-8 months when training 5-6 days per week, equating to around 80-200 sessions (Arnold et al., 2003). This is substantially longer than the 12-27 sessions required for training rats on the SDT in Chapter 3, although stability requirements were not matched. In addition, session duration on the SDT is under 15min, while on 5CSRTT it was >20min and is reported as >30min for SAT. With a limited number of chambers, more sessions and therefore more rats can be tested in a day using the SDT. This may be an important constraint to studies incorporating multiple groups where sufficient numbers are needed to attain statistical power. In addition, the use of such tasks in preclinical drug studies has been limited due to the extensive investment required. In practice, the changes
implemented in the SDT make it more accessible to researchers constrained by limited
time, funding or access to operant chamber facilities. Ultimately the quality of the
experimental design and selection of the most appropriate task are paramount, however to
improve uptake and make this type of research more feasible, consideration must be given
to the investment of time and money required.

8.3 Implications of findings

Firstly, the results of this thesis demonstrate that even subtle changes to protocol design
can have a significant impact on cognitive performance outcomes and an animal’s ability
to acquire a task. Secondly, genetic and environmental factors are important in
determining a rodent’s behavioural phenotype and cognitive performance. This study
highlights the importance of describing home cage details in rodent studies as minor
differences, such as the inclusion of shelter, may influence behavioural traits. Thirdly,
repeated operant testing commonly leads to habitual responding, which may be related
more strongly to striatal functioning and is often desirable when stable performance is
needed (such as within-animal drug studies). However, manipulations may be needed to
reinvigorate the activity of passive cognitive circuitry and increase task demands. Finally,
the importance of considering individual differences was addressed. It is likely that
individual characteristics are an important determinant of cognitive performance and drug
response. When examining drug effects, using a cohort mean value may wash out effects
due to non-responders or potentially opposing effects in different individuals. By
considering baseline performance when analysing performance after drug administration,
a more accurate picture of drug efficacy may be evident. In addition, these studies may
reveal important neurobiological alterations between individuals relating to their cognitive
performance and drug response. Studies of this nature parsing responders from non-
responders may prove useful given the non-responsive nature of many patients to certain
medications. Alternative medications may be better suited to certain sub-groups of
individuals based on performance characteristics, however preclinical studies are typically
designed to measure group effects rather than stratifying cohorts. This may be a fruitful
avenue for future research, not just for psychostimulant treatment of attention deficits, but
across many cognitive domains and drug classes.

In addition, this thesis contributes to the field in a number of ways. Having established the
validity of the SDT, this task could be used by researchers to investigate attentional
deficits in a range of animal models relevant to neuropsychiatric disorders. The results presented in Chapter 6 and Chapter 7 demonstrates the SDT is well suited to measuring procognitive effects of the psychostimulant, amphetamine. Therefore, the SDT is a prime candidate for screening other drugs with similar action, such as methylphenidate or atomoxetine, and potentially for discovering novel agents. Much of the literature aiming to understand the neurobiological mechanisms of psychostimulant effects on attention have relied on imaging studies in humans. Using the SDT, a more comprehensive assessment of molecular factors related to attention can now be made in rodents. This may lead to new insights into the physical manifestation of attentional deficits and how different risk factors are responsible for the development of attentional disorders. When compared to other available tasks, there are similarities and contrasts in protocol design, however few studies have demonstrated improved in attention in rats after amphetamine. Predictive validity is potentially the most valuable asset for a preclinical task and this finding suggests the SDT may provide a novel read-out for screening procognitive compounds.

These results also provide evidence for the use of rodent models to investigate cognitive deficits relevant to neuropsychiatric disorders. Rodent models are beneficial as they allow the experimenter to control for many extraneous factors. Also the potential to use more invasive techniques will allow questions to be addressed that cannot easily be undertaken in human studies. Humans and rodents can be compared across many behavioural and cognitive domains; however it is their shared neuroanatomical function and response to pharmaceuticals that further validates the use of rodents for investigating brain disorders (Young et al., 2009a). Rodent models allow invasive manipulations of different brain regions, genetic manipulations and probing circuitry functions. Although some questions will be limited when comparing rodent and human cognition, this model provides a ‘simpler’ brain for asking questions about fundamental shared functions, such as attention.

Other benefits of using rodent models include the cost of housing, ease of breeding and continuous technical advances that provide new tools for probing brain functions (such as optogenetic approaches, genetic sequencing and advances in imaging). To improve treatment outcomes for patients we need successful clinical trials, which in many cases are preceded by preclinical animal studies. Therefore, we need preclinical tasks to indicate which treatment targets are potentially useful and to reject those that will be ineffective. The SDT was designed to provide a new tool for this purpose. Having shown that the SDT can detect the procognitive effects of a drug that improves attention in humans, it is hoped
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this task can be used to screen new compounds. As attentional deficits occur in a number of neuropsychiatric disorders, the discovery of novel drug targets may lead to new treatments for disorders such as schizophrenia, ADHD and dementia.

The momentum for this project originated in the need for better treatments for cognitive deficits in schizophrenia. The main purpose was to develop a tool that could be used to screen novel compounds and investigate animal models relevant to schizophrenia. This thesis covers the first steps in this process by selecting a task that is sensitive to cognitive deficits in schizophrenia and developing a similar paradigm in rats. This was followed by a series of experiments to characterise the effect of core manipulations, such as genetic, environmental and pharmacological interventions. Although the primary purpose of this task was for the assessment of cognitive deficits relevant to schizophrenia, the SDT may be used to measure attentional processing in a number of disorders, just like the human CPT.

8.4 Limitations

In considering the richness of cognitive functioning in both rodents and humans it is clear that no single task can be used to measure ‘cognitive deficits’. In fact, a single task cannot fully evaluate the scope of a single domain such as ‘attention’. Each task is better considered as a single tool among a large suite of tasks, where researchers can select the most appropriate tool for the question at hand. The use of several tasks will be especially necessary to unpick and elucidate the nature of mild cognitive deficits. For this reason, the use of a battery of tasks is often suggested. The changes made to increase throughput on the SDT will aid the use of this task in such batteries. However, the investment required for a thorough examination of cognitive function should be far more beneficial in the long-term than the use of simple, stand-alone tasks that may not accurately measure cognitive performance.

Compared to other tasks that measure attention, the SDT presents stimuli in a predictable location and with a certain degree of temporal certainty. This has allowed a fast presentation rate, but limits the requirement for attention to be maintained over spatial locations or durations when the stimulus may appear unpredictably. This has been a trade-off in moving the task from humans to rodents. If the stimulus is spatially/temporally unpredictable, the trial rate is slower and the animal may not be engaged in the task consistently across trials. This is not a major problem in humans as they can follow
instructions to stay on task. Alternatively, if the stimuli are presented with spatially/temporally predictability, the rat can perform with an increased trial rate and will stay engaged in the task for longer. Uncertainty is used to drive the ‘sustained’ component of sustained attention. However, in my experience, rodents take breaks in these conditions. Allowing the rat to set the task pace results in faster trial pace and more consistency in task engagement. Therefore, their behaviour appears more similar to the sort of responding observed in humans on the CPT. In addition, a detection paradigm was used rather than requiring discrimination between visual stimuli or requiring working memory. However, these adaptations could be made to the SDT if required. For example, a delay could be imposed between stimulus presentation and the ability to make a response to measure short-term memory.

There are still significant inherent differences in the assessment of attention in rodents in comparison to human tasks. In contrast to human CPT testing, rodents cannot be instructed on how to perform a task. In addition, they have relatively poor visual acuity compared to the more advanced visual system in humans. And although human subjects may be motivated to perform a task for a variety of reasons (course credit, money, psychological assessment), rodents must be rewarded throughout the session and are typically further motivated by food restriction. In addition, the visual system is poorly developed in rodents compared to humans. However, processing of visual information by the primary visual cortex may be relatively similar between species. Whether the simple stimuli used in the SDT requires processing in the primary visual cortex was not directly tested. These differences cannot easily be modified (if at all), but must be considered when comparing rodent and human testing. Rats and mice also differ somewhat in their preferred testing conditions. Nose poking was selected as the response action for the SDT as both mice and rats are more willing and learn to nose poke faster than lever press. In a study separate from this thesis, we have used the SDT in two strains of mice with very few changes. In addition, we have begun piloting a reverse translational SDT in humans. This will allow the translational testing of the SDT across mice, rats and humans.

It should be noted that performance on the SDT could be measured using signal detection theory indices. There are four response options: hit (correct signal trial), correct rejection (correct non-signal trial), false alarm (incorrect signal trial) and miss (incorrect non-signal trial). Therefore, the probability of a hit or false alarm can be derived, along with additional measures of detection sensitivity (d’) and response bias (β). Some of the results from this
thesis were analysed using this method, however the interpretations and conclusions were the same as when using % correct for signal and non-signal trials. Results have generally been presented using % correct as these results are suitable for the main objective of each chapter and provide consistency throughout the thesis. However in future studies signal detection theory outcomes may be more informative where either a response bias or sensitivity differences are expected.

When comparisons were made between 5CSRTT and the SDT, there were a number of factors that could have altered task acquisition and performance. These include the number of training steps required, trial rate, luminance of the light stimuli, the predictability of the stimulus location/timing and the number of response options. The purpose of the comparison in this thesis was to assess differences in the standard versions of each task. However, it would be interesting to test each of these components separately to determine which has the greatest impact on the measures that differed between tasks.

The majority of studies were performed in male SD rats, although females and LE rats were included in some studies. The majority of measures were the same for female and male rats, as observed in Chapter 6. Subtle sex differences were only observed after amphetamine administration and this may be due to differences in drug metabolism kinetics. In some models, females may be of greater interest than males and my results indicate the SDT would be a suitable task for measuring attention in both male and female rats. Unfortunately, the source of LE rats was closed during my PhD candidature, preventing selection of this strain for subsequent studies. This strain is commonly used in behavioural studies and it would be interesting to see what the effects of amphetamine are on attention in LE or other rat strains. This thesis demonstrates some key findings using the SDT covering a range of research topics. From these studies, there are a number of future directions that could be explored.
8.5 Future directions

- **Cross-species translation:** The translation of this task in mice and humans would be useful to directly address the translational validity of the SDT. In collaboration with other researchers, such studies have commenced and initial results in both humans and mice has been promising. Future comparative research could investigate similarities and differences in performance between species.

- **Animal models of neuropsychiatric disorders:** In addition, the SDT can now be applied to other animal models of schizophrenia or attentional deficits. For example models in which genes (such as COMT or DISC1) are known to produce cognitive deficits. Additionally adverse environmental exposures (chronic social stress model) could be examined. It would be particularly interesting to assess animal models that have previously been tested using the 5CSRTT or SAT to compare attentional deficits across these tasks. It is expected that like the human CPT, deficits on the SDT would not be disease specific but the task should be sensitive to detect deficits in a range of animal models with cognitive dysfunction. Therefore, animal models from a wide range of neuropsychiatric disorders, such as dementia, depression, traumatic brain injury or ADHD, could be examined using the SDT.

- **Pharmacology studies:** When considering the importance of developing better therapeutics, the SDT may be most useful for improving our understanding of the procognitive actions of psychostimulants on attention. In schizophrenia patients, procognitive treatments would be provided chronically and in combination with antipsychotic medications. Therefore, it would be beneficial to also investigate the chronic administration of low dose amphetamine, and the effects of co-treatment with antipsychotic drugs. Other drugs used to improve cognitive performance could also be tested, such as modafinil, methylphenidate and atomoxetine.

- **Mechanism of action:** The amphetamine results in Chapters 6 and 7 have not been previously reported and suggest much could be learnt about the neurobiology of individual differences in attentional performance and how this is associated with psychostimulant response in rodents. Future research should focus on functional assessment of neurotransmitter release or neural activity during task performance and after systemic drug administration in low and high performing individuals.
8.6 Conclusion

In conclusion, the work presented in this thesis provides a unique way to assess attentional performance in rodents, and to preclinically screen drugs to improve cognitive impairments in disorders such as schizophrenia. Through a diverse series of experiments the face, construct and predictive validity of the SDT has been measured. Important factors in cognitive performance, such as genetic, environmental, neurobiological and pharmacological perturbations have been used to demonstrate the utility of the SDT. Critically, the SDT has demonstrated reliable predictive validity for the procognitive effects of a low dose psychostimulant on attention. This task may now be used for future studies exploring attentional deficits in animal models relevant to a wide range of neuropsychiatric disorders and for improving our understanding of procognitive drug actions.
Amitai N, Markou A (2010) Disruption of performance in the five-choice serial reaction time task induced by administration of N-methyl-D-aspartate receptor antagonists: relevance to cognitive dysfunction in schizophrenia. Biol Psychiatry 68:5-16.
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Young JW, Geyer MA, Rissling AJ, Sharp RF, Eyler LT, Asgaard GL, Light GA (2013b) Reverse translation of the rodent 5C-CPT reveals that the impaired attention of people with schizophrenia is similar to scopolamine-induced deficits in mice. Transl Psychiatry 3:e324.


# Appendix I

## Animal Ethics Approval Certificate

Please check all details below and inform the Animal Welfare Unit within 10 working days if anything is incorrect.

### Activity Details

**Chief Investigator:** Associate Professor Thomas Burne, Queensland Brain Institute  
**Title:** Novel testing of cognitive deficits relevant to schizophrenia in rats  
**AEC Approval Number:** QBI/417/11/NHMRC  
**Previous AEC Number:**  
**Approval Duration:** 24-Feb-2012 to 24-Feb-2015  
**Funding Body:** NHMRC  
**Group:** Anatomical Biosciences  
**Other Staff/Students:** Darryl Eyles, Kyna-Anne Conn, John McGrath, James Peak, Karly Turner, Suzanne Alexander, Katrina Geary, Trish Hitchcock  
**Location(s):** St Lucia Bldg 76 - Chemistry (SCMB)  
St Lucia Bldg 79 - Queensland Brain Institute

### Summary

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### Permits

**Previous Approval Details**

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31 Dec 2012 Use in 2012 (from 2013 MAR)  46  46
31 Dec 2013 Use in 2013 (from 2014 MAR)  92  138
Rats - Outbred (Sprague Dawley, Mix, Adults, Institutional Breeding Colony)
  24 Feb 2012 Initial approval  -32  106
  13 Jan 2012 Modification #3  8 May 2013 Modification #7
  8 May 2013 Modification #7  60  166
  11 Sep 2013 Modification #8  96  262
  31 Dec 2013 Use in 2013 (from 2014 MAR)  75  117
  12 Feb 2014 Modification #9  -220  42
  12 Mar 2014 Modification #10  45  162

Please note the animal numbers supplied on this certificate are the total allocated for the approval duration.

Please use this Approval Number:
1. When ordering animals from Animal Breeding Houses
2. For labelling of all animal cages or holding areas. In addition please include on the label, Chief Investigator's name and contact phone number.
3. When you need to communicate with this office about the project.

It is a condition of this approval that all project animal details be made available to Animal House OIC.
(UAEC Ruling 14/12/2001)

The Chief Investigator takes responsibility for ensuring all legislative, regulatory and compliance objectives are satisfied for this project.

This certificate supersedes all preceding certificates for this project (i.e. those certificates dated before 20-Oct-2014)
Appendix II

Supplementary Table 6.1. Same as previous but split for performance group although there was no main effect or interaction with Performance group for any of these measures to justify independent group t-tests.

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<td># Trials Initiated</td>
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<td>3.7±0.9</td>
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<td>152.9±19.0</td>
<td>139.3±17.7</td>
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<td>84.6±10.5</td>
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