Neurocognitive deficits in adult ADHD: preclinical and clinical studies

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A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

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ABSTRACT

University of Manchester
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Doctor of Philosophy (PhD)
Thesis title: Neurocognitive deficits in adult ADHD: preclinical and clinical studies
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In this thesis the neurocognitive deficits in adult ADHD and the effects of ADHD medication have been investigated in animals and in humans. Firstly, by utilising a translational behavioural paradigm we have characterised of a novel animal model of the core symptoms of adult ADHD. In the first study, the 5-choice continuous performance task (5C-CPT) was used to examine different forms of attention and impulsivity in female Lister-hooded adult rats. Subsequently rats were separated into subgroups according to their baseline levels of attention and impulsivity in the 5C-CPT. The low-attentive; LA subgroup and the high-attentive; HA subgroup were selected based on levels of sustained attention and vigilance. The second subgroups include animals with varying levels of motor impulsivity and response inhibition (high-impulsive; HI and low-impulsive; LI subgroups). This allowed for examination of the effects of ADHD medication (methylphenidate and atomoxetine) on attention and impulsivity in the subgroups of animals modeling the inattentive subtype (ADHD-I), and the impulsive symptoms in the combined (ADHD-C) and impulsive-hyperactive (ADHD-IH) subtypes. Both drugs significantly improved sustained attention and vigilance in LA animals only. In HI animals methylphenidate decreased motor impulsivity, however in LI also increased motor impulsivity. Atomoxetine decreased motor impulsivity and response disinhibition in HI animals only. The second animal study extended this by selecting a group of animals with combined deficits in both attention and impulsivity (ADHD-C group). This separation (ADHD-C) allowed for the investigation of potential novel therapeutic targets, revealing the cognitive effects of tolcapone and A-412997. Tolcapone increased vigilance and sustained attention and reduced response disinhibition in ADHD-C animals only, while A-412997 increased vigilance and reduced response disinhibition also in ADHD-C animals only.

The first clinical study evaluated the core neurocognitive deficits, including emotion recognition abilities in medicated and unmedicated adult ADHD patients, compared with a group of healthy controls. The back-translational cognitive tasks used for the evaluation were taken from the Cambridge Automated Neuropsychological Test Battery
Unmedicated adults with ADHD showed core deficits in sustained attention, attentional set-shifting, response inhibition and spatial working memory. Medicated patients showed no impairments compared with controls; highlighting the importance of ADHD medication for improving these cognitive deficits in ADHD. In the second study, the emotion recognition ability of each group was assessed and compared to each other. The second study also examined if the emotion recognition impairments were as a result of a general cognitive dysfunction or are a specific impairment in social perception. The unmedicated ADHD patients showed deficits in the correct recognition of the negative emotions including; fear, anger, sadness and disgust compared with controls. The group of patients followed-up after starting treatment with methylphenidate showed significant improvements in the recognition of all four negative emotions. This improvement was improved to a level comparable to healthy controls. Interestingly, in the unmedicated ADHD group, anger recognition proved to be a specific deficit in social perception whereas sadness, disgust and fear were influenced by deficits in attention and working memory. Following treatment with methylphenidate, improvements in attention accounted for the improvements in sadness, fear and disgust recognition but not anger recognition.

In conclusion the animal studies have shown that animals from within a normal population could be selected according to variations in levels of attention and impulsivity. The ADHD drugs had different effects on attention and impulsivity depending on the natural baseline levels of behaviour of the adult rats. These findings highlight the need for a patient stratification approach in adult ADHD; as different responses are dependent of differences in symptom expression. They also show some potential new therapeutic targets in the animal model, which warrant further exploration. The clinical studies highlight the range of neurocognitive deficits, including emotion recognition deficits in adult ADHD. Together these results highlight the importance of pharmacotherapy in ADHD, not only to treat the core symptoms of ADHD (inattention, impulsivity and hyperactivity) but also to improve the disabling emotion recognition deficits of this disorder.
DECLARATION

No portion of the work referred to in the thesis has been submitted in support for another degree or qualification of this or any other University or other institution of learning.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>5-CSRTT</td>
<td>5 choice serial reaction time task</td>
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<tr>
<td>5C-CPT</td>
<td>5 choice continuous performance task</td>
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<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>5HTTLPR</td>
<td>long variant serotonin transporter</td>
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<tr>
<td>ACH</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
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<tr>
<td>ADHD-C</td>
<td>ADHD combined subtype</td>
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<tr>
<td>ADHD-I</td>
<td>ADHD inattentive subtype</td>
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<tr>
<td>ADHD-HI</td>
<td>ADHD hyperactive-impulsive subtype</td>
</tr>
<tr>
<td>ATMX</td>
<td>atomoxetine</td>
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<tr>
<td>CAARS</td>
<td>Conners’ Adult ADHD Rating Scale</td>
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<td>CANTAB</td>
<td>Cambridge automated neuropsychological test battery</td>
</tr>
<tr>
<td>CF</td>
<td>conversion factor</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyl-transferase</td>
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<tr>
<td>DA</td>
<td>dopamine</td>
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<tr>
<td>D-AMP</td>
<td>dextroamphetamine</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
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<tr>
<td>DBH</td>
<td>dopamine beta hydroxylase</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DRD1</td>
<td>dopamine one receptor</td>
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<td>DRD2</td>
<td>dopamine two receptor</td>
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<td>DRD4</td>
<td>dopamine four receptor</td>
</tr>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders Version IV</td>
</tr>
<tr>
<td>DSM-V</td>
<td>Diagnostic and Statistical Manual of Mental Disorders Version V</td>
</tr>
<tr>
<td>ERP</td>
<td>Event related potential</td>
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<tr>
<td>ERT</td>
<td>Emotion recognition task</td>
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<td>GWAS</td>
<td>genome-wide association study</td>
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<td>HA</td>
<td>high-attentive</td>
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<td>Abbreviation</td>
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<tr>
<td>HI</td>
<td>high-impulsive</td>
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<tr>
<td>HTR 1B</td>
<td>serotonin one b receptor</td>
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<tr>
<td>IED</td>
<td>intra-extra dimensional shift</td>
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<tr>
<td>LA</td>
<td>low-attentive</td>
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<tr>
<td>LH</td>
<td>lateral hypothalamus</td>
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<tr>
<td>LI</td>
<td>low-impulsive</td>
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<tr>
<td>LPFC</td>
<td>lateral prefrontal cortex</td>
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<tr>
<td>MPH</td>
<td>methylphenidate</td>
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<tr>
<td>MPOA</td>
<td>medial preoptic area</td>
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<tr>
<td>MSA</td>
<td>medial septal area</td>
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<tr>
<td>NA</td>
<td>noradrenaline</td>
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<td>NET</td>
<td>noradrenaline transporter</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<td>RCT</td>
<td>randomised control trial</td>
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<td>RI</td>
<td>responsivity index</td>
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<td>RVIP</td>
<td>rapid visual information processing</td>
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<td>SERT</td>
<td>serotonin transporter</td>
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<td>SNAP 25</td>
<td>synaptic protein</td>
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<td>spontaneously hypertensive rat</td>
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<td>sensitivity index</td>
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<td>SSD</td>
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<tr>
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<td>stop-signal task</td>
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<tr>
<td>WKY</td>
<td>Wistar-Kyoto</td>
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<td>SWM</td>
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<td>VBM</td>
<td>voxel-based morphometry</td>
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<td>VMAT</td>
<td>vesicular monoamine transporter</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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1. INTRODUCTION

1.1 Setting the scene

Compared to studies in children with ADHD, studies in adults with ADHD are limited. Studies do show that ADHD persists into adulthood in approximately half of the individuals diagnosed with childhood ADHD (Faraone et al., 2006b). Many children with ADHD do show improvements as they mature through to adulthood; however a significant number still continue to have problems. Approximately 15% of children with ADHD by age 25 years still retained the full ADHD diagnosis, and approximately 65% fulfilled criteria for ADHD or ADHD in partial remission (Faraone et al., 2006b). There are also a significant number of adults who were not recognised and formally diagnosed with ADHD until adulthood. Adults with ADHD are more likely to have comorbidities including mental health disorders (depression, anxiety, bipolar disorder, drug or alcohol misuse) and physical health conditions (asthma) (Kessler et al., 2006). Under-recognition of ADHD in adulthood remains a problem and results in significant morbidity and greater costs to society. The lack of knowledge about adult ADHD in the general public and amongst professionals is beginning to decline, as is the resistance to accept ADHD as a valid condition (Zaman and Muller, 2014). Further research is still required to continue educating and raising awareness, and to gain an understanding into the biological underpinnings to enable the development of improved treatments.

1.2 Motivations

It is estimated that in the U.S, 89% of adults living with ADHD are untreated (Kessler et al., 2007), and similarly in Europe only a small number of adults with ADHD are treated (Aragones et al., 2010). The distinct lack of treatment can seriously affect psychosocial functioning and quality of life. There is considerable interest in designing a treatment for ADHD without the adverse effects of the current treatment. Little is understood about the exact mechanisms by which drug treatments exert their effects in ADHD. It could be the case that the different ADHD drugs reduce symptoms to different extents, dependent on the baseline level of that symptom, i.e. one drug may affect inattention to a greater extent in individuals with higher levels of inattention compared to individuals with lower baseline levels.

More research is needed to better understand the biological mechanisms and clinical presentation of the disorder in adulthood. The presentation of symptoms of ADHD
changes from childhood into adulthood, and because of this many patients receive other diagnoses and ineffective treatment. It is vital at this time that more research focuses on the specific symptoms and presentations in adult ADHD. ADHD has long been associated with cognitive developmental impairments (executive dysfunction) in children and adults (Seidman, 2006). Adult ADHD has principally been associated with poorer control executive functions, such as, memory, self-regulation of affect and motivation. These cognitive deficits have been shown to affect academic, social and overall quality of life (Brown, 2008; Simon et al., 2009). There are many neuropsychological measures of cognitive deficits that have been used in ADHD and that target specific cognitive domains. Some studies have suggested that neuropsychological assessments have a role in clinical situations, including the diagnostic process (Stavro et al., 2007). However others have found that executive function impairments are better identified by self-reports and clinical interviews (Brown et al., 2009). Further clarification is required to establish if neuropsychological test results correlate with self-report measures in adults with ADHD. The effects of drug treatment in adult ADHD on cognitive deficits are beginning to emerge with insight from imaging studies. The specific cognitive deficits, including social cognition deficits still need to be fully established in adults with ADHD for future treatment targets.
1.3 The structure of the thesis

The material presented in this thesis addresses a number of aspects concerning adult ADHD and the associated cognitive deficits. These findings have been assembled in the alternative format whereby work has been incorporated into sections that are suitable for submission in a peer-review journal or a conference communication. Within this framework, the structure of the report has been organised into two main sections:

Background theory

- Chapter 2 contains an introduction to the clinical profile and diagnostic requirements for adult ADHD.
- Chapter 3 provides a discussion of the ADHD drugs, their mechanism of action and their adverse effects.
- Chapter 4 describes animal models of ADHD, with a clear explanation of the validity of current models.
- Chapter 5 introduces the core cognitive deficits in adult ADHD, including deficits in social cognition specifically focusing on emotion recognition abilities.
- Chapter 6 outlines the main aims and objectives of this thesis.

Main contributions

- Chapter 7 describes the development of novel rat models of the inattentive subtype and impulsive symptoms of adult ADHD using a translational rodent task of attention and impulsivity. The effects of methylphenidate and atomoxetine on different behaviours were investigated in these subtypes and are also reported in this chapter.
- Chapter 8 builds upon and extends the previous work and combines the symptoms of inattention and response inhibition to model the combined subtype of adult ADHD in the rat. Using this model, a number of novel therapeutic targets were explored.
- Chapter 9 translates the cognitive variability in rats to the clinic and assesses the cognitive deficits in a clinical sample with ADHD, and the effects of medication on these cognitive domains.
- Chapter 10 explores the social cognitive deficits resulting from cognitive dysfunctions, specifically facial emotion recognition in individuals with adult ADHD and the impact of ADHD treatment.
BACKGROUND THEORY
2. CLINICAL FEATURES AND DIAGNOSIS OF ADULT ADHD

Adult attention deficit hyperactivity disorder (ADHD) is becoming a more recognised psychiatric condition, yet it still remains under-researched and poorly understood (Asherson et al., 2010). The prevalence rate of adult ADHD is estimated to be approximately 3-5% (Fayyad et al., 2007; Kessler et al., 2005; Simon et al., 2009) with approximately two-thirds of children with ADHD continuing to have debilitating levels of ADHD symptoms in adulthood (Faraone et al., 2006a).

Adults living with ADHD suffer a lower quality of life. Socially; adults with ADHD are twice as likely to separate/divorce compared with adults without ADHD, and educationally; are less likely to finish college (Biederman et al., 2006). Adult ADHD is associated with poor outcomes in occupational functioning. Compared with colleagues, individuals with ADHD have more days out of a work-role, are less productive, are less likely to be promoted and have reduced work-performance quality (de Graaf et al., 2008). As well as frequent job changes, adults with ADHD on average have lower household incomes compared to adults with ADHD (Biederman et al., 2006). ADHD symptom severity and specific executive deficits have been associated with overall work impairments in individuals with ADHD (Barkley and Murphy, 2010). ADHD has also been associated with higher than normal rates of negative driving outcomes with an average relative risk of 1.54 (Jeroma et al., 2006). Individuals with ADHD are more likely to be involved in road traffic accidents, speeding offences, trait driving anger and aggression (associated with risky driving) for meta-analysis see: (Jeroma et al., 2006). Adult ADHD is also associated with other ‘risky’ behaviours such as an increased rate (2-3 times higher) of cigarette smoking/substance abuse (Biederman et al., 1995; Kollins et al., 2005).

2.1 Diagnosis

Diagnosis of ADHD is made on the observation of a cluster of symptoms and the presence of a clinically significant impairment. ADHD is separated into three separate symptom clusters: inattentive, hyperactive, and impulsive as classified by the DSM-IV (DSM-IV; American Psychiatric Association, 1994). The work presented in this thesis uses the DSM-IV classification system; however since undertaking this work the DSM-V has now been produced. The key changes in the adult ADHD diagnostic criteria between the earlier edition and the new DSM-V include: firstly an increase in the age at
which an individual must have ADHD present, from age 7 years to age 12 years; and secondly the DSM-V is written to assist clinicians with the diagnosis of adults as well as children (DSM-IV, focused mainly on children), and more accurately characterises the experience of adults. As in the DSM-IV, symptoms will be divided into two categories of inattention, and hyperactivity and impulsivity.

ADHD core symptoms (DSM-IV):

1. **Inattention** is used to describe the inability to sustain attention. Symptoms and features include; disorganised and forgetful behaviour, easily distractible, poor task completion and frequent mistakes in tasks that require sustained attention.
2. **Impulsivity** can be defined as actions without reflection; symptoms and features include premature, unprepared and poorly timed behaviours.
3. **Hyperactivity** includes symptoms such as fidgeting, moving, inability to play quietly, or sit still in adults, and continually interrupting. Hyperactivity is recognised most often in children at about four to five years of age, when the child starts school. However, many parents complain about excessive restlessness in infancy.

Diagnostic criteria for ADHD in the DSM-IV (DSM-IV; American Psychiatric Association, 1994) separate ADHD into three distinct subtypes. The Predominantly Inattentive Type (ADHD-I) includes symptoms of inattention with few symptoms of hyperactivity-impulsivity, the Predominantly Hyperactive-Impulsive Type (ADHD-H) includes symptoms of hyperactivity-impulsivity, and the Combined Type (ADHD-C) which is defined by all three core symptoms. ADHD-I is the most prevalent of the subtypes in adulthood, and ADHD-HI is argued not to exist as a clinical condition in adulthood (Gibbins et al., 2010).

A common criticism of the DSM-IV is its lack of a firm biological basis; diagnosis is made based on superficial behavioural signs and verbal reports (Hugh, 2005). The current diagnostic criteria for ADHD are still the topic of debate and are continually being refined and altered (Paule et al., 2000). There is an ever-increasing need for a more objective, biological assessment tool to assist in diagnosis.
2.2 Clinical features adult ADHD

The clinical profile and manifestations of ADHD evolve with age, the core symptoms of inattention, impulsivity and hyperactivity well recognised in childhood ADHD can have different and more subtle expressions in adulthood (Kooij et al., 2010). Hyperactivity becomes less obvious in adulthood, with a decline in gross motor activity (Adler, 2004; Biederman et al., 2000). Adults with ADHD are more likely to experience constant mental activity and feelings of inner restlessness, rather than physical hyperactivity most commonly experienced in childhood (Faraone et al., 2006a; McCarthy et al., 2009; Wender et al., 2001). Although adults do show a decline in frequency of impulsive behaviours over time, there still remains a significant link between impulsive action and response disinhibition in adult ADHD (e.g. road traffic accidents, quitting a job, substance misuse and having an affair) and overall potentially devastating impairments (Faraone et al., 2006a). The prevalence of ADHD-I is greater in women in adulthood (Biederman et al., 1994), with women showing higher rates of comorbidities such as depression, anxiety, substance abuse and dependence disorders, and cognitive impairment (Biederman et al., 1994).

2.3 Epidemiology of adult ADHD

Relatively little is known about the prevalence of ADHD among adults. Epidemiological studies of ADHD have been hampered by the difficulties in diagnosis of adult ADHD, due to the changes in the definition and diagnostic criteria over the past 30 years. Epidemiological data has largely been generated from family studies, follow-up studies and population-based studies. Family studies cannot be generalised as they use a strongly selected sample, excluding a group with a strong genetic predisposition – parents of children with ADHD (Faraone and Biederman, 2005). A recent meta-analysis demonstrates that published estimates of the prevalence of adult ADHD vary greatly (Simon et al., 2009). Results from follow-up studies have shown that ADHD persists in 4-66% of cases in adulthood. This variability could be due to methodological differences in studies, including but not limited to; small sample sizes and the use of different diagnostic criteria. The same meta-analysis reported that population-based studies estimate the prevalence rate of adult ADHD to be between 1 and 7.3% applying DSM-IV criteria (meta-analysis. (Simon et al., 2009). Overall the pooled prevalence rate for adult ADHD in the most recent meta-analysis was reported to be 2.5% (95% CI 2.1-3.1), with no significant gender effect. However studies not included in the meta-analysis suggest a higher prevalence of adult ADHD in males.
(Fayyard et al., 2007; Kessler et al., 2006). The prevalence of ADHD has been shown to decline with age although this effect can be dependent on the gender composition of the samples used (Simon et al., 2009). Overall more research is required to establish the exact prevalence and the factors affecting prevalence throughout the lifespan. Improvements in diagnostic criteria, i.e. publication of the DSM-V now provides new opportunities to improve the methodology (and validity) of future studies. Community-based epidemiological studies are severely lacking and should be the focus of future work, with improved study designs. However at this time epidemiological studies are restricted by the limited understanding of the aetiological and pathophysiological basis of the disorder. Improvements in this area will lead to improvements in diagnostic criteria which in turn should lead to improvements in detection and identification of ADHD and thus more accurate epidemiological data.

2.4 Genetic and environmental risk factors for ADHD

Genetic factors play an important role in the aetiology of ADHD (Wallis et al., 2008). Family studies have consistently shown a familial association in ADHD (Biederman et al., 1992; Biederman et al., 1990; Faraone and Doyle, 2001). Twin (monozygotic and dizygotic) studies have been used to determine the heritability of ADHD. Twin studies have demonstrated heritability estimates varying between 60% and 90% (Biederman et al., 1990), with an average heritability of 80% in children and adolescents (Kieling et al., 2008); meaning that approximately 80% of the aetiologic contribution is genetic. This makes ADHD as heritable, if not more so, than schizophrenia (75% heritability). One reason for the variation in heritability estimates of ADHD lies in the differences between the number of items used to calculate heritability, from 2 or 3 items from the Rutter A scale to all 14-18 items in the ADHD criteria of DSM. In general, the studies that used more items of ADHD diagnostic criteria found higher heritability compared to the studies that used the 2 or 3 items. Therefore theoretically, the heritability of ADHD may be even greater than 80%. However, interestingly heritability estimates for ADHD in adults are significantly lower, approximately 30% (Boomsma et al., 2010)

To date no single gene has been identified as contributing a major role to the aetiology of ADHD. However a number of genes have been found to be associated with ADHD symptomatology (OMIM 143465 (genetic database)). Genetic association studies examining putative risk genes have mainly focused on genes encoding for dopamine and noradrenaline neurotransmitter systems. The most consistent evidence for genetic associations to ADHD phenotype has been shown for markers of the dopamine
receptors, D4 (DRD4), D5 (DRD5), dopamine transporter (SLC6A3/DAT1), serotonin receptor 1B (HTR1B), serotonin transporter (SLC6A4/5HTT), and synaptosomal-associated protein genes (SNAP-25) (meta-analyses: (Cheuk and Wong, 2006; Faraone et al., 2005b; Gizer et al., 2009; Li et al., 2006; Mick and Faraone, 2008; Smoller et al., 2006)). A number of these genes are discussed in more detail in chapter 8. Currently there is no biomarker for ADHD; genetic studies may provide the possibility of the development of a diagnostic marker for ADHD in the future.

No single aetiology has been identified for ADHD, no single cause or event can be identified in the pathophysiology of the disorder. However certain environmental associations have been established, including both prenatal and postnatal factors. Prenatal factors contributing to the aetiology of ADHD include smoking (Linnet et al., 2003; Ohlmeier et al., 2007), drinking alcohol (Linnet et al., 2003), cocaine-use during pregnancy (Linares et al., 2006); maternal stress during pregnancy (O'Connor et al., 2003) and anticonvulsant use (Steinhausen et al., 1994). These prenatal factors have been shown to increase the risk of the offspring developing ADHD in humans. However the link between ADHD and stress during pregnancy disappeared when controlling for maternal genetic factors, indicating this association may be accounted for by genetic factors and not stress exposure (Rice and Thapar, 2010). Animal studies have strengthened the findings and provided an insight into the pathophysiology of ADHD developed from the factors outlined above (see chapter 4; animal models of ADHD, for more details).

Postnatal risk factors include neonatal anoxia (Pineda et al., 2007), severe head injury, brain disease (minor injury is more often the result of hyperactivity than a cause) (Taylor and Warner-Rogers, 2005). A study examining the effects of extreme deprivation in orphanages in Romania, found that children that had been exposed to such factors (noted above) showed higher rates of pervasive and persistent over activity and inattention later in childhood, even after adoption into English families before the age of 4 years (Kreppner et al., 2001). Diet is a controversial factor often blamed for hyperactivity. There is some literature supporting the role of diet in hyperactivity, but the effect sizes are modest at best (Nigg et al., 2012). The main evidence is derived from therapeutic trials, which suggest that a range of foodstuffs (cow’s milk, wheat flour, eggs and artificial colourings and additives) can have a negative impact in individual children (Bateman et al., 2004). There is a great need for clarification of the exact extent of these aetiological factors; future studies need to be more carefully conducted i.e. the use of more randomised controlled trials (RCTs).
2.5 Neurobiology of ADHD

Research conducted in the past two decades has revealed various CNS abnormalities in ADHD patients, confirming the neurobiological basis of the disorder. The regions that are traditionally linked with ADHD include regions and structures of the frontal-striatal circuits, frontocerebellar circuit, regions involved in reward processing and motivation, and emotional control. Brain morphology in ADHD is suggested to differ from healthy controls.

2.5.1 Clinical neuroimaging

The ADHD functional imaging literature has grown substantially over the past two decades with more frequent use of the neuropsychological paradigms (Cortese et al., 2012). Meta-analyses of structural MRI studies suggest that the regions with maximal volume reduction in children with ADHD are the cerebellar vermis, total cerebellum, total brain and the right (and left (Frodl and Skokausas, 2012)) caudate nucleus (Valera et al., 2007). A meta-analysis of structural MRI studies have shown that gray matter volume reductions in ADHD are evident in the right putamen and the globus pallidus (Frodl and Skokausas, 2012), and the right anterior cingulate cortex (in untreated children only). Other groups have reported reductions in the volume of the amygdala, thalamus, frontal-prefrontal and premotor regions. Results from a whole-brain exploratory technique – voxel-based morphometry (VBM) has reported that adults with ADHD show reductions in the right inferior frontal gyrus and reductions in gray matter in the left superior frontal cortex, anterior cingulate and the orbitofrontal cortex (Depue et al., 2010). Associations have been found between prefrontal cortex volume reductions and severity of outcome in adolescents with ADHD (Shaw et al., 2006), however in adults there were no associations between severity of outcome and volume reductions (Proal et al., 2011). A recent meta-analysis has shown a different pattern of brain dysfunction during response inhibition tasks in children and adults with ADHD. Children with ADHD showed hypoactivation of the supplementary motor area and basal ganglia, and adults with ADHD showed hypoactivation of the inferior frontal cortex and thalamus (Hart et al., 2013). When undertaking a working memory task, functional imaging fMRI has revealed reduced functional connectivity between the ventrolateral prefrontal cortex, the anterior cingulate, the superior parietal cortex and the cerebellum. At the same time, connectivity was increased between the inferior frontal gyrus, the anterior cingulum, the superior frontal gyrus and cuneus (Wolf et al., 2009).
The dorsal frontostraital connections are involved in response inhibition, planning and set-shifting. Reduced dorsolateral prefrontal cortex activity has been shown in ADHD patients during cognitive control tasks involving response inhibition, working memory and planning (Willcutt et al., 2005a). However, interestingly another study reports not finding the same cognitive deficits in all of the ADHD groups, not even the majority, suggesting that response inhibition is not a key abnormality in ADHD (Nigg et al., 2005). Further research is needed to elucidate the key areas of cognitive deficit in ADHD. This thesis presents studies in adults with ADHD tested on cognitive tasks with and without medication.

The frontocerebellar network is linked with the ability of time estimation, and time predictions (Duerden et al., 2012). Impairments in the ability to estimate time and in behavioural control has been described in children with ADHD (Zelaznik et al., 2012). Frontoparietal and cerebellar hypoactivation, along with reduced frontocerebellar functional connectivity are apparent during time discrimination or synchronisation of motor activity paradigms (Hart et al., 2013), and during cognitive paradigms (Suskauer et al., 2008). These results suggest a complex involvement of the frontocerebellar dysfunction in the symptoms of ADHD.

The ventrolateral-orbitofrontal-striatal network is involved in reward and motivational processes. Patients with ADHD have impairments in delayed reward anticipation, and prefer a small immediate reward compared with a larger delayed reward, showing reduced motivation and increased impulsivity (Durston et al., 2011). Patients with ADHD show more frequent and faster responses immediately after a rewarding stimulus and an inability to inhibit responding when reinforcers are removed (i.e. reduced inhibitory control) (Sagvolden et al., 1998). During reward anticipation, ADHD patients show impairments in activity in the ventral striatum, which supports the hypothesis of a reward system dysfunction in ADHD (Scheres et al., 2007).

The dorsal anterior midcingulate cortex, the dorsolateral and ventrolateral prefrontal cortex and the parietal cortex together with the cerebellum, the thalamus and the striatum are involved in the attention and executive function network. This network is key in ADHD and dysfunction may lead to deficits in motor response inhibition, impulsivity and impairments in using feedback for behaviour modification (Bush, 2011).

Finally changes in the amygdala are present in groups of patients with ADHD (Plessen et al., 2006), which is supported by the observation that there are higher rates of anxiety
comorbidities (Jarrett and Ollendick, 2008) and emotional dysfunction in ADHD (see chapter 5.3 for further details).

In summary, neuroimaging studies have revealed a number of brain regions to be affected in ADHD including; frontal and parietal cortices, basal ganglia, cerebellum, hippocampus, and corpus callosum (Giedd and Rapoport, 2010) (Cortese et al., 2012). These core regions have been associated with functional networks involved in ADHD (fig 1) (Purper-Ouakil et al., 2011). These findings suggest that ADHD involves both cortical dysfunction and abnormal connectivity.

2.5.2 Attention

It is widely accepted that attention is not a unitary phenomenon but a cognitive system composed of several neural networks that perform specific computations (Posner and Peterson, 1990; Raz and Buhle, 2006). Attention can be conceptualised into four networks including: 1. alerting network; 2. orientating network; 3. executive control; and more recently 4. self-regulation (Peterson and Posner, 2012).

The alerting system is involved in maintaining alertness from arousal and then producing differentiated components, thus maintaining optimal vigilance during task performance. The locus coeruleus which is the source of noradrenergic innervation has
been strongly implicated in alertness (Aston-Jones and Cohon, 2005). Activity in the locus coeruleus has been observed during periods of warning signals, these warning-signal effects can be blocked by alpha-2 adrenoceptor agonists (guanfacine and clonidine) and enhanced by alpha-2 adrenoceptor antagonists (Beane and Marrocco, 2004). The locus coeruleus-noradrenaline system has two distinguishable modes of activity – phasic and tonic. The phasic mode involves a burst of activity in the locus coeruleus and produces widespread but temporally specific release of noradrenaline, enhancing cortical processing and facilitating task-appropriate behaviour. The event-specific nature of this phasic response acts as a temporal attentional filter, filtering relevant processing and ignoring distracting events. Tonic mode alertness involves dissociation and search for alternate stimuli in a changing environment (exploration), correlating to sustained attention and vigilance respectively. An established approach to tonic alertness involves using a long boring task to measure sustained vigilance. These tasks rely heavily on the functioning of the right cerebral cortex. Major projections to the locus coeruleus from the orbitofrontal cortex and anterior cingulate cortex are important in generating tonic locus coeruleus activity (Aston-Jones and Cohon, 2005).

The orienting network involves the dorsal (top-down visuospatial) system and the ventral (bottom-up reorienting) system (Corbetta and Shulman, 2002). The dorsal system involves the frontal eye field and interparietal sulcus when there is a move in the spotlight of attention. When switching the target location (breaking attention and refocusing) the temporoparietal junction and the ventral frontal cortex become activated (Corbetta, 1998). Cholinergic innervations arising from the basal forebrain were suggested to play a critical role in orienting, however further evidence suggests that the site of effect may involve the superior parietal lobe (Davidson and Marraccco, 2000). Studies in rats and in monkeys have confirmed the involvement of ACh in orienting (Davidson and Marraccco, 2000; Everitt and Robbins, 1997; Stewart et al., 2001). The ventral network has been identified as part of a network responsive to sensory events. This network is predominantly right-lateralised with interactions of the temporoparietal junction with the frontal and dorsal brain regions (Shulman and Corbetta, 2012).

Executive control functions have been suggested to involve two separate systems. The first system involves the anterior cingulate cortex in monitoring conflict and the second network involves the frontal areas to resolve conflict (Botvinick et al., 2001). The second system involves two networks. These two networks are suggested to work relatively independent from each other in producing top-down control. The first
involves the cingulo-opercular control system (including the dorsal anterior cingulate/medial superior frontal cortex, anterior insula/frontal operculum and the anterior prefrontal cortex) and acts to maintain a background focus during trials. On the other hand the frontoparietal system (including the dorsolateral prefrontal cortex and the posterior parietal cortex) is suggested to be related to task switching and initiation and to adjustments during performance in a task (Peterson and Posner, 2012) (Dosenbach et al., 2008).

Self-regulation, also known as self-control (mainly in adults) can be defined as the ability to control our thoughts, feelings and behaviour, thus controlling our reflexive or dominant response to select the less dominant ones (Peterson and Posner, 2012). Stoop-tasks are commonly used to assess self-regulation, neuroimaging studies have widely reported anterior cingulate gyrus activation during the Stoop effect. The dorsal area of the anterior cingulate appears to be involved in strict cognitive tasks, whereas the ventral region appears to be more involved in emotion-related tasks (Botvinick et al., 2001; Bush, 2011). Imaging studies have also shown the involvement of the anterior insula (Bush et al., 2000), and in certain situations (inhibition of a dominant response) the prefrontal cortex, in self-regulation mechanisms (Sridharan et al., 2007; Sridharan et al., 2008).

2.5.3 Impulsivity

Impulsivity represents a multidimensional construct, broadly defined as poor self-control, with fast decision-making without forethought or regard for potential consequences (Dalley et al., 2011). The concept of impulsivity has been recently defined and divided into separate domains including: 1. Motor impulsivity: impaired ability to stop a motor response, often reflected in stop-signal reaction time tasks and the rodent 5-choice serial reaction time task (5-CSRTT). 2. Decision making impulsivity: inefficient balancing of options and taking appropriate risks based on all the available information, tested by gambling tasks (i.e. Iowa Gambling Task). 3. Choice impulsivity: difficulty in delaying gratification and opting for small but immediate rewards despite long-term negative consequences, assessed by delay-discounting tasks. Finally, reflection impulsivity: reduced ability to obtain information from the environment before making a choice, reflected in information sampling tasks (Fineberg et al., 2014). Dalley and colleagues have extensively explored both impulsive choice (decision-making impulsivity) and impulsive action (motor impulsivity) showing profound differences between the two (Jupp et al., 2013). Impulsive choice has been
measured in impulsive individuals and results have shown that the more impulsive individuals prefer immediate rewards even if they are smaller than those offered later on (Richards et al., 1999). Impulsive individuals have also shown increases in motor impulsivity (impulsive action) in the go/no-go task, indicated by more rapid but incorrect responses (Riccio et al., 2002).

Clinical and preclinical studies have largely implicated monoaminergic corticostriatal systems in the underlying mechanisms of impulsivity. Neuroimaging studies in individuals with ADHD and high levels of impulsivity have shown structural and functional differences compared with controls (Carmona et al., 2009; Wilbertz et al., 2012). The main regions of interest include the prefrontal cortex and linked corticostriatal circuitry. Results from FMRI studies, lesion (frontal lobe) studies and preclinical studies have shown that motor impulsivity is under the control of the right inferior frontal gyrus (RIFG) and sub-cortical (including sub-thalamic) network connections (Potenza and de Wit, 2010; Rubia et al., 2001). Whilst serotonin does not appear to be involved in motor impulsivity (Clark et al., 2005; Rubia et al., 2003), the noradrenaline system has been widely implicated in motor impulsivity (Chamberlain et al., 2007; Chamberlain et al., 2006a; Chamberlain and Robbins, 2013). Drugs influencing noradrenaline levels (atomoxetine and methylphenidate) have both improved response inhibition deficits in patients with ADHD, and both normalised the under-activation observed in the left prefrontal cortex (Cubillo et al., 2014). Higher doses of atomoxetine have been shown to increase error-signaling in the bilateral inferior frontal cortices and the pre-supplementary motor area (Graf et al., 2011). These results suggest that individuals with higher levels of motor impulsivity (ADHD patients), have a fronto-striatal dysfunction (Fineberg et al., 2014). It is important to note that these results were extrapolated from individuals with pathologically high levels of impulsivity as they all had ADHD, therefore the structural abnormalities cannot exclusively be related to impulsivity alone but could be part of this heterogeneous disorder. Further research is needed to focus on groups of individuals representing the spectrum of levels of impulsivity not solely pathologically high levels, as in ADHD.

Impairments in decision-making have been associated with the orbitofrontal and related cortical circuitry, which is suggested to be under the control of the serotonergic system (Rogers et al., 1999). Decision-making has also been shown to involve subcortical networks involving both serotonergic and dopaminergic systems (Baarendse et al., 2013; Zeeb et al., 2009).
During delayed-discounting tasks, impulsive choice can be observed and measured in rats and in humans. In rats, both serotonergic and dopaminergic differences have been observed in impulsive-choice tasks. Increases in serotonin were observed in the medial prefrontal cortices, and increases in the dopamine metabolite (DOPAC) were observed in the orbitofrontal cortex suggesting a double dissociation (Winstanley et al., 2006b). Others have suggested three distinct networks to be involved including; the ventromedial PFC and medial OFR, ventral striatum and the posterior cingulate cortex (specifically involved in the subjective discounted value of future rewards). The lateral PFC and nucleus accumbens have been suggested to be involved in delayed-discounting, and the hippocampus has been proposed to potentially be involved in the prospective outcomes of decisions (Peters and Buchel, 2011).

2.5.4 Recent models of emotional dysregulation in ADHD

Emotion regulation can be defined as an individual’s ability to alter an emotional state to allow for adaptive, goal-oriented behaviours (Thompson, 1994). Emotion dysregulation is clearly an important dimensional trait in the disorder but is not unique to ADHD. Many psychological and physiological processes may be responsible for the connection between ADHD and emotion dysregulation. The most recent models, described in a recent meta-analysis (Shaw et al., 2014) explain the underlying neural processes as either “bottom-up” processes or “top-down” processes. The “bottom-up” processes that affect emotion regulation at a basic-level, refer to orienting to emotional stimuli and reward valuation. The “top-down” psychological processes include the allocation of attention to emotional stimuli. Deficits in these processes and in other cognitive processes including response inhibition and working memory may contribute to the emotional dysregulation in ADHD.

The neural regions involved in the “bottom-up” responses to emotional stimuli include the amygdala, ventral striatum, and the orbitofrontal cortex. Numerous functional imaging studies have reported the involvement of the amygdala in regulating emotion dysfunction in ADHD, specifically emotion perception and recognition (Brotman et al., 2010; Posner et al., 2011b). In a study of 18 children with ADHD and no comorbidity, Brotman et al (2011) showed that when rating neutral faces as fearful, this group had left amygdala hypoactivity compared with healthy controls. Posner et al (2011) found greater right amygdala activity and enhanced connectivity with the lateral PFC in medication-naïve ADHD adolescents, compared with controls. Interestingly stimulants normalised these activity and connectivity differences in ADHD adolescents (Posner et
al., 2011b). However others have failed to find significant alterations in amygdala functioning in response to emotion stimuli (i.e. fearful face presentation) compared with controls (Herpertz et al., 2008; Malisza et al., 2011; Marsh and Blair, 2008). This difference could be due to sample differences including size; limiting the power to detect effects, and characteristics i.e. ADHD children with comorbidity (conduct disorder). There is a distinct lack of studies examining amygdala activity in adults with ADHD, more research in large samples is needed to examine if these differences are present in adults with ADHD. The amygdala is a region of particular importance in ADHD, stemming from not only deficits in early processing of visual emotional stimuli but also the deficits in reward processing observed in ADHD (Scheres et al., 2007). This is also supported by studies showing amygdala structural abnormalities e.g. dopamine receptor density (Plessen et al., 2006). A very recent study has elegantly shown that polymorphisms in the DAT gene (SLC6A3) influence amygdala reactivity, this is particularly relevant to ADHD as DAT is the molecular target of stimulants (Bergman et al., 2014).

The second region of interest in ADHD involved in “bottom-up” processes is the orbitofrontal cortex (Shaw et al., 2014). The orbitofrontal cortex has important connections with the amygdala, the thalamus, and several cortical regions and is vital in emotion regulation and reward processing (Knutson et al., 2001; Phillips et al., 2008). Plessen et al. (2006) demonstrated, reductions in the size bilaterally across the area of the basolateral complex using surface analysis of the amygdala in an anatomical MRI study of 51 children and adolescents with ADHD. They also showed disrupted connectivity between the amygdala and the orbitofrontal cortex in individuals with ADHD compared to controls. The authors conclude that the disruptions in these connections may play a role in behavioural disinhibition (Plessen et al., 2006). In agreement with these findings, others have shown structural differences in the orbitofrontal cortex and atypical activation in response to reward anticipation in children with ADHD (Overmeyer et al., 2001).

The third region involved in the “bottom-up” circuitry is the ventral striatum. The ventral striatum is of particular importance in ADHD because of its vital role in mediating positive affect and reward processing (Knutson et al., 2001). Medication naïve adults with a history of childhood ADHD showed decreased activity in the ventral striatum and the subgenual cingulate during the perception of positive and negative emotions (Schlochtermeier et al., 2011). Functional imaging studies have also shown ventral striatal involvement in the anticipation and receipt of rewards, thus playing a
role in the aversion to delay in ADHD in both adults and children (Scheres et al., 2007; Strohle et al., 2008). Connectivity differences have also been found during resting brain activity in children with ADHD. Tomasi et al (2012) have shown that children with ADHD had decreased connectivity in the superior parietal cortex and precuneus and in the cerebellum, and increased connectivity in reward-processing regions – the ventral striatum and orbitofrontal cortex compared with controls (Tomasi and Volkow, 2012). Costa Dias et al. (2013) in a functional connectivity MRI study showed connectivity differences in ADHD subjects experiencing appropriate rewarding stimuli. They also showed that increased connectivity between the NAcc and PFC was associated with greater impulsive decision making in a delay-discounting task (Costa-Dias et al., 2013).

In summary, results from imaging studies suggest the involvement of a network including the amygdala, ventral striatum and orbitofrontal cortex in emotion processing, thus alterations in this network contribute to emotion dysfunction as observed in ADHD.

Regions mediating the “top-down” processes include the cortical regions responsible for the allocation of attentional resources in emotionally arousing situations (Nigg and Casey, 2005; Phillips et al., 2008). The prefrontal cortex is at the interface of cognition and emotion, specifically the ventrolateral, medial prefrontal and the anterior cingulate cortical regions. Typically, in healthy individuals when an emotion element is included in a cognitive task this would increase top-down prefrontal cortical activation and would reduce subcortical activity (Phillips et al., 2008). These important patterns do not appear to be completely present in ADHD (Shaw et al., 2014). In a functional imaging study of 14 adolescents with ADHD, Passarotti et al. (2010a) have shown that ADHD individuals show increased activation in the DLPFC and reduced activation in the ventral and medial PFC in response to negative stimuli added to a working memory task compared to healthy controls. However, when shown positive stimuli in the working memory task ADHD patients showed hyperactivation of ventral and medial PFC (Passarotti et al., 2010b). Other studies have also found similar patterns of hypoactivation in the right medial and ventrolateral PFC in response to negative distractors and hyperactivation in the left medial PFC in response to positive distractors during the emotional Stroop task in ADHD (Passarotti et al., 2010a; Posner et al., 2011a). These results taken together suggest dysregulation of attentional control in ADHD when emotional stimuli are also present. More work is needed in adults with ADHD to confirm whether or not these processes are still dysregulated in adulthood, and if treatment in adulthood ameliorates these dysfunctions.
3. PHARMACOTHERAPY OF ADHD

Neuroimaging studies have provided compelling evidence for a neurobiological underpinning in ADHD. Medication used in the treatment of ADHD (methylphenidate, dextroamphetamine and atomoxetine) act to enhance brain catecholamine levels. This thesis examines the effects of ADHD medication on the different symptoms in ADHD, including inattention, impulsivity and social cognition.

3.1 Stimulants

The stimulants used in the treatment of ADHD are methylphenidate and dextroamphetamine (D-amphetamine), and more recently L-lysine-dextroamphetamine dimesylate (pro-drug of D-amphetamine). A recent meta-analysis of efficacy outcomes for medication studies of adults with ADHD has estimated a mean effect size of 0.86 for short-acting stimulants and a mean effect size of 0.73 for long-acting stimulants (Faraone and Glatt, 2009). A review of meta-analyses has shown that compared to other psychiatric medication, stimulants are more efficacious in ADHD than antipsychotics in schizophrenia, antidepressants in depression and mood stabilizers in bipolar disorder (Leucht et al., 2012).

3.1.1 Methylphenidate

Methylphenidate has remained the mainstay of treatment for ADHD. Methylphenidate is equally efficacious (Cornforth et al., 2012; Gunther et al., 2010; Rucklidge, 2010) in both sexes even though the psychostimulant may be metabolised differently in males and females (Cornforth et al., 2012). There are a limited number of controlled studies assessing the efficacy of methylphenidate in adults with ADHD.

Small-scale acute dose studies suggest that therapeutic doses of methylphenidate improve fronto-executive functions in children and adults diagnosed with ADHD (Chamberlain et al., 2010; Mehta et al., 2004b; Turner et al., 2005) and also in healthy subjects (Elliot et al., 1997; Koelega, 1993; Mehta et al., 2000), indicating that these effects are not pathognomonic for ADHD. Methylphenidate has been reported by others to only affect specific neurocognitive domains including impulsive control, working memory, and attention, and that these domains interact with methylphenidate in a baseline performance-dependent manner (Chamberlain et al., 2010; Clatworthy et al., 2009; Naylor et al., 1985; Turner et al., 2003). Other researchers have reported lack of cognitive-enhancing effects of MPH on executive function in children with ADHD.
More research is needed to establish the precise effects of methylphenidate on specific cognitive processes, with a particular focus on adults as the research to date is limited, relative to research in children.

Methylphenidate exerts therapeutic effects by increasing extracellular levels of dopamine and noradrenaline by blocking their reuptake (Zetterstrom et al., 1988). The dopamine transporter (DAT) is the main target for ADHD stimulant medication, in line with the suggestion that ADHD patients have increased DAT density (Spencer et al., 2005). Interestingly, Volkow and colleagues have recently challenged this idea by examining adult medication-naïve ADHD patients on a case-control basis using PET imaging, finding that ADHD patients have reduced DAT and D2/D3 receptor availability in subcortical regions of the left hemisphere (including; nucleus accumbens, caudate nucleus and midbrain) (Volkow et al., 2009; Volkow et al., 2007a, b). Clearly more imaging research is required in larger samples in medication-naïve patients and in those previously medicated in adulthood. Therapeutic oral doses of methylphenidate have been shown to block more than 50% of DAT in healthy subjects (Volkow et al., 1998) resulting in an increase in extracellular dopamine in the striatum (Volkow et al., 2001). DAT density is high in the striatum and low in the frontal cortex. Microdialysis studies in animals have shown that dopamine increases are not specific to the striatum but are also found in extrastriatal regions such as the frontal cortex (Moghaddam et al., 1993). This finding is thought to be due to involvement of the noradrenaline transporter (NAT), NAT density is high in the cortex and low in the striatum (opposite to DAT). Dopamine has a higher affinity for NAT in PFC than DAT and it is the noradrenergic system that is responsible for the termination of dopamine neurotransmission in the frontal cortex (Moron et al., 2002). When methylphenidate saturates DAT in the nucleus accumbens this can lead to euphoria, and continued abuse due to elevated DA levels.

3.1.2 Dextroamphetamine

Researchers have shown that d-amphetamine has the same effects in “normal” children and adults; decreasing impulsivity, decreasing total activity, and increasing attentiveness in a dose-specific manner (Castells et al., 2011). It is important to note that these effects are dose-specific as stimulants are often abused at higher doses, to achieve the “high” associated with stimulant-abuse. Both d-amphetamine and methylphenidate enhance performance in various tests such as the continuous
performance task (CPT) in normal adults at therapeutic doses, with impairments in performance with high doses.

Dextroamphetamine significantly increases extracellular levels of dopamine and noradrenaline, via a slightly different mechanism than methylphenidate: d-amphetamine inhibits the reuptake of dopamine and noradrenaline and also increases the release of these neurotransmitters into the extraneuronal space and inhibits the catabolic activity of monoamine oxidase (Kuczenski and Sengal, 1975). Amphetamine is a competitive inhibitor at DAT (methylphenidate is a non-competitive inhibitor), thus amphetamine is transported into the dopaminergic terminal and synaptic dopamine levels increase. Amphetamine (unlike methylphenidate) is also a competitive inhibitor of the vesicular monoamine transporter (VMAT), thus amphetamine can be packaged into vesicles and at high levels (high doses) will displace dopamine from the vesicles into the dopamine terminal. Once a threshold of dopamine has been reached within the terminal, dopamine will be released from the terminal via two mechanisms; channel opening and the reversal of DAT, leading to a massive release of dopamine into the synapse. This rapid release of dopamine into the synapse will lead to the euphoric effect experienced after amphetamine use, and its high abuse potential.

3.1.3 Stimulants and abuse

Treatment of ADHD is dependent on psychostimulant drugs (amphetamine, lisdexamphetamine and methylphenidate) but despite their known clinical efficacy, the exact mechanism of action of these treatments remains poorly understood. Psychostimulants have the potential to potently enhance arousal, increase motor-activity; they also have reinforcing effects and importantly the potential for abuse. The beneficial short-term effects on ADHD symptoms/behaviours have to be carefully weighed against potential adverse effects (Graham and Coghill, 2008; Singh, 2008). There have been concerns regarding the long-term use of stimulants and the risk of developing substance abuse (Humphreys et al., 2013; Singh, 2008). This includes reports of behavioural sensitisation (increased response to a psychoactive agent after repeated exposure) (Boileau et al., 2008) to stimulants and their abuse (Kollins and MacDonald E, 2001; Wilens et al., 2008). However many studies have found no effects of prescribed ADHD medication use on subsequent substance abuse (Barkley et al., 2003; Faraone and Wilens, 2007; Humphreys et al., 2013; Wilens et al., 2003). Some studies have even reported protective effects of prescribed ADHD medication on subsequent substance misuse and abuse (Biederman et al., 2008; Chang et al., 2013).
At low doses psychostimulants are highly effective in reducing ADHD symptoms and enhancing executive function in healthy humans and animals and in individuals with ADHD (Arnsten and Pliszka, 2011). At low therapeutic doses, psychostimulants preferentially target PFC catecholamines, leading to large increases in extracellular levels of dopamine and noradrenaline in the PFC and only modest increases outside of the PFC (Berridge et al., 2006). An inverted-U shape relationship can be used to describe signaling processing in the PFC; low doses enhance signal processing in the PFC, whereas high doses weaken this (Devilbiss and Berridge, 2008). At low doses, stimulants are hypothesised to enhance activity in the prefrontal cortex by facilitating stimulation of dopamine receptors specifically; postsynaptic D1 receptors, and noradrenergic receptors; specifically alpha2A adrenoceptors (Arnsten et al., 2007; Berridge et al., 2006; Devilbiss and Berridge, 2008). These results demonstrate that at low doses, psychostimulants do not necessarily act as ‘stimulants’ but rather as cognitive enhancers, targeting PFC dopamine and noradrenaline.

When using stimulants therapeutically it is usually preferable to obtain a slow-rising, constant, steady-state level of the drug. Under these conditions the firing pattern of dopamine will be tonic and regular. At high doses, psychostimulants stimulate dopamine and noradrenaline efflux (Florin et al., 1995), leading to the reinforcing and motor-activating effects. Dopamine and noradrenaline levels need to be consistently maintained without over- or under-stimulation. Psychostimulants exert their effects through the medial septal area (MSA), medial preoptic area (MPOA) and lateral hypothalamus (LH) to promote arousal (Berridge et al., 1999), presumably via elevated noradrenaline signaling. Elevations in dopamine signaling have also been shown to contribute to the arousal-promoting actions of psychostimulants (Berridge and Arnsten, 2013).

Larger doses mean higher concentrations of psychostimulant per unit time which can cause rapid DAT blockade also thought to cause the reinforcing and abuse-related effects (Volkow, 2006; Volkow et al., 1998). Thus high doses of stimulants may induce the excessive release of dopamine and noradrenaline and so lead to an over-stimulation of other dopamine and noradrenaline receptors (including D1, alpha1, and/or beta1 adrenoceptors) and amplification in phasic firing leading to euphoria and abuse. Over time, stimulants may cause reward conditioning and lead to craving between doses. At the same time there is a lack of pleasurable phasic dopamine firing with only residual tonic firing. This is the point of addiction, and higher doses are now required to induce the pleasurable phasic firing. The next stage will occur when tonic firing is further
reduced and the cravings are replaced with withdrawal symptoms (anhedonia and sleepiness), gradually increasing to aggression and impulsive behaviours. The final stage is reached when depletions of dopamine may lead to irreversible alterations to dopaminergic neurons including cell death and axonal degeneration (Stahl, 2011).

As well as dose, the route of administration of stimulants can also modify their reinforcing effects. Thus when stimulants are abused for their reinforcing effects they are often snorted or injected, but when administered orally at therapeutic doses they have attenuated reinforcing effects (Volkow and Swanson, 2003).

3.1.4 Lisdexamphetamine

Lisdexamphetamine (Elvanse or Vyvanse) was recently developed to address the abuse potential and the limited duration of action of methylphenidate and d-amphetamine. It is the first Food and Drug Administration (FDA) approved pro-drug for the treatment of ADHD in adults and children. Lis-dexamphetamine is therapeutically inactive until it is converted by enzymatic hydrolysis to the active drug – d-amphetamine and l-lysine (Adler et al., 2008a; Weber and Siddiqui, 2009). The duration of effects are suggested to be 13 to 14 hrs. and thus requires once daily dosing, reducing the potential for abuse and diversion (Adler et al., 2013).

3.2 Non-Stimulants

3.2.1 Atomoxetine

Atomoxetine (Strattera) is the first non-stimulant-based medication with proven efficacy in ADHD (Caballero and Nahata, 2003; Kratochvil et al., 2002) and to be approved by the FDA. Atomoxetine has been shown to be effective in children and adults with ADHD (Adler et al., 2008b; Fredrikson et al., 2012). Interestingly, a recent study has revealed sex differences in the efficacy of atomoxetine with a greater improvement in outcome in females compared with males (Marchant et al., 2011). The same study also showed women improved more in terms of emotional and hyperactivity symptoms compared with males. Atomoxetine is the only drug in the UK licenced for use in adults with ADHD, however it is licenced only for adults who were diagnosed with ADHD in childhood.

The role of noradrenaline in the pathophysiology of ADHD has long been investigated (Arnsten et al., 1996; Zametkin and Rapoport, 1987). Noradrenergic neurons project mainly from within the locus coeruleus to regions such as the prefrontal cortices which
are involved in high-level cognitive functions that are often impaired in ADHD such as working memory and inhibitory response control (Chamberlain et al., 2010; Robbins and Arnsten, 2009). There is limited innervation of the striatum by noradrenergic neurons so there is much less of a correlation between noradrenaline and any striatal alterations in ADHD, i.e. reduced stimulant effects.

Atomoxetine acts via a different mechanism to the psychostimulants, as a presynaptic noradrenaline reuptake inhibitor (NRI). Atomoxetine is a selective NAT blocker, and has no known affinity for DAT (Swanson et al., 2006). The primary site of action for atomoxetine is the prefrontal cortex, where it acts to raise noradrenaline levels by blocking noradrenaline reuptake (Swanson et al., 2006). Inhibiting NAT (which also has affinity for dopamine, as described above) will result in an increase in synaptic dopamine levels as well as noradrenaline levels, and thus leads to a further enhancement of the diffusion radius. Animal studies have shown that atomoxetine increases cortical dopamine and noradrenaline levels several-fold following systemic administration, without significant effects on the subcortical dopamine system (Bymaster et al., 2002). The limited effect of atomoxetine on subcortical dopamine is a clinical advantage as it is the subcortical dopamine pathway that has been proposed to be responsible for the drug-abuse potential of psychostimulants (Volkow, 2006).

Atomoxetine is an effective medication for adults with ADHD, it is generally well tolerated and can be administered as a single daily dose or separated into two equal doses. However, unlike the rapid response with stimulants, some patients require 3 to 4 weeks of treatment with atomoxetine before improvements are present (Adler et al., 2008b).

3.3 Summary ADHD pharmacotherapy

Whilst the stimulants are the mainstay of the pharmacological treatment of adults (and children) with ADHD (Biederman et al., 2004; Faraone and Buitelaar, 2010) and appear to be more effective than nonstimulants (Faraone and Glatt, 2010), the time-limited duration of action and abuse potential can limit their utility (Faraone and Wilens, 2007). The relatively high rates of stimulant abuse highlight the need to develop treatment with reduced abuse liability to prevent abuse. Lis-dexamphetamine, is proving a useful alternative with limited abuse potential, however as with all of the current ADHD medication, it is contraindicated in patients with cardiovascular disorders (hypertension, arteriosclerosis, cardiomyopathy, heart rhythm abnormalities). Common side effects of the stimulants can lead to poor adherence; the most common side effects include
anorexia, insomnia, anxiety, palpitations, tachycardia, dry mouth and gastric irritability (Pliszka, 2007). Atomoxetine can cause similar side effects, along with mild increases in blood pressure and heart rate. The more severe, but rare adverse effects of atomoxetine that can limit its use include liver toxicity and increased suicidal thoughts. Atomoxetine also has the problem of the initial time period it takes before the benefits become apparent. This can lead to issues with continued compliance during the initial stages of treatment.

There is a great need for a new selective compound with an improved side effect profile and no abuse potential to treat ADHD. This requires the identification of specific targets that improve the cortical cognitive processes and the striatal motor processes of ADHD. This thesis examines two novel targets for ADHD treatment – the dopamine 4 receptor (DRD4) and catecholamine-O-methyl-transferase (COMT) in a novel animal model of adult ADHD.
4. ANIMAL MODELS OF ADHD

Research over the past 10 years has been hampered and is in need of a vital overhaul of translational research. There is an imminent crisis in mental health drug development (Insel et al., 2012) and it is now imperative to redirect and coordinate preclinical research. Animal models are used as a means of gaining insight into a disease/disorder and as a critical stage in the discovery and development of novel therapeutic strategies. However many pharmaceutical companies are withdrawing from drug discovery in psychiatry (Nutt and Goodwin, 2012), and one suggested reason for this is that the animal models are not predicting viable molecules that will be efficacious in the clinic. Animal model validity in psychiatric research has traditionally been considered in terms of 3 types of validity. 1. Predictive validity; the sensitivity of the proposed model to treatments affecting the disorder in humans; 2. Face validity; the ability of the proposed model to recapitulate behavioural and other symptoms in the human disorder; 3. Construct validity; the relevance of the proposed model to the disorder/disease etiology/pathology in humans (Goldstein et al., 2011). There is now a crucial need for more valid and sensitive animal models for psychiatric disorders. To date, many animal models of ADHD have been proposed, varying from neurotoxin lesions to genetically manipulated models to behavioural models.

4.1 Genetic rat models

4.1.1 Spontaneously Hypertensive Rat

The spontaneously hypertensive rat (SHR) developed in the 1960’s (Okamoto and Aoki, 1963) has been extensively studied as an animal model of ADHD (Sagvolden, 2000; Sagvolden et al., 1998; Sagvolden et al., 1992). The SHR was developed in Japan by inbreeding Wistar-Kyoto (WKY) rats and selecting the rats with hypertension. Hypertension was also noted to produce unexpectedly high spontaneous motor activity (Moser et al., 1988). As well as motor hyperactivity in a novel environment, the SHR exhibits several behavioural characteristics of ADHD including excessive responses under a fixed-interval/extinction schedule, and difficulty acquiring operant tasks (Sagvolden, 2000; Wong et al., 2000; Wyss et al., 1992). Impulsivity is reported to develop over time and is seen as an inability to inhibit a response during the extinction stage of an operant task-impulsive action and an inability to delay a response to gain a larger reward-impulsive choice (Bergh et al., 2006; Bull et al., 2000; Sagvolden, 2000).
These characteristics correlate with the clinical features of ADHD including hyperactivity, impulsivity and learning deficits.

The SHR as a model of ADHD does display elements of construct validity as the behavioural and cognitive abnormalities in the SHR are responsive to stimulant drugs (methylphenidate and D-amphetamine) (Myers et al., 1982; Sagvolden et al., 1992). However the SHR does not respond to methylphenidate in certain behavioural tests (Bergh et al., 2006). The model also shows strong similarities to support the face validity of the SHR as an animal model of ADHD, but the model fails to show sex differences. The SHR and WKY have been strongly criticised, the WKY strain (normotensive control rat) does not perform to the same standard as other strains in various behavioural tasks and has been reported to be less active than other rat strains (Bergh et al., 2006; Bull et al., 2000). The results from studies investigating the performance of the SHR are largely inconsistent and seem to be dependent upon the demands of the task (Sagvolden et al., 2005a; Sagvolden et al., 2005b). Despite the weaknesses of the model, valuable information has been gained from investigations comparing WKY and SHR, specifically the differences in certain behaviour in operant tasks and their neurochemistry. The SHR provides a basis for deficient dopamine-mediated strengthening of neural circuits, and alterations in the noradrenergic neurotransmitter system (Russell, 2001; Russell and Wiggins, 2000). The SHR remains the most characterised model but is limited by the development of hypertension which occurs in early adulthood, therefore does not reflect a model of adult ADHD (Coogan et al., 2012).

4.2 Lesion rat models

4.2.1 6-OHDA lesion

Juvenile rats with neonatal dopamine lesions are widely used to model the hyperactivity observed in ADHD and assess the efficacy of ADHD treatments (Davids et al., 2002a). Neurochemical lesions of dopamine neurons in neonatal rats have been achieved by administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) (Archer et al., 1988; Cole and Robbins, 1989). 6-OHDA injected intra-cisternally is toxic to noradrenergic and dopaminergic neurons leading to a reduction in both dopamine and noradrenaline neurotransmission in the brain. Neuroprotection of the noradrenergic neurons is achieved by pre-treating animals with a noradrenaline reuptake inhibitor such as desmethylimipramine, allowing for the study of dopamine loss alone (approximately 80% of dopamine fibres are lesioned).
The 6-OHDA lesion leads to locomotor hyperactivity during development that persists into adulthood (Luthman et al., 1989). Others have only shown temporary hyperactivity lasting between postnatal day 12 and 22 (Kostrzewa et al., 2008; Shaywitz et al., 1976). Lesioned animals also show impaired learning in a spatial discrimination task, memory deficits (Archer et al., 1988) and impairments in delay discounting (Freund et al., 2014). Hyperactivity, learning and memory deficits are found in children with ADHD, which diminish during adolescence and early adulthood, indicating that the model shows some face validity for aspects of ADHD in childhood. The model supports the notion of a motivational dysfunction in ADHD for behavioural symptoms, including impairments in delay discounting and higher sensitivity to rewards, suggesting the involvement of hypofunction in the PFC (Luman et al., 2005; Sonuga-Barke, 2003). Interestingly sex differences have been observed in the 6-OHDA model, PFC lesions enhanced locomotor activity at postnatal day 60 in males but not in females (Boyce and Finlay, 2009). Freund et al., 2014) have recently extended these findings and reported increases in cortical D1 receptors in 6-OHDA lesioned females but not males, and increases in novelty preference in 6-OHDA lesioned females but not males.

The 6-OHDA lesion model displays good predictive validity; the hyperactivity induced by neonatal 6-OHDA lesions is significantly reduced by amphetamine (Shaywitz et al., 1976) and methylphenidate (Davids et al., 2002b; Heffner and Seiden, 1982; Luthman et al., 1989).

Alterations to the dopaminergic system of relevance to ADHD include correlations between hyperactivity and increases in D4 receptor binding in the striatum. The hyperactivity in this model was enhanced by treatment with a D4 receptor agonist and attenuated by a D4 receptor antagonist (Zhang et al., 2002b; Zhang et al., 2001). Interestingly DAT inhibitors had no effect on hyperactivity (Davids et al., 2003). Clinically increasing attention is being given to the involvement of the DRD4 gene in ADHD (Russell, 2007), see chapter 8.

Loss of dopamine during early neurodevelopment leads to profound changes in the serotonin system, including serotonergic hyper-innervation of the striatum but not the cerebral cortex (Zhang et al., 2002b), as well as noradrenergic changes. The serotonergic system has been implicated in the regulation of impulsive behaviour in ADHD. Impaired serotonergic transmission can have significant modulatory effects on behavioural inhibition (Dalley and Roiser, 2012). Antagonising both serotonin and noradrenaline transporters reduced motor hyperactivity in 6-OHDA lesioned animals.
(Davids et al., 2002b). Similarly dopamine and serotonin depleted animals treated with 5-HT2 receptor agonists showed a decrease in hyperactivity (Brus et al., 2004). These findings indicate that psychostimulant medication may reduce hyperactivity in the 6-OHDA lesioned rats by inhibiting NAT and SERT (Russell, 2007). In support of this, a recent PET scan study using monkeys has reported that, at therapeutic doses, atomoxetine occupies both NET and SERT (Ding et al., 2014).

4.3 Behavioural rat models

4.3.1 Poor performers in the 5-choice serial reaction time task (5-CSRTT)

The 5-choice serial reaction time task (5-CSRTT) is a widely employed rodent operant cognitive task adopted from the human continuous performance task (CPT) that measures aspects of attention and motor impulsivity (Bari et al., 2008; Robbins, 2002). The neural circuitry and neurochemistry underlying performance of the CPT and 5-CSRTT provide a reasonable degree of correlation between the two species-dependent paradigms (Robbins, 2002). Human CPTs have been used to study the attention deficits in ADHD (Corkum and Siegel, 1993; Sonuga-Barke et al., 2008). CPTs are sensitive in detecting attention deficits in ADHD (Riccio and Reynolds, 2001; Teicher et al., 2004) and require the subject to respond to a signal and inhibit from responding to non-signal stimuli, assessing impulsivity and response inhibition. The human CPT is an accepted measure of sustained attention and vigilance (Riccio and Reynolds, 2001). Vigilance defines the ability to remain alert toward impending stimulus information (Collings, 2003) often referred to as phasic alertness (Posner and Peterson, 1990). Clinical studies have highlighted the importance of differentiating between sustained attention and vigilance when assessing subjects with ADHD-I and ADHD-C using the CPT, as the two subtypes differ in their attentional profiles (Egeland et al., 2009; Tucha et al., 2009). ADHD-C subjects show less of a deficit in sustained attention but are less vigilant, whereas ADHD-I subjects had a sustained attention deficit and normal vigilance (Egeland et al., 2009).

The 5C-SRTT has been proposed as a preclinical analogue of the human continuous performance test (CPT) (Robbins, 2002). In 5-CSRTT the animals are required to learn to nose-poke into one of five apertures after a brief visual stimulus (simple cue light) has been presented in that aperture, a correct nose-poke results in a food reward. The 5-CSRTT requires animals to attend closely as the stimulus presentation is brief (typically 1-10 seconds).
The 5-CSRTT is regarded as a test that can measure attention and impulsivity. Disturbances in attention and inhibitory control over behaviour play a central role in the symptomatology of ADHD. Using the 5-CSRTT, it was noted that some apparently normal Lister-hooded rats displayed attentional deficits and impulsivity (Puumala et al., 1997a; Puumala et al., 1997b; Puumala et al., 1996). By selecting the rats within a normal “population” that display deficient sustained attention, poor choice accuracy towards the end of training sessions, and demonstrate impulsiveness (measured by the number of premature responses) these have been suggested to provide a useful model of ADHD (Puumala et al., 1996; Robinson et al., 2009). The rats selected using these measures are generally selected for poor overall performance. Poor performers of the 5-CSRTT were found not to be hyperactive and therefore may be proposed as an animal model of aspects of the inattentive subtype of ADHD (Puumala et al., 1996).

Trait-like impulsivity in rats, determined by high levels of anticipatory responses made during the ITI in the 5-CSRTT has been elegantly used to investigate the neurobiology underlying impulsivity (Dalley et al., 2007; Robinson et al., 2009). Rats selected for spontaneously high levels of impulsivity have been shown to also exhibit high levels of impulsive decision-making in a delay-of-reward task (Robinson et al., 2008). The same animals, were however unimpaired in a task requiring inhibition of an already initiated motor response (stop-signal task) (Robinson et al., 2009). These animals have been proposed as a model for the impulsive symptoms in ADHD, specifically modeling the aspect of impulsivity relating to the inability to wait, not the form of impulsivity involved in stopping responses once initiated. 5-CSRTT poor performers as an animal model of ADHD has been shown to fulfill some aspects of predictive validity.

4.3.1.2 5-CSRTT and ADHD pharmacological agents

Methylphenidate has produced varied results in the 5-CSRTT, marginally improving sustained attention performance (measured by choice accuracy) in poor performers, but at higher doses (1mg/kg) increasing the number of premature responses (a measure of impulsivity) (Puumala et al., 1996). Atomoxetine has been shown to reduce impulsivity, specifically impulsive action (measured by premature responses) in a sub-population of rats selected for a high level of premature responding in the 5-CSRTT and when enhancing the demands of the 5-CSRTT (Blondeau and Dellu-Hagedom, 2007; Gaalen et al., 2006; Navarra et al., 2008; Paterson et al., 2011; Robinson et al., 2008; Sun et al., 2011). Administration of amphetamine has been shown to increase premature responding in the 5-CSRTT (Cole and Robbins, 1987, 1989; Gaalen et al., 2006;
The stimulant and non-stimulant agents that affect catecholamine reuptake (methylphenidate and atomoxetine) or enhance the release of monoamine neurotransmitters (amphetamine) have been shown to improve attentional performance in the 5-CSRTT, but their effects appear to depend on the task conditions and baseline levels of performance.

### 5-CSRTT- Lesions

Quinolinic acid lesions in the medial prefrontal cortex, peri- and post-genual anterior cingulate cortex, and the parietal cortex in rats produced deficits in accuracy in the 5-CSRTT. The medial PFC effects were long-lasting, these rats still showed impairments following a reduced stimulus duration, they also showed increased latencies suggesting an exchange of speed for accuracy. Lesions of the antero-dorsal PFC also produced lengthened latencies when the stimuli were presented unpredictably. Rats with anterior-cingulate cortex lesions showed increases in premature responding and normal attentional performance (Muir et al., 1996). Interestingly Muir et al. (1996) have also shown that attentional measures in the 5-CSRTT were not affected by parietal cortex lesions. These findings suggest that the parietal cortex is not primarily involved in attention, or that the 5-CSRTT is not an appropriate task for assessing attention associated with the parietal cortex. It is evident that the anterior cortex is important for the control of performance in the 5-CSRTT, which is supported by the lack of effects of hippocampal lesioning on performance in 5-CSRTT trained rats (Kirkby and Higgins, 1998).

Lesions to the lateral striatum produce significant performance deficits in the 5-CSRTT, and these rats only complete a very small number of trials (Rogers et al., 2001). Lesions of the medial striatum result in a substantial disruption in 5-CSRTT performance, but is not however as disrupted compared with the lateral striatal lesions, the animals do still manage to complete a larger number of trials. However these rats do show major impairments in response accuracy, increased latencies and increases in premature and preservative responding. Interestingly the medial PFC and the medial striatum have shown to display a functional interaction. Studies have shown that lesions to the medial PFC in one hemisphere combined with a lesion to the medial striatum in the opposite hemisphere produces behavioural impairments that are as prominent as that of bilateral lesions to either cortical or striatal structures, and cannot be seen by just one lesion of either structure alone or in the same hemisphere.
The dorsal and ventral striatum have been shown to be functionally dissociable. Dopaminergic lesions of the ventral striatum did not affect performance accuracy but did reduce response latencies (Robbins et al., 1986).

Bilateral lesions of the nucleus accumbens have also produced performance impairments in the 5-CSRTT, opposite to the effects produced by lesions of the dorsomedial and dorsolateral striatum (Rogers et al., 2001). Lesions of the nucleus accumbens produced no effects on accuracy but did affect perseverative behaviour, only observed after a positive reinforcement or timeout (Cardinal et al., 2001; Christakou and Robbins, 2004; Winstanley et al., 2003). The timeout period, signaled by a period of darkness (following a failed trial), does not relate directly to a positive reinforcement but does indicate to the animal a delay in the opportunity of reinforcement. These findings suggest that the nucleus accumbens is part of networks involved in the modulation of attentional performance by affective outcome. Christakou et al (2004) have elegantly shown how combined unilateral lesions of the medial prefrontal cortex and the core sub-region of the nucleus-accumbens in opposite hemispheres (disconnection) significantly affected aspects of response control related to affective feedback.

4.3.2 5 choice continuous performance task (5C-CPT)

Previously, the 5C-SRTT has been proposed as a preclinical analogue of the human continuous performance test (CPT) (Robbins, 2002). The 5C-SRTT measures sustained attention and impulsivity, however the 5C-SRTT lacks non-target trials (used in human CPTs) requiring response inhibition. This is a limitation of the 5C-SRTT as this limits the accuracy of the task in measuring vigilance in a way consistent with human CPTs (Barnes et al., 2012b). To effectively assess vigilance a number of factors must be taken into consideration including; inattentiveness, stimulus discrimination inability, and poor impulse control (Eagle et al., 2008). Assessing response inhibition in the 5C-SRTT is limited by containing only target-trials (Young et al., 2009). Response inhibition has emerged as one of the principle paradigms for studying ADHD (Aron and Russell, 2005), and impairments of response inhibition an important etiological marker of the disorder (Castellanos and Tannock, 2002).

As a result of the translational limitations of the 5C-SRTT, the 5-choice continuous performance test (5C-CPT) was developed. The 5C-CPT has been validated in mice (Young et al., 2009), rats (Barnes et al., 2012a, b), and, importantly, has recently shown
cross-species validity to humans (McKenna et al., 2013; Young et al., 2013). The 5C-CPT is more consistent with the human CPT paradigm compared with the standard 5C-SRTT. Like the human CPT, the 5C-CPT presents both target and non-target trials, allowing for the same measurements of various responses including; false alarm responding, hits, misses, correct rejections (Riccio et al., 2002). Vigilance can be measured by correct rejections and false alarms to non-target trials, and as hits and misses to target trials. The 5C-CPT allows for detailed analysis of vigilance using signal detection theory (SDT), which is often used in human CPT analysis (Huang-Pollock et al., 2012). Behavioural response inhibition can be measured, like the human CPT, by false alarm responding to non-target trials (Young et al., 2011). This is an important measure when testing an animal model of ADHD as enhanced false alarm responding often accompanies or determines impaired CPT performance in clinical ADHD (Crosbie et al., 2008; Groman et al., 2009; Kuntsi et al., 2006).

The 5C-CPT therefore, may provide a useful tool in finding novel targets for improved pharmacotherapy for adult ADHD. This thesis explores the 5C-CPT as a tool for modelling adult ADHD in rats and to test novel targets.
5. NEUROCOGNITIVE DEFICITS IN ADHD

Neurocognitive deficits, particularly involving attention and executive function have been reported in children with ADHD (Barkley et al., 1992; Barnett et al., 2001; Burden and Mitchell, 2005; Grodzinsky and Diamond, 1992; Kempton et al., 1999; Oosterlaan et al., 2005; Pennington and Ozonoff, 1996; Rhodes et al., 2005; Seidman et al., 1997; Tannoch et al., 1995; Williams et al., 2000) and more recently in adults with ADHD (Bekker et al., 2005; Boonstra et al., 2005; Gallagher and Blader, 2001; Johnson et al., 2001; Lijffijt et al., 2005; Tsal et al., 2005; Woods et al., 2002). Children with ADHD have also shown deficits in planning, working-memory, set shifting and response inhibition, which are associated with dorsolateral and medial prefrontal cortex function (Kain and Perner, 2003). The deficits in executive function reported were only mild and not consistently observed in ADHD (Willcutt et al., 2005b).

5.1 Executive Function

Executive function (EF) refers to a wide range of higher order cognitive processes that regulate behaviour, emotion and cognition. ADHD has been associated with impairments in executive function across the lifespan (Doyle, 2006), however others have shown inconsistent results (Sonuga-Barke, 2005). The most apparent and consistent EF deficits in ADHD include: response inhibition, vigilance, spatial working memory and planning (Willcutt et al., 2005a). Distinct executive function impairment profiles for ADHD subtypes in adults have not yet been established. In a meta-analysis of 83 studies in children with ADHD, the authors failed to find differences in executive function between ADHD-C and ADHD-I subtypes (Willcutt et al., 2005a).

5.1.2 Spatial working memory

Working memory is the process by which individuals retain information no longer present in the immediate environment that is not required for later use (Baddeley, 2003). Working memory has been suggested to be particularly impaired in adults with ADHD in comparison to other psychiatric disorders with EF impairments (Gallagher and Blader, 2001). A recent study comparing adults with ADHD and adults with borderline personality disorder have supported these findings showing greater impairments in spatial working memory (SWM) in the ADHD group (Dowson et al., 2004). SWM impairments have also been correlated with ADHD self-ratings in adults with ADHD.
Many EF tasks require non-EF ability, working memory tasks involve general intelligence, short-term maintenance memory and executive ability (Baddeley, 2003; Sergeant et al., 2003).

Treatment with methylphenidate has been shown to improve SMW in healthy adults (Clatworthy et al., 2009; Elliot et al., 1997; Mehta et al., 2000) and in adults (Ni et al., 2013) and children (Barnett et al., 2001; Bedard et al., 2004; Kempton et al., 1999; Mehta et al., 2004a) with ADHD. Impaired performance on SWM tasks, typically involving increased errors and a disturbed strategy score, has been found in patients with frontal lobe lesions (Owen et al., 1990). The main areas of interest include the dorsolateral and ventrolateral prefrontal cortex (Owen et al., 1990; Owen et al., 1996) and the posterior parietal cortex (Ikkai and Curtis, 2011). Improvements by methylphenidate in SWM have been shown to occur with task-related reductions in regional cerebral blood flow in the dorsolateral prefrontal cortex and posterior parietal cortex in healthy adults (Mehta et al., 2001). Clatworthy et al. (2009) reported that methylphenidate had significant effects on SWM, predicted by changes in ventral striatal D2/D3 receptor availability in healthy adults. A recent study in rats has demonstrated how direct injection of methylphenidate directly into the dorsolateral PFC (dorsal prelimbic and anterior cingulate cortex) improves working memory (Spencer et al., 2012) suggesting the PFC as the site of action for the cognitive enhancing effects of methylphenidate. Acute treatment (single dose) of atomoxetine had no effects on SWM in adults with ADHD. However longer-term treatment (8-10 weeks) improved SWM in adults (Chamberlain et al., 2007) and children (Gau and Shang, 2010b) with ADHD.

5.1.3 Attentional set-shifting

Set-shifting is described as the ability to move back and forth between numerous tasks, operations and mental sets (Miyake et al., 2000). Set-shifting has recently been described as a potential endophenotype for ADHD (Boonstra et al., 2008). However despite several studies investigating set-shifting in ADHD the results to date have proven largely inconclusive (Barkley et al., 2008; Piek et al., 2007; Rohlf et al., 2012). A recent study in a large sample of adults with ADHD showed set-shifting deficits in this group even after controlling for working memory and intelligence (Halleland et al., 2012). The authors used Color Word Inference Test (CWIT) taken from the Delis Kaplan Executive Function System (D-KEFS) (Halleland et al., 2012). Others have used the intra-extra dimensional shift task taken from the Cambridge
Neuropsychological Test Automated Battery (CANTAB), and shown deficits in set-shifting in children with ADHD (Gau and Shang, 2010a; Goldberg et al., 2005; McLean et al., 2004; Vance et al., 2003). Most of the research to date has focused on children and adolescents; more studies are needed to elucidate the role of set-shifting deficits in adults with ADHD.

Treatment with methylphenidate and atomoxetine have produced mixed results. In children with ADHD methylphenidate improved accuracy in the CANTAB set-shifting task (Mehta et al., 2004a), however others have shown no change (Coghill et al., 2007; Elliot et al., 1997; Rhodes et al., 2006); these differences in results can may be explained by different age groups and task difficulties. Methylphenidate has failed to improve accuracy in set-shifting in healthy adult control subjects (Elliot et al., 1997; Turner et al., 2003). However interestingly, a recent RCT has reported improvements in set-shifting in adult ADHD patients after methylphenidate treatment (Ni et al., 2013). These results from ADHD patients and healthy controls indicate that baseline levels of performance are related to the level of improvement observed before treatment. There are a limited number of studies examining the effects of atomoxetine on set-shifting in ADHD, to date groups have shown improvements in shifting flexibility and flexibility of attention in set-shifting tasks (Chamberlain et al., 2007; Gau and Shang, 2010b). However studies in adults with ADHD are not in line with this finding, and have shown no improvement in set-shifting (Ni et al., 2013). The cognitive effects of both atomoxetine and methylphenidate seem dependent on baseline levels of performance and thus baseline neurotransmitter activity (Chamberlain et al., 2010). Studies have shown that many different regions of the PFC play critical roles in set-shifting functions (reviewed in: Bissonnette et al., 2013).

5.1.4 Response inhibition

One aspect of the complex construct of impulsivity includes response inhibition. Response inhibition is the ability to suppress or inhibit inappropriate motor responses. Adults with ADHD frequently show deficits in response inhibition; largely assessed by stop-signal tasks (SST), rapid visual neuropsychological tasks or CPTs. Treatment with atomoxetine has improved performance (response inhibition) in the CANTAB SST, in adults with ADHD (Chamberlain et al., 2007) and healthy control adults (Chamberlain et al., 2009; Chamberlain et al., 2006a). Methylphenidate at low doses has improved
response inhibition deficits in adults (Aron et al., 2003a) and children (DeVito et al., 2009) with ADHD.

The stop-signal reaction task (SSRT) has been hypothesised to be under the control of the noradrenergic system and has been shown to be unaffected by manipulations of the serotonin and dopamine systems in animals and humans (Chamberlain et al., 2006a; Chamberlain et al., 2006b). Response inhibition has been shown to be dependent on the right inferior frontal gyrus, bilateral anterior cingulate cortices and supplementary motor area (Aron et al., 2007; Chamberlain et al., 2010; Hampshire et al., 2010; Rubia et al., 2001; Winstanley et al., 2006a).

5.1.5 Rapid visual information processing

Sustained attention can be measured by rapid visual information processing (RVP) tasks, which are similar to the CPT and generate the A’- prime measure (Gau and Huang, 2014). Impaired sustained attention and increased reaction times are related to the core symptoms of ADHD. These two characteristics have been assumed to be associated with dopaminergic dysregulation in frontal circuits. The RVP task engages activation of the bilateral inferior frontal gyrus, parietal cortex, fusiform gyrus and right frontal superior gyrus (Coull et al., 1996). RVP task performance requires two cognitive processes; working memory and sustained attention. Functional imaging studies have revealed two distinct neural circuits underlying these cognitive processes (Garavan, 2003). Sustained attention is related to the right fronto-parietal network, and working memory involves left frontal activation (Coull et al., 1996).

Studies have shown that children with ADHD have impairments in RVP performance (Gau and Huang, 2014; Hammerness et al., 2014) compared with health controls. Gau and Huang (2014) have suggested impairments in RVP performance as a cognitive endophenotype of ADHD. One study reports no difference in RVP performance in adults with ADHD compared with controls (Chamberlain et al., 2007). Despite research in children and adolescents, studies in adults are lacking. Studies have shown that treatment with methylphenidate in healthy controls does not alter accuracy performance in the RVP i.e. no enhancement of sustained attention (Elliot et al., 1997; Turner et al., 2003). Furthermore methylphenidate treatment in individuals with ADHD does appear to improve accuracy (sustained attention) both in children (Hammerness et al., 2014) and in adults (Turner et al., 2005). Treatment with atomoxetine has provided mixed
results, in adults with ADHD Chamberlain et al. (2007) found no improvements in sustained attention following a single dose. However longer-term treatment in adults with ADHD has produced improvements in RVP measures, including sustained attention (Ni et al., 2013). More research is required to determine the long-term effects of methylphenidate and atomoxetine on RVP performance.

5.2 Neurocognitive assessment

Cognitive function is commonly evaluated with neuropsychological tests and validated rating scales. Cognitive tests commonly used for individuals with ADHD include the Stroop task (selective attention, inhibition), the Continuous Performance Test (CPT; sustained attention, processing speed), the Trail Making Test (psychomotor speed, visual search, working memory, set shifting), the Go-No-Go Test (response inhibition), Wisconsin Card Sorting Test (abstract reasoning, concept formation, working memory, set shifting), the Controlled Word Association Test (verbal fluency), and the Tower of London (planning ability).

A recent approach used to improve the measurement of neurocognitive abilities is the use of computerised assessment batteries. One of the most widely used batteries is the CANTAB (Fray et al., 1996). This thesis presents findings using CANTAB tests, this battery was chosen as CANTAB tests have been validated in clinical trials, academic research and healthcare provision. CANTAB tests are designed to be culturally and language independent, and are sensitive to disorder related cognitive impairments. CANTAB tests are supported by over 1,300 peer-reviewed articles (Cambridge Cognition Ltd).

5.3 Social cognition

Social cognition describes the ability to understand the mind and feelings of other people (Uekermann et al., 2008; Uekermann and Daum, 2007) and is imperative for successful social interaction. Social cognitive functions including; emotion recognition and theory of mind, are viewed to be critical for the development of effective social functioning (Frith and Singer, 2008).
5.3.1 Social functioning

Impairments in interpersonal and social functioning in individuals with ADHD has been widely recognised in children (Nixon, 2001) and in adults (Schutte and Petermann, 2006). The early concept of ADHD – minimal brain dysfunction included emotional symptoms as a central feature of the disorder (Barkley, 2006). Currently, ADHD assessment rating scales and diagnostic criteria including the Utah Criteria for ADHD include temper, mood instability, and emotional over-reactivity as key features of ADHD (Wender, 1995). The Conners’ Adult ADHD Rating Scale and the Brown Adult ADHD Rating Scale also include emotion symptoms (Brown, 1995; Conners et al., 1999). All versions of the DSM since 1968 have included emotional traits as associated features of ADHD but not a core feature of the disorder (Association, 2000). Barkley proposes that these emotional traits should be considered as a core feature of ADHD and not as only associated symptoms (Barkley, 1997). Impairments in social cognition in ADHD during childhood have been shown to be significant predictors of serious negative outcomes in adolescence and adulthood (Green et al., 1997; Mrug et al., 2012). Interestingly, only a limited number of studies examining social cognition in adults with ADHD have been conducted (Gonzalez-Gadea et al., 2013; Lis et al., 2013; Uckermann et al., 2010).

5.3.2 Emotional regulation

Emotional self-regulation refers to the regulation of physiological arousal driven by emotions, inhibition of inappropriate behaviour to positive and/or negative emotions, and refocusing attention from strong emotions (Surman et al., 2011). Deficits in emotional self-regulation have been reported in adults with ADHD (Barkley, 2010; Barkley and Murphy, 2010; Barkley et al., 2008; Reimherr et al., 2005; Reimherr et al., 2007) and in the siblings of adults with ADHD with deficient emotional self-regulation (Surman et al., 2011). Surman et al (2011) suggest these deficits may represent a subtype of ADHD based on the findings of familial inheritance of these deficits. Deficient emotional self-regulation traits can include easily and quickly angered, temper outbursts, low frustration tolerance and being easily excited in response to emotional reactions (Surman et al., 2011). Barley et al (2008) found that up to 60% of adults with ADHD in a clinical sample had traits of deficient emotional self-regulation compared to 15% of control participants. Deficient emotional self-regulation in ADHD has been strongly associated with the impact on quality of life (Surman et al., 2013). Recently,
treatment with methylphenidate in adults with ADHD has been shown to improve emotional symptoms as measured by the observer rated 10-item Emotional Dysregulation Scale (EDS) derived from the Wender-Reimherr Adult Attention Deficit Disorder Scale (WRAADDS) and a self-report, 6-item Emotional Lability Scale (ERS) extracted from the long version of the Conners’ Adult ADHD Self-Report Scale (CAARS:S:L) (Rosler et al., 2010).

5.3.3 Emotion recognition

Facial emotion recognition is imperative for normal socialisation and personal interaction. Emotion recognition can be measured by presenting an individual with faces conveying an emotion, then asking the individual to name the emotion conveyed by the face. Impairments in the ability to correctly recognise different emotions from facial expressions can be associated with low social competence, low popularity in peer groups and may result in antisocial behaviours such as aggression (Marsh and Blair, 2008). Distress related cues (sadness and fearful expressions) have been shown to play a vital part in inhibiting antisocial behaviour (Marsh and Blair, 2008). Impairments in recognition in social cues have been proposed as an independent risk factor for interpersonal disturbances in ADHD. Children with ADHD as well as having a high number of social problems also show reduced social competence (Lee et al., 2012), and have also been found to be less accepted by peers (DuPaul et al., 2004; Hoza et al., 2005). Research has suggested that children with ADHD have deficits in overall emotion-processing (Da Fonseca et al., 2009), and deficits in facial emotion recognition (Boakes et al., 2008; Collin et al., 2013; Kagan et al., 1964; Passarotti et al., 2010b; Pelc et al., 2006; Schwenck et al., 2013; Shin et al., 2009; Sinzig et al., 2008), indicating impairments in social cognition in childhood. Others have suggested that the symptoms of ADHD appear to have an impact on facial emotion recognition in children with ADHD and children with autism and ADHD (Sinzig et al., 2008). Children with ADHD seem to have particular difficulty in recognising negative emotions (Cadesky et al., 2000; Singh et al., 1998; Williams et al., 2008). Others have not found such deficits in emotion recognition (Downs and Smith, 2004; Greenbaum et al., 2009).

Studies investigating social cognition, specifically emotion recognition in adults with ADHD are lacking and have presented mixed findings (Cadesky et al., 2000; Miller et al., 2011; Rapport et al., 2002). Research is particularly limited with respect to differences in emotion recognition deficits between different ADHD subtypes in
adulthood. To the author’s knowledge, only one study has evaluated emotion recognition in adult ADHD subtypes; Miller et al (2011) have shown deficits in nonverbal affect recognition in adults with ADHD, with a specific link to inattentive symptoms. Children with the inattentive subtype of ADHD, have also been shown to be socially less aggressive and less likely to develop oppositional defiant disorder or conduct disorder, than children with the combined or hyperactive/impulsive subtypes (Eiraldi et al., 1997; Maedgen and Carlson, 2000; Willcutt et al., 1999).

Successful emotion recognition has been suggested to play a key role in social malfunctioning (Dadds et al., 2012), and is clearly a target of significant importance for future ADHD therapy with particular focus on adulthood. The studies outlined in this thesis compare treated and untreated adults with ADHD, comparisons are made between treatment groups and healthy controls. This thesis also assesses the correlations between specific emotion recognition deficits and symptom expression in adults with ADHD.

5.3.4 Theoretical models of emotion processing in ADHD

Several lines of evidence support the presence of emotion recognition deficits in individuals with ADHD. These deficits may reflect a primary social cognitive deficit or may be secondary to a more general cognitive dysfunction (e.g. inattention or impulsiveness). It is vital to take these concepts into consideration when investigating emotion dysfunction and ADHD, as cognitive deficits are defining symptoms of the disorder. It is also important for therapeutic purposes to distinguish whether or not the emotion recognition deficits are emotion-specific or due to inattention or impulsivity. Thus, if the deficits were due to social perception difficulties then one should target treatment towards this aspect specifically i.e. employ a social cognitive training approach. Whereas if the deficits were due to inattention or poor inhibitory control, then treatment should aim to improve these general cognitive deficits which should, theoretically, lead to a subsequent improvement in emotion recognition abilities.

Emotion recognition deficits explained by a specific impairment in processing information about emotions, is a theoretical hypothesis proposed by socio-cognitive approaches. There are several studies in support of this hypothesis conducted in children and/or adolescents with ADHD. Yuill and Lyon (2008) investigated the question of whether or not emotion recognition deficits were a specific social cognitive or general cognitive impairment. The study included boys aged five to eleven with ADHD and a
control group of ADHD boys who performed the same face-processing task as the first group but with the inclusion of an ‘inhibitory scaffolding’ method, which was used to prevent impulsive responding. The authors showed that ADHD boys had a selective difficulty in facial expression processing compared with controls. Interestingly the emotion matching was not markedly improved by the ‘inhibitory scaffolding’; suggesting a specific socio-cognitive impairment in ADHD (Yuill and Lyon, 2007). In accordance with these findings, Da Fonseca et al (2009) using an emotion recognition task and an emotion recognition task based on conceptual cues have found impaired emotion processing in children and adolescents with ADHD (n=27). Importantly the authors demonstrated that these deficits are unrelated to a general cognitive dysfunction e.g. impulsivity, suggesting a specific emotion-processing deficit (Da Fonseca et al., 2009). The results from work in adolescent boys with ADHD, also suggest a specific emotion recognition pattern not due to attention problems. This conclusion was drawn, as overall emotion recognition did not alter during the test, indicating that changes in selective attention toward the emotion stimuli were unaffected (Aspan et al., 2014). Findings in adult ADHD are also in agreement with these findings. Rapport et al (2002) demonstrated that adults with ADHD (n=28) performed worse than healthy controls on recognition tasks involving affective stimuli, in contract to when responding to tasks with non-affective stimuli recognition. The authors assert that the emotion recognition deficits observed in adults with ADHD are not due to fundamental deficits in attention and/or impulsivity (Rapport et al., 2002).

In contrast to the findings above are the results from studies concluding that emotion recognition deficits only occur as a result of a general cognitive dysfunction (e.g. attention and impulsivity). Visual attention deficit has been suggested to be a moderator of emotion recognition in children with ADHD. Again, the majority of studies are in children and adolescents with ADHD, with only one study in adults. Cadesky et al (2000) examined how individuals with ADHD perceive emotion using a reasonably sized sample (n=86) of children with ADHD. They showed that ADHD children had deficits in emotion recognition (more random errors than a clear bias) and this was due to problems in encoding affective stimuli due to a general cognitive dysfunction (e.g. attention) (Cadesky et al., 2000). In agreement with these results are those from studies using attentional tasks including the CPT alongside a facial emotion recognition task. These show that inattention accounted for or contributed to the emotion recognition deficits seen in children with ADHD (Shin et al., 2009; Sinzig et al., 2008). Shin et al (2009) demonstrated that visual-attention is important in the
contribution of errors in children with ADHD during an emotion recognition task. Sinzig et al (2008) also highlighted an effect of response inhibition on emotion recognition deficits. This finding has been replicated in a recent study using an emotional Go/No-Go task in children with ADHD (Kochel et al., 2014). Other studies accounting for attention in social perception assessment have also shown that attention can contribute to inaccurate or incomplete encoding (Corbett and Glidden, 2010) and interpretation (Fine et al., 2008) of affective stimuli. In agreement with these findings is the only study in adults with ADHD (n=51) in which patients were separated into groups based on their DSM-IV ADHD subtype classification. The authors found, contrary to the results of Rapport et al (2002), that the deficits in emotion recognition were only present in the ADHD-I group. This suggests that the deficits are largely related, predominately to inattentive symptoms and not a specific underlying deficit in emotion processing (Miller et al., 2011). The authors highlight the need for continued research across the life span in ADHD, and acknowledge their limitations including the small sample size and the highly intelligent participants. The results from these studies suggest a wider origin of social dysfunction, not a specific part of social behaviour. This thesis will address this by using a larger sample from two distinct geographical regions with a range of intelligence across the group. These studies also highlight the importance of future studies controlling for inattention and response inhibition. The work outlined in this thesis will use various measures of different cognitive processes including; attention, working memory and response inhibition, in an attempt to control for the possible contributing effects of cognitive deficits on emotion recognition difficulties. This thesis aims to explore the issue of whether or not emotion recognition deficits are due to a specific socio-cognitive problem or a general cognitive dysfunction.
6. GENERAL AIMS, OBJECTIVES AND HYPOTHESES

Detailed aims can also be found at the beginning of each chapter (7, 8, 9, 10) at the end of the introduction section.

Main hypothesis

The overall modulatory effects of dopaminergic compounds are affected by individual differences in attention and response inhibitory control, thus the compounds modulation of behaviour is baseline-dependent.

The current animal models of ADHD involve artificial manipulations, e.g. genetic manipulation and brain lesions. Taking a translational approach to model endophenotypes rather than attempting to recapitulate the entirety of such a complex, heterogeneous disorder is most likely to provide enhanced understanding of the disorder and therapeutic approaches.

i. To establish an animal model of the core symptoms of adult ADHD using a translational behavioural paradigm.

To establish an animal model of adult ADHD, these studies will utilise the translational behavioural paradigm – the 5C-CPT to evaluate inattentive and impulsive behaviour in some detail. After training to criterion, the variability of behaviour (attention and impulsivity) of a group of adult female Lister-hooded rats will be assessed. The rats’ behavioural performance will be assessed in terms of two forms of attention (sustained attention and vigilance) and two forms of impulsivity (motor impulsivity and response inhibition). The highly inattentive rats will be selected to form one group (low-attentive; LA), and the remaining rats with normal to high levels of attention will form the comparison group (high-attentive; HA). The same procedure will be followed for assessing impulsivity and selecting for impulsive rats; the impulsive rats will form one group (high-impulsive; HI) and the normal to low impulsive rats, the comparison group (low-impulsive; LI). The LA group will form the model of the ADHD-I clinical subtype and the HI group will form the model of the impulsive symptoms in the ADHD-C and ADHD-HI clinical subtypes of adult ADHD. The behaviours will then be combined and animals with impairments in
response inhibition, sustained attention and vigilance will then be selected to model the ADHD-C subtype of adult ADHD.

**Hypothesis:** Previous research utilising the 5-CSRTT has shown that rats can be separated based on sustained attention (accuracy) and motor impulsivity (premature responses) into distinct groups (high and low impulsive and poor performers). Therefore it is hypothesized that the rats in the studies in this thesis will also show the same individual variations in the same measures (accuracy and premature responding) and the additional domains assessed by the 5C-CPT – response inhibition and vigilance.

Limited research exists comparing the effects of methylphenidate and atomoxetine in the different endophenotypes (subtypes) of adult ADHD. Methylphenidate and atomoxetine have not been assessed previously using the 5C-CPT.

**ii. To assess the selective effects of standard ADHD medication on the different behavioural phenotypes using animal models.**

The effects of atomoxetine and methylphenidate on the different behavioural symptoms will be assessed in two ADHD symptom models (LA and HI) and in the two comparison groups (HA and LI). Each drug will be administered at three doses to enable a dose-response evaluation, and a comparison vehicle group will be used to evaluate overall effects. Following drug treatment the animals will be challenged in the 5C-CPT by enhancing the attentional load. The behavioural outcome measures used for evaluation will include; sustained attention, vigilance, response bias, motor impulsivity, response inhibition and response speed.

**Hypothesis:** Previous research investigating the effects of methylphenidate and atomoxetine has provided largely, inconsistent results. The most consistent finding is the improvement in sustained attention following treatment with methylphenidate and reduction in premature responding (motor impulsivity) following treatment with atomoxetine in the 5-CSRTT. The majority of these studies have be conducted in male rats however, it is predicted that female rats will respond in a similar manner. Previous studies have also highlighted the difference in responses to these drugs, to be dependent on the baseline level of
behaviour. Thus it is predicted that in the 5C-CPT, inattentive rats (with normal baseline levels of impulsivity) will respond to methylphenidate by increasing sustained attention and vigilance. Atomoxetine is predicted to reduce impulsivity in highly impulsive rats both in terms of response inhibition and motor impulsivity.

Current medications used in treatment of ADHD (stimulants and non-stimulants) have many unwanted side effects, and the stimulants have the potential for abuse. New therapeutic agents are required for adult ADHD. The generation of more selective therapeutic agents, with improved side effect profiles requires the identification of a specific target that improves the cognitive processes impaired in ADHD.

iii. To investigate potential novel therapeutic targets for treating adult ADHD.

Two compounds targeting the dopaminergic system will be evaluated in a group of animals (ADHD-C) modeling the combined subtype of adult ADHD, compared with a group of ‘normal’ animals (HA group). A selective DRD4 agonist and a COMT inhibitor will be administered at a range of doses to allow for a dose-response evaluation. Both compounds will be tested in the 5C-CPT under conditions of increased attentional load. The behavioural measures outlined above (ii) will be compared with a vehicle treated group, and with the HA control group.

Hypothesis: The DRD4 receptor has been suggested as a target to improve symptoms in ADHD. DRD4 agonists have been shown to have procognitive effects in normal rats and in the SHR rat. Recent work in D4 knockout mice has shown that these mice have attenuated response inhibition and attention deficits in the 5C-CPT. Thus, it is hypothesized that, a DRD4-agonist will improve response inhibition subsequently leading to improvements in attention in rats with baseline levels of inattention and response inhibition impairments (ADHD_C rats). COMT inhibitors in humans have been shown to improve various cognitive measures including attention, executive function and working memory. Therefore it is predicted that the COMT inhibitor used in these studies
will improve attention deficits with minimal or no effects on impulsivity in ADHD-C animals.

There are a limited number of large sample studies in adult ADHD using the CANTAB neuropsychological test battery to determine the core cognitive deficits in adults with ADHD. To date no single study has examined all four cognitive domains using the CANTAB tests – RVIP, SST, IED and SWM in adult ADHD. Also to date, gender differences in cognitive performance in unmedicated adults with ADHD using CANTAB tasks have not been investigated.

iv. **To evaluate the core cognitive deficits in treated and untreated adult patients with ADHD.**

In these studies, a sample of adults with ADHD will be recruited and divided into groups depending on whether or not they are stable on ADHD medication (medicated group), or are not yet taking medication (unmedicated group). Different domains of cognition including spatial working memory, sustained attention, response inhibition and attentional set-shifting will be assessed using the RVIP, SST, IED and SWM CANTAB tasks. To determine the deficits; the outcome measures will be compared between groups and with a group of healthy participants without ADHD. The unmedicated group will be split based on gender, and the results of females across each cognitive domain will be compared to males. The control group will also be split based on gender and the same comparisons made, to establish if there are any cognitive differences across the same cognitive domains in healthy participants.

**Hypothesis:** It is well documented that adults with ADHD have deficits in executive function, response inhibition and working memory. However results using the CANTAB battery are inconsistent and are generally from smaller samples. It is hypothesized, in line with research using other neuropsychological tasks, that unmedicated adults with ADHD will have impairments in all domains assessed – sustained attention, response inhibition, attention set-shifting and spatial working memory, compared with healthy controls. It is also predicted that medicated patients will perform to a comparable level as healthy controls. There is less research examining
differences between males and females, some research using working memory tasks demonstrates that females perform worse than males. Thus, in this thesis it is hypothesized that females in all 3 groups (ADHD, unmedicated, ADHD medicated and controls) will perform worse than males on the spatial working memory task.

Currently there are no reports using the CANTAB emotion recognition task (ERT) to assess emotion recognition abilities in adults with ADHD. There are a limited number of studies using different facial emotion recognition tasks in adult ADHD. Those existing studies have produced conflicting findings using modest sample sizes.

v. **To investigate specific emotion recognition impairments in adults with ADHD.**

By using the CANTAB ERT, two groups of adults with ADHD will undertake an assessment of emotion recognition ability. The groups will consist of 1. Patients stable on medication and 2. Patients not yet taking medication. The task will consist of different emotions including both positive (surprise and happiness) and negative (fear, disgust, anger and sadness) emotions. Scores reflecting the correct recognition of each emotion will be compared between the medicated and unmedicated ADHD groups and with a group of healthy controls.

**Hypothesis:** In agreement with previous findings utilising facial emotion recognition tasks it is hypothesized that unmedicated ADHD patients will have impairments in the recognition of fear and anger.

Currently the evidence reported in the literature is divided between theoretical models of emotion recognition in ADHD. These models include; a ‘social-perception’ hypothesis proposed by socio-cognitive approaches and a ‘general cognitive dysfunction’ hypothesis proposed by cognitive behaviour models.

vi. **To investigate whether the emotion recognition deficits in adults with ADHD are emotion-specific deficits or are as a result of a general cognitive dysfunction**
The two groups of adults with ADHD will complete the ERT and CANTAB tasks of attention and impulsivity (as outlined above). The results will be analysed in a way to incorporate the measures of attention and impulsivity in the model by using them as covariates. This model of analysis will allow for the control of attention and impulsivity, and identify if the emotion deficits remain as a specific deficit or are due to inattention and/or impulsivity.

**Hypothesis:** At this point this remains an exploratory study; currently there is evidence to support both theories of emotion recognition. Interestingly only one study exists in adults, and lends support to a specific deficit in emotion recognition, not resulting from an attention deficit, however this is in a smaller sample than those used in the studies in this thesis.

A small number of studies have investigated the effects of methylphenidate in adolescents with ADHD on emotion recognition abilities and have reported intriguing findings. However, to date, no studies exist examining the effects of ADHD medication on emotion recognition in adults with ADHD.

**vii. To investigate the effects of short-term treatment with a stimulant medication on emotion recognition in adults with ADHD.**

A group of adults with ADHD will be assessed using the CANTAB ERT to establish a ‘before treatment’ baseline measurement of emotion recognition abilities. This group will be followed-up 2-3 months after starting treatment with methylphenidate and re-tested to obtain the ‘after treatment’ measurement. The emotion recognition scores for each emotion will be compared before and after treatment to assess the effects of treatment. The results from both time points will also be compared with a group of healthy controls to establish if the deficits are normalised following treatment.

**Hypothesis:** Only one study exists examining the effects of methylphenidate on emotion recognition, however this study was in a group of adolescents with ADHD. It is predicted that adults (used in the studies in this thesis) will respond in a similar way to the adolescents in that study – and that methylphenidate will improve the emotion recognition deficits observed.
MAIN CONTRIBUTIONS
7. PAY ATTENTION TO IMPULSIVITY: MODELLING LOW ATTENTIVE AND HIGH IMPULSIVE SUBTYPES OF ADULT ADHD IN THE 5-CHOICE CONTINUOUS PERFORMANCE TASK (5C-CPT) IN FEMALE RATS.

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Abstract

Varying levels of attention and impulsivity deficits are core features of the three subtypes of adult attention deficit-hyperactivity disorder (ADHD). To date, little is known about the neurobiological correlates of these subtypes. Development of a translational animal model is essential to improve our understanding and improve therapeutic strategies. The 5-choice continuous performance task (5C-CPT) in rats can be used to examine different forms of attention and impulsivity. Adult rats were trained to pre-set 5C-CPT criterion and subsequently separated into subgroups according to baseline levels of sustained attention, vigilance, premature responding and response disinhibition in the 5C-CPT. The behavioural subgroups were selected to represent the different subtypes of adult ADHD. Consequently, effects of the clinically used pharmacotherapies (methylphenidate and atomoxetine) were assessed in the different subgroups. Four subgroups were identified: low-attentive (LA), high-attentive (HA), high-impulsive (HI) and low-impulsive (LI). Methylphenidate and atomoxetine produced differential effects in the subgroups. Methylphenidate increased sustained attention and vigilance in LA animals, and reduced premature responding in HI animals. Atomoxetine also improved sustained attention and vigilance in LA animals, and reduced response disinhibition and premature responding in HI animals. This is the first study using adult rats to demonstrate the translational value of the 5C-CPT to select subgroups of rats, which may be used to model the subtypes observed in adult ADHD. Our findings suggest that this as an important paradigm to increase our understanding of the neurobiological underpinnings of adult ADHD-subtypes and their response to pharmacotherapy.
7.1 Introduction

Adult attention deficit hyperactivity disorder (ADHD) is becoming a recognised psychiatric condition with a prevalence of 3-5% in the general population (Fayyad et al., 2007; Kessler et al., 2005; Simon et al., 2009) however its neurobiology remains poorly understood (Asherson et al., 2010). The DSM-V (DSM-V; American Psychiatric Association, 2013) suggests three subtypes, predominantly inattentive, predominantly hyperactive-impulsive and the combined type. Predominantly inattentive is the most prevalent subtype in adulthood, and hyperactive-impulsive has been argued not to exist as a clinical condition in adulthood (Gibbins et al., 2010).

Animal models with enhanced construct validity and translation to the clinic are essential to improve our understanding of the underlying pathophysiology and to develop improved therapeutic strategies. Selecting animals from within a normal population that display extreme behavioural traits may provide an opportunity to model subtypes of the disease in humans (Blondeau and Dellu-Hagedom, 2007; Dalley et al., 2007; Puumala et al., 1997b; Puumala et al., 1996; Puumala and Sirvio, 1998). This is of particular translational relevance to ADHD as the core symptoms of inattention, impulsivity and hyperactivity may represent extreme manifestations of normal behaviours. Blondeau et al (2007) demonstrated that subtypes in rats could mimic aspects of those observed in children. However to date no animal model of adult ADHD exists, nor have studies focused on the continuation of symptoms into adulthood; i.e. reduced motor hyperactivity, inattention and impulsivity. A focus on single symptomatic phenotypes (endophenotypes) rather than attempting to recapitulate the entirety of such a complex, heterogeneous condition has been recommended, taking a translational approach (Coogan et al., 2012).

Such a model must utilise a task that has demonstrated translational validity and should use adult subjects. Previously, the 5 choice serial reaction time task (5-CSRTT) has been proposed as a preclinical analogue of the human continuous performance test (CPT) (Robbins, 2002). The CPT is sensitive to detection of attention deficits in ADHD (Riccio and Reynolds, 2001; Teicher et al., 2004) and requires the subject to respond to a signal stimulus and inhibit from responding to non-signal stimuli, assessing impulsivity and response inhibition. Previous groups have utilised the 5-CSRTT to separate rats into subgroups using accuracy as a measure of sustained attention and premature responses as a measure of impulsive behaviour (Bari et al., 2008; Dalley et
al., 2007; Puumala et al., 1996; Puumala and Sirvio, 1998; Robinson et al., 2008). The 5-CSRTT lacks non-target trials (used in human CPTs) requiring response inhibition and so the 5-CSRTT may not assess vigilance in a manner consistent with human CPTs (Young et al., 2009). Response inhibition has emerged as one of the principle paradigms for studying ADHD symptomatology (Aron and Russell, 2005). Indeed, impairments of response inhibition are an important etiological marker of the disorder (Castellanos and Tannock, 2002).

To improve the translational validity, the 5-CSRTT was modified to include target and non-target trials, in a procedure called the 5-choice continuous performance test (5C-CPT), which has been validated in both mice (Young et al., 2013; Young et al., 2009; Young et al., 2011), and rats (Barnes et al., 2012a, b; Byrne et al., 2013). The 5C-CPT is potentially more convergent with the human CPT and has recently been reverse translated to humans (McKenna et al., 2013; Young et al., 2013). As the 5C-CPT presents both target and non-target trials, a number of parameters including false alarm responding, hits, misses and correct rejections are produced (Riccio et al., 2002). These indices allow for detailed analysis of vigilance using signal detection theory (SDT), often used in human CPT analysis (Huang-Pollock et al., 2012). Behavioural response inhibition can also be measured, as in the human CPT, by false alarm responding to non-target trials (Young et al., 2011). This is an important measure when developing an animal model of ADHD as enhanced false alarm responding often accompanies or determines impaired CPT performance in the clinic (Crosbie et al., 2008; Groman et al., 2009; Kuntsi et al., 2006).

To date preclinical studies have focused on ADHD as a childhood disorder. Studies utilising the selection of spontaneously occurring extreme behaviours of relevance to ADHD (inattention, impulsivity and hyperactivity) have utilised the 5-CSRTT to assess behaviour. No studies to date have examined the effects of psychostimulant (methylphenidate) and non-stimulant (atomoxetine) pharmacotherapeutic agents in rats with extreme behavioural traits from within a normal population using the 5C-CPT paradigm.

The dual aims of the present study were: to demonstrate the value of the 5C-CPT as a translational test for selecting subgroups of high and low attentive, and impulsive adult rats under baseline conditions and; to compare the effects of methylphenidate and atomoxetine in the selected subgroups to examine the validity of this model for the
inattentive subtype of adult ADHD and impulsive symptoms in the combined and predominately hyperactive-impulsive subtypes of adult ADHD.

7.2 Experimental Procedures

7.2.1 Subjects and housing conditions

Subjects were 40 adult female Lister-hooded rats (Charles River, UK; weighing 240 ± 10g at the start of training) housed in groups of five on a reversed 12 hr. light: dark cycle (lights on at 19:00 h). We used female rats as the 5C-CPT has been carefully validated in female rats in our laboratory (Barnes et al. 2012a; 2012b) see supplement 1 for further justification for using female rats. All animals were housed in a temperature (21 ± 2°C) and humidity (55 ±5%) controlled environment. Animals had free access to food (Special Diet Services, UK) and water until one week prior to training when food restriction was initiated. Thereafter rats were maintained at approximately 90% of their free-feeding body weight (fed 10g rat chow/rat/day). Water was available for the duration of this study ad libitum. All experiments took place in the dark phase of the light: dark cycle under red light, illumination of the light in the chamber was a punishment following an omission or an incorrect or premature response. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and University ethical guidelines.

7.2.2 Apparatus

The 5-choice test apparatus consisted of eight 25 cm x 25 cm aluminium chambers, each enclosed within a wooden sound attenuating box. Within each box there was a low-level fan to provide ventilation and mask extraneous background noise. The rear wall of the testing chamber contained nine individual apertures, four of which were occluded, leaving apertures 1, 3, 5, 7 and 9 free for presentation of light stimuli. All eight chambers were connected to a PC and data collection and initial analysis was controlled by K-limbic software (Conclusive Solutions), which generated an Excel spread sheet (Microsoft) containing the raw data for detailed statistical analysis.

7.2.3 5C-CPT behavioural procedure

The 5C-CPT procedure (Young et al., 2009) is an extension of the standard 5-CSRTT procedure (Bari et al., 2008; Robbins, 2002); both methods include target trials in which the animal must respond by nose-poking. The 5C-CPT procedure also includes non-
target trials in which the animal must withhold from responding to a stimulus in order to receive a food reward.

The training procedure used was similar to the original mouse 5C-CPT procedure (Young et al. 2009), with one alteration – initially placing greater emphasis on non-target trials by adjusting the proportion of target and non-target trials. The procedure is well established in our laboratory and has been described in detail previously by Barnes et al. 2012a; 2012b. See table 1 for a full description of 5-CSRTT and 5C-CPT measures. Training sessions consisted of 120 trials and lasted 30 min, the session ended when either 120 trials had been completed or 30 min reached. Training began with stimulus duration (SD) and limited hold duration (LH) set at 10 s. The proportion of target and non-target trials per session was 77 target trials and 43 non-target trials. When the rats successfully discriminated between trial-type (p[HR] > p[FA] – approximately 16 weeks), the number of target trials was increased to 84 and non-target trials decreased to 36. As animals’ performance improved, the SD was gradually reduced to the target parameter of 2 s SD, and the LH reduced to 2 s. The SD was reduced for individual rats in stages (10, 8, 4 and 2 s). The inter-trial interval (ITI) and time out (TO) remained constant throughout training (TO 5 s and ITI variable mean 5 s). Animals had to satisfy set criteria to progress to the next stage of training until reaching the target criterion of >70% accuracy, <25% omissions, >65% correct rejections for two consecutive days. Animals progressed through the eight stages of training and were fully trained when they reached the target parameters under the standard training conditions (2 s SD, 5 s ITI, 2 s LH) for two consecutive days. Two animals were excluded at this point, as they did not achieve criteria. Animals were trained Monday to Friday and took approximately 24 weeks to reach target test criterion. Once test criterion was achieved, animals were trained 3 times per week to prevent over-training and to enable other animals to reach criterion.
### Table 1: Description of the behavioural measures used in the 5C-CPT

<table>
<thead>
<tr>
<th>Measure &amp; Description</th>
<th>Measure &amp; Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5-CSRTT</strong></td>
<td><strong>5C-CPT</strong></td>
</tr>
<tr>
<td><strong>Target trials</strong></td>
<td><strong>Non-target trials</strong></td>
</tr>
<tr>
<td><strong>Accuracy:</strong> Correct responses/(correct + incorrect) x 100.</td>
<td>Percent correct rejections: Correctly withheld/(correctly withheld + false alarms) x 100</td>
</tr>
<tr>
<td>A measure of sustained attention.</td>
<td><strong>Hit rate, p[HR]:</strong></td>
</tr>
<tr>
<td><strong>Percent correct:</strong> Correct responses/total number of trials x 100.</td>
<td>Correct</td>
</tr>
<tr>
<td><strong>Percent omission:</strong> Omissions/(correct + incorrect + omissions) x 100.</td>
<td>Correct + Incorrect + Omission</td>
</tr>
<tr>
<td><strong>Premature response:</strong> Inappropriate responses made during the ITI. A measure of motor impulsivity.</td>
<td>The proportion of target trials correctly detected.</td>
</tr>
<tr>
<td><strong>Perseverative response:</strong> Inappropriate repeat response following a correct response.</td>
<td><strong>False alarm rate, p[FA]:</strong></td>
</tr>
<tr>
<td><strong>Correct latency:</strong> Time taken to make a correct response.</td>
<td>( FA )</td>
</tr>
<tr>
<td><strong>Incorrect latency:</strong> Time taken to make an incorrect response.</td>
<td>( FA + CR )</td>
</tr>
<tr>
<td><strong>Magazine latency:</strong> Time taken to retrieve food reward. Measure of motivational state.</td>
<td><strong>Sensitivity index, SI:</strong></td>
</tr>
<tr>
<td>2(p[HR] - p[FA]) / (2(p[HR] + p[FA]) - (p[HR] + p[FA])^2)</td>
<td>Non-parametric measure of the ability to discriminate between target and non-target trials. Measure of vigilance</td>
</tr>
<tr>
<td><strong>Responsivity Index, RI:</strong></td>
<td>( p[HR] + p[FA] - l ) / ( l - (p[HR] - p[FA])^2 )</td>
</tr>
<tr>
<td>( p[HR] + p[FA] - l ) / ( l - (p[HR] - p[FA])^2 )</td>
<td>Non-parametric measure of strategy or response bias</td>
</tr>
</tbody>
</table>

Table 1: adapted from Barnes et al. (2012a). Further information regarding the formulation of these measures may be found in Amitai et al. (2007) and Young et al. (2009)

#### 7.2.4 Experimental design part I- Selection of subgroups

#### 7.2.4.1 The attentive subgroups

Rats must have achieved the set criterion for two consecutive days to progress to the subgroup selection stage. Variability between attentional and impulsivity measures was observed in individual animals and was used to formulate the subgroups. Following acquisition of the 5C-CPT (approximately 24 weeks) 38 rats were screened for 5 consecutive days under standard training conditions. The means of the attentional measures (accuracy, and SI) were calculated for the 5 days for individual animals. Using the calculated attentional means, the rats were then assigned to either a high-attentive (HA) or a low-attentive (LA) subgroup, according to the following parameters:
HA (>90% accuracy, and SI >0.3) and LA (<90% accuracy, and SI <0.3). The six animals that showed significant fluctuations in attentional performance over the 5 days (i.e. means meeting highest and lowest quartiles) were excluded, giving 16 of each HA and LA.

7.2.4.2 The impulsive subgroups

The same 40 rats were also screened for impulsivity over 5 consecutive days, following the attention (LA and HA) experiments under standard training conditions. The means of impulsivity measures (premature responses and p[FA]) were calculated for the 5 days for individual animals. Using the calculated impulsivity means, the rats were then assigned to either a high-impulsive (HI) or low-impulsive (LI) subgroup, according to the following parameters: HI (>10 premature responses, >0.3 p[FA]) and LI (<10 premature responses, <0.3 p[FA]). Six animals showing significant fluctuations in impulsivity measures over the 5 days (i.e. means meeting highest and lowest quartiles) were excluded giving 16 of each HI and LI. These 6 animals were not the same as animals excluded from the attentive subgroups.

7.2.5 Experimental design part 2 – behavioural testing

7.2.5.1 Drugs

Methylphenidate hydrochloride (Tocris Cookson, Bristol, UK) and atomoxetine hydrochloride (Kemprotec Limited, Middlesbrough, UK) were dissolved in 0.9% NaCl and administered intraperitoneally (IP) in a volume of 1 ml/kg, 30 and 20 min before behavioural testing respectively. Vehicle (0.9% NaCl) was administered IP at the same time as drug treatments. Each test day was separated by a three-day washout period (no drugs or testing).

7.2.5.2 Behavioural testing

Methylphenidate and atomoxetine were tested in HA, LA, HI and LI rats using the 5C-CPT. For all experiments, drugs were administered according to a Latin-square within subjects design with a minimum of 72 h between drug challenge sessions. Animals were tested on a Tuesday and Friday and trained under standard conditions in between test days for one day (Wednesday). On the remaining days (Monday and Thursday) the animals were not trained or tested. Experiments were separated by a 1-week washout period.
Methylphenidate and atomoxetine low and high attentive

After animals were separated into two subgroups HA (n=16) and LA (n=16) they were then randomised to either receive atomoxetine or methylphenidate (half of each subgroup received each drug, i.e. n=8). Atomoxetine (0.5, 1.0, 2.0 mg/kg) or vehicle (0.9% NaCl) was administered to HA (n=8) and LA (n=8) subgroups. Methylphenidate (0.5, 1.0, 2.0 mg/kg) or vehicle (0.9% NaCl) was administered to HA (n=8) and LA (n=8) subgroups. Doses of both compounds were selected based on previous studies in male rats utilising the 5-CSRTT (Paterson et al 2011, Robinson et al 2008). Animals were tested in the 5C-CPT at an increased variable ITI (10 s), 2 s SD and 2 s LH. The ITI was increased from 5 s (variable) to 10 s (variable) to challenge their performance (see appendix 1 for detailed explanation of ITI used).

Experiment 2 – methylphenidate and atomoxetine high and low impulsive subgroups

After animals were separated into two subgroups: HI (n=16) and LI (n=16), the same procedure as above was followed for drug administration. However the ITI was increased from 5 s (variable) to 20 s (variable) to challenge the animal’s performance. This was because the same animals from experiment 1 were used in experiment 2, to ensure the animals remained challenged, a longer variable ITI (20 s) was utilised.

7.2.6 Data and statistical analysis

Data were expressed as mean ± SEM and analysed using SPSS version 16.0. The data were checked for normality and where appropriate (normally distributed) were analysed using parametric measures. Overall subgroup performance using individual measures (e.g. accuracy, premature responses etc.) were normally distributed and analysed by one-way between-subjects ANOVA followed by the post-hoc test Fisher’s Least Significant Difference test.

Analysis of 5C-CPT performance following drug treatment was conducted by two-way repeated measures ANOVA with Dose as the repeated measure and Group the between-subjects factor. Where appropriate; when the overall ANOVA was significant, post-hoc comparisons were made with Fisher’s Least Significant Difference (LSD) analysis. A level was set to 0.05.
7.3 Results

7.3.1 Comparison of low-attentive and high-attentive subgroups

One-way ANOVA followed by Fishers LSD analysis showed that accuracy was significantly reduced \([F(1,38)=8.61, p<0.001]\) in LA compared with HA animals, fig 1A, table 2. The same group showed a significant deficit in SI \([F(1,38)=7.21, p<0.001]\) compared with the HA group, fig 1B and table 2.

Table 2: Baseline performance for attentive and impulsive sub-groups.

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>HA</th>
<th>LI</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>115.25 ± 2.75</td>
<td>110.84 ± 2.62</td>
<td>111.31 ± 2.68</td>
<td>115.14 ± 4.78</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>84.24 ± 1.42***</td>
<td>96.54 ± 0.77</td>
<td>92.60 ± 2.30</td>
<td>87.71 ± 3.31</td>
</tr>
<tr>
<td>% Omission</td>
<td>41.79 ± 5.32***</td>
<td>25.82 ± 2.23</td>
<td>30.59 ± 3.86</td>
<td>30.74 ± 4.69</td>
</tr>
<tr>
<td>Correct latency</td>
<td>0.69 ± 0.03</td>
<td>0.65 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.64 ± 0.03</td>
</tr>
<tr>
<td>Incorrect latency</td>
<td>0.83 ± 0.08</td>
<td>0.79 ± 0.11</td>
<td>0.84 ± 0.09</td>
<td>0.78 ± 0.10</td>
</tr>
<tr>
<td>Magazine latency</td>
<td>1.52 ± 0.16</td>
<td>1.45 ± 0.12</td>
<td>1.45 ± 0.14</td>
<td>1.44 ± 0.17</td>
</tr>
<tr>
<td>Premature responses</td>
<td>14.54 ± 3.30</td>
<td>9.64 ± 3.03</td>
<td>5.49 ± 1.37</td>
<td>20.69 ± 2.84***</td>
</tr>
</tbody>
</table>

Correct rejections (%) | 50.40 ± 3.23*** | 78.54 ± 2.18 | 71.76 ± 4.42 | 59.57 ± 7.98
p[HR]                 | 0.26 ± 0.02*** | 0.50 ± 0.01  | 0.45 ± 0.03  | 0.38 ± 0.14  |
p[FA]                 | 0.50 ± 0.03*** | 0.21 ± 0.02  | 0.27 ± 0.03  | 0.40 ± 0.05*** |
Incorrect latency     | 0.67 ± 0.05   | 0.64 ± 0.03  | 0.65 ± 0.03  | 0.64 ± 0.05  |
Magazine latency      | 1.94 ± 0.34   | 1.75 ± 0.19  | 1.76 ± 0.28  | 1.72 ± 0.28  |
SI                    | -0.24 ± 0.05***| 0.32 ± 0.04  | 0.20 ± 0.09  | 0.01 ± 0.11  |
RI                    | -0.26 ± 0.03  | -0.31 ± 0.02 | 0.23 ± 0.02  | -0.26 ± 0.02 |

The subgroups are high-attentive (HA) and low-attentive (LA); and low-impulsive (LI) and high-impulsive (HI). Measures are shown as observed mean ±SEM. Statistically significant different values are in bold (***p<0.001) LA compared with HA, and HI compared with LI.

7.3.1.1 Effects of methylphenidate and atomoxetine following an increased ITI (10 s) in LA and HA subgroups (experiment 1)

Methylphenidate

Repeated measures two-way ANOVA revealed a Group x Dose interaction for accuracy \([F(3,42)=4.75, p<0.01]\). Vehicle HA SI was significantly greater than in LA animals \((p<0.001, \text{Fig 2b})\). Post-hoc analysis revealed that accuracy was lower in vehicle LA compared with HA animals \((p<0.01, \text{Fig 2a})\). Methylphenidate (2 mg/kg) improved performance specifically in LA animals, compared with vehicle \((p<0.01, \text{Fig 2a})\), to a level equivalent to that of control HA animals. A significant interaction between Group and Dose was not observed for SI \([F(3,42)=1.95, \text{NS}]\). However, planned comparisons
revealed that 0.5 and 1.0 mg/kg significantly increased SI in LA animals ($p<0.01$, Fig 2b).

*Atomoxetine*

Repeated measures two-way ANOVA revealed a Group x Dose interaction for accuracy [$F_{(3,42)}=4.46$, $p<0.01$]. Post-hoc analysis revealed that accuracy was lower in vehicle LA animals compared with vehicle HA animals ($p<0.05$, Fig 3a). Atomoxetine (2 mg/kg) significantly improved performance in LA animals compared with vehicle ($p<0.05$, Fig 3a), to a level comparable to control HA animals. However, 1.0 mg/kg significantly reduced accuracy in HA animals ($p<0.01$, Fig 3a). ANOVA also revealed a Group x Dose interaction for SI [$F_{(3,42)}=4.69$, $p<0.05$]. SI was significantly lower in vehicle LA compared with HA animals ($p<0.05$, Fig 3b). Doses of 1.0 and 2.0 mg/kg improved performance in LA animals only ($p<0.01$, Fig 3b). Repeated measures two-way ANOVA revealed a Group x Dose interaction for go magazine latency [$F_{(3,42)}=3.40$, $p<0.05$]. Post-hoc analysis revealed that go magazine latency was increased at 2.0 mg/kg in LA and HA animals compared with vehicle animals ($p<0.05$). ANOVA did not reveal any significant interactions for the remaining measures.
**Figure 1:** Subgroup differences in attention. (A) Subgroup differences in target trial accuracy in the 5C-CPT under standard conditions, (B) Subgroups differences in vigilance (SI). The subgroups are high-attentive (HA) and low-attentive (LA). Data are expressed as the mean ± SEM over 5 days, n=16 per sub-group. One-way ANOVA followed by post-hoc analysis showed a significant reduction in accuracy and SI; SI denotes sensitivity index; the non-parametric measure of vigilance (***p<0.001) compared with HA.

**Figure 2:** Effects of methylphenidate (0.5, 1.0 and 2.0 mg/kg, i.p. 20 min prior to testing) on overall performance in the 5C-CPT with an enhanced attentional load.
(variable ITI 10 s) in HA and LA subgroups. (A) Attentional measures in target-trials (accuracy). (B) Vigilance in the two subgroups; SI denotes sensitivity index; the non-parametric measure of vigilance and the ability to discriminate between target and non-target trials. All data are expressed as mean ± SEM, asterisks (**p<0.01) indicate significant differences compared to vehicle, crosses (+++p<0.001) indicate significant differences between vehicle HA and vehicle LA animals. All data were analysed using repeated measures two-way ANOVA followed by planned post-hoc pairwise comparisons.

**Figure 3:** Effects of atomoxetine (0.5, 1.0 and 2.0 mg/kg, i.p. 30 min prior to testing) on overall performance in the 5C-CPT with an enhanced attentional load (variable ITI 10 s) in HA and LA subgroups. (A) Attentional measures in target-trials (accuracy). (B) Vigilance in the two subgroups; SI denotes sensitivity index; the non-parametric measure of vigilance and the ability to discriminate between target and non-target trials. All data are expressed as mean ± SEM, asterisks (*p<0.05, **p<0.01) indicate significant differences compared to vehicle, crosses (+p<0.05) indicate significant differences between vehicle HA and vehicle LA animals. All data were analysed using repeated measures two-way ANOVA followed by post-hoc comparisons.

7.3.2 Comparison of high-impulsive and low-impulsive subgroups

One-way ANOVA followed by planned LSD analysis showed that HI animals made significantly more premature responses \([F(1,38)=10.87, p<0.001]\) and had significantly increased p[FA] \([F(1,38)=10.69, p<0.001]\), compared with LI animals, figs. 4a, 4b and table 2.

7.3.2.1 Effects of methylphenidate and atomoxetine following an increased ITI (20 s) challenge in HI and LI subgroups

**Methylphenidate**

Repeated measures two-way ANOVA revealed a Group x Dose interaction for premature responding \([F(3,42)=4.70, p<0.05]\). Premature responding was higher in vehicle HI compared with LI animals \((p<0.05, \text{fig 5a})\). Methylphenidate at 0.5 and 1.0 mg/kg significantly reduced premature responses in HI animals compared to vehicle \((p<0.05; p<0.01)\), to a level comparable with vehicle LI animals, (fig 5a). Methylphenidate (2.0 mg/kg) significantly increased premature responses in LI animals.
(p<0.05, fig 5a). No significant interactions between the remaining measures were observed.

Atomoxetine

Repeated measures two-way ANOVA revealed a Group x Dose interaction for premature responding [F(3,42)=8.39, p<0.001]. Premature responding was significantly higher in vehicle HI compared with vehicle LI animals (p<0.001, fig 6a). Atomoxetine at all doses significantly reduced premature responding in HI animals compared with vehicle (p<0.001), to a level comparable with vehicle LI animals (fig 6a). A significant Group x Dose interaction for [pFA] was evident [F(3,42)= 4.47, p<0.01]. Post-hoc analysis revealed that 1.0 and 2.0 mg/kg significantly reduced FA responding in HI animals (p<0.01; p<0.05) compared with vehicle, (fig 6b). Atomoxetine at 2.0 mg/kg also significantly reduced the [pFA] in LI animals compared with vehicle (p<0.05, fig 6B). Repeated measures two-way ANOVA revealed a Group x Dose interaction for no/go magazine latency [F(3,42)=3.39, p<0.01]. Magazine latency was significantly higher following treatment with atomoxetine in LI animals at all three doses (p<0.01; p<0.05; p<0.05, respectively) compared with vehicle LI animals.

ANOVA revealed a Group x Dose interaction for SI [F(3,42)=3.45 p<0.05]. Doses of 1.0 and 2 mg/kg of atomoxetine significantly improved performance in HI animals compared to vehicle (p<0.01; p<0.05, fig 6c). No significant interactions in the remaining measures were observed.
Figure 4: Subgroup differences in impulsivity. (A) Subgroup differences in the total number of premature responses in both target and non-target trials and (B) Subgroup differences between the proportion of incorrectly rejected non-target trials; false alarm rate (p[FA]) in the 5C-CPT under standard conditions. The subgroups include; HI and LI and data are expressed as the mean ± SEM, over 5 days n=16 per group. One-way ANOVA followed by post-hoc analysis showed a significant increase (**p<0.01) in impulsivity (premature responses and p[FA]) in HI compared with LI subgroups.

Figure 5: Effects of methylphenidate on impulsivity in the 5C-CPT in response to the challenge of an increased variable ITI (20 s). (A) Impulsivity measures in the 5C-CPT (premature responses). (B) The false alarm rate (response inhibition) in HI and LI subgroups. All data are expressed as mean ± SEM, (*p<0.05, **p<0.01) significant differences compared to vehicle, crosses (+p<0.05) indicate significant differences.
between vehicle HA and vehicle LA animals. All data were analysed using repeated measures two-way ANOVA followed by post-hoc comparisons.

Figure 6: Effects of atomoxetine on impulsivity in the 5C-CPT in response to the challenge of an increased variable ITI (20 s). (A) Impulsivity measures in the 5C-CPT (premature responses). (B) The false alarm rate (response inhibition) in HI and LI subgroups. (C) Vigilance in two subgroups; SI denotes sensitivity index; the non-parametric measure of vigilance and the ability to discriminate between target and non-target trials. All data are expressed as mean ± SEM, (**p<0.01, *p<0.05) indicate significant differences compared to vehicle, crosses (+++p<0.001) indicate significant differences between vehicle HA and vehicle LA animals. All data were analysed using repeated measures two-way ANOVA followed by post-hoc comparisons.

7.4 Discussion

The subgroups identified in this study may represent the first animal model of the inattentive subtype (ADHD-I) of adult ADHD, and the impulsive traits in the combined (ADHD-C) and predominately hyperactive-impulsive (ADHD-HI) subtypes of adult ADHD. The LA subgroup demonstrated deficits in sustained attention and vigilance and the HI subgroup were more impulsive and lacked response inhibition. Interestingly, although the numbers of animals in each subgroup were equal this was not a median split in performance as animals were allocated to subgroups based on individual performance measurements. Each of the subgroups showed behavioural extremes of impulsivity or inattention. Methylphenidate, the first line National Institute for Health and Clinical Excellence (NICE) recommended adult ADHD treatment option, increased sustained attention and to a limited extent - vigilance, in inattentive animals, and reduced motor impulsivity in impulsive animals. However methylphenidate also increased motor impulsivity in low impulsive animals. The second-line NICE recommended treatment, atomoxetine, increased sustained attention and vigilance in LA animals and reduced motor impulsivity and response disinhibition in HI animals with no effects in LI animals.

Our studies, in addition to demonstrating impairments in sustained attention as supported by previous studies (Blondeau and Dellu-Hagedom, 2007; Paterson et al., 2011; Puumala et al., 1996), have uniquely used the 5C-CPT to select a LA subgroup showing deficits in sustained attention and vigilance, thereby enhancing translation to the ADHD-I subtype in the clinic. The LA animals were unable to sustain attention and
respond vigilantly, an effect not caused by enhanced impulsive behaviour, consistent with clinical findings. Individuals classified as having ADHD-I do not display high levels of impulsivity (Biederman et al., 2000). Hence these deficits mimic those in sustained attention and vigilance observed in the clinical ADHD-I subtype (Faraone et al., 2006a) again suggesting enhanced translation to the clinical population.

Selecting high-impulsive rats on the basis of high premature responding is consistent with previous studies (Blondeau and Dellu-Hagedom, 2007; Dalley et al., 2007; Robinson et al., 2008). However, impulse control and importantly response inhibition remain critical to the cognitive impairments in adult ADHD (Faraone et al., 2006a). Therefore our selection of a HI subgroup using the 5C-CPT, including animals with both high motor impulsivity and deficits in response inhibition (as assessed by false alarms) provides enhanced translation to the ADHD-HI and ADHD-C clinical subtypes.

Both methylphenidate and atomoxetine increased accuracy and thus increased sustained attention in LA animals only. In agreement with this finding, Puumala (1996) with methylphenidate and Robinson et al. (2008) with atomoxetine, have also shown increased sustained attention in poor 5-CSRTT performing rats. In addition to assessing sustained attention, the 5C-CPT also generates the sensitivity index (SI) which corresponds with d' commonly used in human CPTs as a measure of vigilance. SI has previously been used to measure vigilance in the 5C-CPT in rats (Barnes et al., 2012a, b), and in mice (Young et al., 2009). D' is consistently reduced in ADHD (Collings, 2003), indicating that deficits in vigilance are a core symptom of ADHD. This is the first study, by using the 5C-CPT, to show that atomoxetine improved vigilance in a baseline-dependent manner, alleviating deficits in LA animals. Atomoxetine also enhanced vigilance in HI animals. These enhancements were independent of changes in the responsivity index (RI) a measure used to determine the response bias of the animal. This supports the suggestion that atomoxetine reliably increases vigilance in both low attentive and high impulsive subtypes. Atomoxetine is postulated to improve sustained attention by facilitating PFC function via stimulation of postsynaptic alpha-2 adrenoreceptors (Arnsten et al., 2007; Bymaster et al., 2002). Atomoxetine has also been shown to increase cortical acetylcholine neurotransmission in rats, indicating a cholinergic mechanism (Tzavara et al., 2006). These effects were dependent upon alpha-1 adrenoceptor and/or D1 receptor activation (Tzavara et al., 2006). Methylphenidate enhances dopamine (DA), noradrenaline (NA) and acetylcholine (Ach) in the PFC, consistent with the role of the these neurotransmitters in the PFC in
the modulation of attention (Robbins, 2002). The differential effects within the subgroups following methylphenidate and atomoxetine may depend on the dopamine enhancing effects of methylphenidate within the dorsal or ventral striatum, as atomoxetine lacks a subcortical dopaminergic mechanism of action.

In addition to assessing attentiveness, animals were also screened for impulsivity using measures of premature response (motor impulsivity) and false alarm responding (response inhibition). In LI animals, methylphenidate increased premature responding, consistent with previous findings (Dalley et al., 2007; Fernando et al., 2012; Jupp et al., 2013). In HI animals, methylphenidate reduced premature responding. Thus, baseline levels of impulsivity are an important determinant of the effects of methylphenidate, as previously shown (Puumala et al., 1996). Dalley and colleagues hypothesise that the effects of NA-modulating drugs are greater in HI animals showing that HI animals are resistant to the striatal DA-enhancing effects of methylphenidate (Fernando et al., 2012).

Our findings in HI animals are also consistent with the observation that methylphenidate reduces impulsivity in human ADHD (Faraone et al., 2006a; Faraone et al., 2000; Swanson et al., 1998). Interestingly methylphenidate had no effect on false alarm responding. However, it is important to note that p[FA] was lower in the vehicle group in the methylphenidate experiment, compared with the atomoxetine experiment, indicating that this effect may depend on baseline p[FA] performance.

Atomoxetine reduced premature responding in HI animals only which supports findings from previous studies using the 5-CSRTT (Blondeau and Della-Hagedom, 2007; Dalley et al., 2007; Navarra et al., 2008; Paterson et al., 2011; Robbins and Everitt, 2007; Robinson, 2012; Robinson et al., 2009; Robinson et al., 2008). For the first time by using the 5C-CPT we have shown that atomoxetine, but not methylphenidate, also improved a second form of impulsivity; response inhibition, in HI animals, and at the highest dose in LI animals. These data suggest that altered NAergic neurotransmission by atomoxetine is an important mechanism for controlling impulsivity (including response inhibition) in HI animals, and perhaps in ADHD-HI and ADHD-C patients. The HI and LI subgroups may differ in DA and NA baseline functioning. Through blockade of the noradrenaline transporter (NET), cortical extracellular levels of DA and NA increase in HI animals to a comparable level of LI animals (Fernando et al., 2012; Robinson, 2012; Robinson et al., 2008). This mechanism could also underlie the clinical response to atomoxetine (Robbins and Arnsten, 2009). Hence, atomoxetine is proposed to rebalance catecholamine function in the PFC (Robbins and Arnsten, 2009;
Robinson, 2012). Others postulate that HI animals may represent endophenotypes of a DA receptor dysfunction (Dalley et al., 2007; Fernando et al., 2012). Further work is required but our findings support the hypothesis that selective inhibition of NET reduced impulsivity (motor and response inhibition) in the HI subgroup. It is important to note that atomoxetine did increase magazine latency in LI, LA and HA animals, possibly indicating a change in motivational state. This may explain the reduction in premature responding at the highest dose in LI animals, however magazine latency was unaffected in HI animals supporting an overall improvement in premature responding is independent of magazine latency.

We have shown that improvements in performance are dependent on the baseline performance of the animal. We postulate that the specific behavioural phenotypes (subgroups) have underlying neurobiological differences, and thus respond differentially to drug treatment, restoring their behaviour to a standard baseline level; i.e. to the baseline level of the “normal” subgroup (LI or HA). This finding could have significant implications for therapeutic strategy in the different ADHD subtypes in the clinic. It is important to note however that hyperactivity is also observed in adult ADHD in the clinic and could be evaluated in these adult animals to increase the translational validity of this model.

In summary, this study demonstrates that, by using the 5C-CPT in combination with the separation of rats into attentive and impulsive subgroups, we can provide a highly translational model of the ADHD-I subtype and impulsive symptoms in the ADHD-HI and ADHD-C subtypes of adult ADHD. Further characterisation of the neurobiological substrates underpinning these endophenotypes is required to further validate this model.
SUPPLEMENT 1

Justification for the use of female rats

The 5C-CPT was validated in female rats, and has not yet been thoroughly validated in male rats however; Young et al (2009) have validated the 5C-CPT in male mice. Although previous work in our laboratory showed that males could be trained as easily as females in this test (Barnes, 2011). Female rats remain smaller than males in long-term studies such as these, females can remain group housed (5 rats per cage) whereas males grow to a larger size and have to be housed in pairs. The training procedure and experimental procedures can last up to a year, therefore females were chosen. Female rats have been shown to behave differently and perform better in certain cognitive tasks including the task of working memory – the novel object recognition task (Sutcliffe et al. 2007). Future research, following on from these preclinical studies should of course include male rats. Future work should include validation of 5C-CPT in males. Whilst there are gender differences in the prevalence of ADHD, the estimated males versus female ratio is reported to be 1.6:1 in the DSM-V, therefore although higher in males, females are still affected by the disorder (Ramtekkar et al., 2011). It is also argued that the reason for the higher prevalence in males may be due to males having more noticeable symptoms including disruptive hyperactive-impulsive symptoms.

Justification for the selection criteria

The selection criteria for LA and HA animals were defined by mapping the distribution of accuracy and sensitivity index scores for each animal n=40. The scores were taken across five training days and a mean score calculated for each animal. The middle data point was taken to be the selection criteria score, i.e. approximately 20 animals were below 90% accuracy and 20 animals above. The same method was followed for LI and HI animals using premature responses and false alarm rate measures. The animals that were excluded from the experiment were excluded after the separation into groups. The animals that were excluded showed fluctuations in the behavioural measures used across the five days (i.e. means meeting highest and lowest quartiles).
Table 1: Descriptive statistics for HA and LA

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>84.24</td>
<td>96.54</td>
</tr>
<tr>
<td>Mode</td>
<td>84.50</td>
<td>95.40</td>
</tr>
<tr>
<td>Median</td>
<td>85.30</td>
<td>96.32</td>
</tr>
<tr>
<td><strong>Sensitivity Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-0.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Mode</td>
<td>-0.21</td>
<td>0.31</td>
</tr>
<tr>
<td>Median</td>
<td>-0.22</td>
<td>0.33</td>
</tr>
</tbody>
</table>

LA denotes low-attentive (n=8); HA denotes high-attentive (n=8)

Table 2: Descriptive statistics for LI and HI

<table>
<thead>
<tr>
<th></th>
<th>LI</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Premature Response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.49</td>
<td>20.69</td>
</tr>
<tr>
<td>Mode</td>
<td>4.40</td>
<td>18.00</td>
</tr>
<tr>
<td>Median</td>
<td>4.80</td>
<td>20.54</td>
</tr>
<tr>
<td><strong>False Alarm Rate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.27</td>
<td>0.40</td>
</tr>
<tr>
<td>Mode</td>
<td>0.20</td>
<td>0.33</td>
</tr>
<tr>
<td>Median</td>
<td>0.23</td>
<td>0.45</td>
</tr>
</tbody>
</table>

LI denotes low-impulsive (n=8); HI denotes high-impulsive (n=8)
The group membership for individual animals (HA, LA, HI and LI) were not strictly independent. In the two experiments animals could be allocated to one group in experiment one, either LA or HA, and also to a second group (HI or LI) in experiment two. Notably the p[FA] was higher in the LA subgroup, however there were no significant differences in premature responses between HA and LA subgroups.

**Figure legends**

Figure 1a and 1b: Histograms showing the distribution of LA and HA animals’ accuracy scores (1a) and sensitivity index (1b), (LA; n=8, HA; n=8). The darker bars represent the HA animals, and the grey bars the LA animals.

Figure 2a and 2b: Histograms showing the distribution of LI and HI animals’ premature responses (1a) and false alarm responses (1b), (LI; n=8, HI; n=8). The darker bars represent the HI animals, and the grey bars the LI animals.
### Table 3: Effect of methylphenidate in the HA subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>81.63 ± 4.75</td>
<td>86.13 ± 6.76</td>
<td>85.13 ± 5.41</td>
<td>88.88 ± 5.92</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>93.03 ± 2.45</td>
<td>91.28 ± 2.22</td>
<td>91.85 ± 2.35</td>
<td>90.17 ± 1.48</td>
</tr>
<tr>
<td>% Omission</td>
<td>24.16 ± 3.32</td>
<td>23.36 ± 4.48</td>
<td>28.03 ± 5.99</td>
<td>26.87 ± 7.16</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.64 ± 0.03</td>
<td>0.67 ± 0.04</td>
<td>0.67 ± 0.03</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.71 ± 0.19</td>
<td>0.66 ± 0.09</td>
<td>0.60 ± 0.14</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.06 ± 0.05</td>
<td>1.14 ± 0.13</td>
<td>1.15 ± 0.10</td>
<td>1.13 ± 0.10</td>
</tr>
<tr>
<td>Premature responses</td>
<td>57.13 ± 6.14</td>
<td>53.13 ± 12.59</td>
<td>57.88 ± 7.51</td>
<td>73.38 ± 17.26</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.71 ± 0.04</td>
<td>0.70 ± 0.03</td>
<td>0.67 ± 0.06</td>
<td>0.67 ± 0.07</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.32 ± 0.07</td>
<td>0.31 ± 0.04</td>
<td>0.40 ± 0.06</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.60 ± 0.03</td>
<td>0.68 ± 0.07</td>
<td>0.68 ± 0.03</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.22 ± 0.24</td>
<td>1.53 ± 0.42</td>
<td>1.68 ± 0.41</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td>% Omission</td>
<td>24.16 ± 3.32</td>
<td>23.36 ± 4.48</td>
<td>28.03 ± 5.99</td>
<td>26.87 ± 7.16</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.64 ± 0.03</td>
<td>0.67 ± 0.04</td>
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</tr>
<tr>
<td>Incorrect latency (go)</td>
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<td>0.60 ± 0.14</td>
<td>0.69 ± 0.07</td>
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<td>Magazine latency (go)</td>
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<td>Premature responses</td>
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<td>73.38 ± 17.26</td>
</tr>
<tr>
<td>p[HR]</td>
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<td>0.70 ± 0.03</td>
<td>0.67 ± 0.06</td>
<td>0.67 ± 0.07</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.32 ± 0.07</td>
<td>0.31 ± 0.04</td>
<td>0.40 ± 0.06</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.60 ± 0.03</td>
<td>0.68 ± 0.07</td>
<td>0.68 ± 0.03</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.22 ± 0.24</td>
<td>1.53 ± 0.42</td>
<td>1.68 ± 0.41</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td>SI</td>
<td>0.39 ± 0.09</td>
<td>0.39 ± 0.06</td>
<td>0.28 ± 0.09</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>RI</td>
<td>0.01 ± 0.09</td>
<td>0.01 ± 0.04</td>
<td>0.06 ± 0.09</td>
<td>0.06 ± 0.11</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold.

*p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 10 s. Target trials are represented as (go) and non-target trials are represented as (no-go).

### Table 4: Effect of methylphenidate in the LA subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>83.63 ± 4.61</td>
<td>77.75 ± 3.99</td>
<td>82.00 ± 1.82</td>
<td>82.65 ± 6.11</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>80.67 ± 1.84</td>
<td>84.45 ± 2.50</td>
<td>87.24 ± 2.30**</td>
<td>90.74 ± 2.31**</td>
</tr>
<tr>
<td>% Omission</td>
<td>51.84 ± 4.20</td>
<td>44.85 ± 4.36</td>
<td>48.11 ± 6.75</td>
<td>52.41 ± 4.05</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.75 ± 0.04</td>
<td>0.74 ± 0.06</td>
<td>0.67 ± 0.05</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.66 ± 0.12</td>
<td>0.55 ± 0.09</td>
<td>0.91 ± 0.21</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.64 ± 0.22</td>
<td>1.48 ± 0.16</td>
<td>1.36 ± 0.10</td>
<td>1.64 ± 0.22</td>
</tr>
<tr>
<td>Premature responses</td>
<td>47.85 ± 4.01</td>
<td>36.38 ± 3.83</td>
<td>49.50 ± 4.57</td>
<td>31.26 ± 5.53</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.52 ± 0.04</td>
<td>0.42 ± 0.05</td>
<td>0.40 ± 0.05*</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.73 ± 0.16</td>
<td>0.72 ± 0.06</td>
<td>0.62 ± 0.07</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>1.65 ± 0.16</td>
<td>1.40 ± 0.16</td>
<td>1.53 ± 0.21</td>
<td>1.70 ± 0.27</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.34 ± 0.24</td>
<td>1.54 ± 0.39</td>
<td>1.56 ± 0.41</td>
<td>1.29 ± 0.22</td>
</tr>
<tr>
<td>SI</td>
<td>-0.14 ± 0.03</td>
<td><strong>0.05 ± 0.04</strong></td>
<td><strong>0.06 ± 0.05</strong></td>
<td>-0.01 ± 0.07**</td>
</tr>
<tr>
<td>RI</td>
<td>-0.09 ± 0.07</td>
<td>-0.12 ± 0.06</td>
<td>-0.15 ± 0.11</td>
<td>-0.17 ± 0.07</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold.

*p<0.05, **p<0.01, ***p<0.001, compared with vehicle. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 10 s.
Table 5: Effect of atomoxetine in the HA subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT measures.

<table>
<thead>
<tr>
<th></th>
<th>Atomoxetine</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 0.5 mg/kg</td>
<td>1.0 mg/kg</td>
<td>2.0 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Processed trials</td>
<td>84.75 ± 3.65</td>
<td>78.00 ± 4.66</td>
<td>84.13 ± 4.89</td>
<td>77.50 ± 5.55</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>96.54 ± 1.05</td>
<td>92.60 ± 2.94</td>
<td>92.84 ± 1.28</td>
<td>94.61 ± 1.47</td>
</tr>
<tr>
<td>% Omission</td>
<td>43.03 ± 6.43</td>
<td>41.11 ± 7.50</td>
<td>33.22 ± 5.76</td>
<td>45.25 ± 6.81</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.68 ± 0.02</td>
<td>0.71 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.56 ± 0.16</td>
<td>0.64 ± 0.12</td>
<td>0.63 ± 0.14</td>
<td>0.85 ± 0.15</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.34 ± 0.09</td>
<td>1.17 ± 0.07</td>
<td>1.40 ± 0.14</td>
<td>1.84 ± 0.31*</td>
</tr>
<tr>
<td>Premature responses</td>
<td>27.13 ± 1.17</td>
<td>29.38 ± 3.86</td>
<td>23.38 ± 3.20</td>
<td>14.88 ± 2.45***</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.55 ± 0.06</td>
<td>0.54 ± 0.07</td>
<td>0.62 ± 0.06</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>% Omission</td>
<td>0.38 ± 0.07</td>
<td>0.59 ± 0.07</td>
<td>0.39 ± 0.06</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.71 ± 0.03</td>
<td>0.67 ± 0.03</td>
<td>0.67 ± 0.04</td>
<td>0.83 ± 0.14</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>2.28 ± 0.51</td>
<td>1.77 ± 0.44</td>
<td>1.38 ± 0.16</td>
<td>1.65 ± 0.25</td>
</tr>
<tr>
<td>SI</td>
<td>0.18 ± 0.07</td>
<td>0.05 ± 0.09</td>
<td>0.24 ± 0.08</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>RI</td>
<td>-0.08 ± 0.11</td>
<td>0.14 ± 0.13</td>
<td>0.01 ± 0.09</td>
<td>0.15 ± 0.13</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold *p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 10 s. Target trials are represented as (go) and non-target trials are represented as (no-go).

Table 6: Effect of atomoxetine in the LA subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT measures.

<table>
<thead>
<tr>
<th></th>
<th>Atomoxetine</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 0.5 mg/kg</td>
<td>1.0 mg/kg</td>
<td>2.0 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Processed trials</td>
<td>78.50 ± 2.23</td>
<td>79.13 ± 3.75</td>
<td>76.50 ± 5.75</td>
<td>80.13 ± 3.87</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>84.84 ± 3.23</td>
<td>86.34 ± 2.14</td>
<td>89.69 ± 3.26</td>
<td>92.14 ± 2.87*</td>
</tr>
<tr>
<td>% Omission</td>
<td>55.63 ± 4.38</td>
<td>51.73 ± 5.97</td>
<td>53.16 ± 6.84</td>
<td>55.63 ± 4.38</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.72 ± 0.05</td>
<td>0.72 ± 0.02</td>
<td>0.72 ± 0.03</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.91 ± 0.19</td>
<td>0.86 ± 0.10</td>
<td>0.68 ± 0.15</td>
<td>1.15 ± 0.17</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.26 ± 0.09</td>
<td>1.31 ± 0.09</td>
<td>1.47 ± 0.23</td>
<td>1.62 ± 0.15*</td>
</tr>
<tr>
<td>Premature responses</td>
<td>28.75 ± 3.18</td>
<td>32.00 ± 6.59</td>
<td>23.63 ± 4.12</td>
<td>16.63 ± 5.04</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.37 ± 0.07</td>
<td>0.42 ± 0.05</td>
<td>0.42 ± 0.06</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>% Omission</td>
<td>0.66 ± 0.04</td>
<td>0.72 ± 0.08</td>
<td>0.71 ± 0.04</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>2.72 ± 0.80</td>
<td>2.03 ± 0.46</td>
<td>3.73 ± 0.76</td>
<td>2.74 ± 1.20</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.78 ± 0.71</td>
<td>1.77 ± 0.89</td>
<td>1.46 ± 0.26</td>
<td>1.67 ± 0.48</td>
</tr>
<tr>
<td>SI</td>
<td>-0.16 ± 0.06</td>
<td>-0.07 ± 0.04</td>
<td>0.13 ± 0.07**</td>
<td>0.05 ± 0.06**</td>
</tr>
<tr>
<td>RI</td>
<td>-0.10 ± 0.10</td>
<td>41.55 ± 5.12</td>
<td>-0.23 ± 0.13</td>
<td>-0.10 ± 0.11</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold *represents p<0.05, ** p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 10 s.
### Table 7: Effect of methylphenidate in the HI subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processed trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.25 ± 1.92</td>
<td>50.50 ± 2.63</td>
<td>49.63 ± 3.46</td>
<td>45.00 ± 2.18</td>
</tr>
<tr>
<td><strong>Accuracy (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.65 ± 1.10</td>
<td>92.87 ± 2.81</td>
<td>89.78 ± 3.72</td>
<td>89.77 ± 3.52</td>
</tr>
<tr>
<td><strong>% Omission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.06 ± 3.12</td>
<td>22.09 ± 2.75</td>
<td>25.13 ± 2.82</td>
<td>20.47 ± 2.52</td>
</tr>
<tr>
<td><strong>Correct latency (go/no-go)</strong></td>
<td>0.77 ± 0.04</td>
<td>0.81 ± 0.03</td>
<td>0.82 ± 0.05</td>
<td>0.77 ± 0.04</td>
</tr>
<tr>
<td><strong>Incorrect latency (go)</strong></td>
<td>0.71 ± 0.14</td>
<td>1.06 ± 0.23</td>
<td>0.97 ± 0.22</td>
<td>1.26 ± 0.18</td>
</tr>
<tr>
<td><strong>Magazine latency (go)</strong></td>
<td>1.18 ± 0.08</td>
<td>1.29 ± 0.18</td>
<td>1.23 ± 0.17</td>
<td>1.13 ± 0.10</td>
</tr>
<tr>
<td><strong>Premature responses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.01 ± 3.31</td>
<td>27.01 ± 2.59*</td>
<td>29.99 ± 2.16**</td>
<td>59.76 ± 6.30</td>
</tr>
<tr>
<td><strong>p[HR]</strong></td>
<td>0.48 ± 0.07</td>
<td>0.44 ± 0.06</td>
<td>0.36 ± 0.07</td>
<td>0.47 ± 0.06</td>
</tr>
<tr>
<td><strong>p[FA]</strong></td>
<td>0.42 ± 0.09</td>
<td>0.49 ± 0.03</td>
<td>0.50 ± 0.06</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td><strong>Incorrect latency (no-go)</strong></td>
<td>0.83 ± 0.05</td>
<td>0.74 ± 0.05</td>
<td>0.82 ± 0.07</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td><strong>Magazine latency (no-go)</strong></td>
<td>1.84 ± 0.68</td>
<td>2.27 ± 0.76</td>
<td>1.82 ± 0.48</td>
<td>2.30 ± 0.92</td>
</tr>
<tr>
<td><strong>SI</strong></td>
<td>0.13 ± 0.12</td>
<td>0.06 ± 0.04</td>
<td>0.14 ± 0.09</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td><strong>RI</strong></td>
<td>-0.13 ± 0.19</td>
<td>-0.07 ± 0.12</td>
<td>-0.14 ± 0.15</td>
<td>0.02 ± 0.09</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 shown in bold compared with the vehicle treated group. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 20 s.

### Table 8: Effect of methylphenidate in the LI subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processed trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.38 ± 1.75</td>
<td>49.38 ± 2.81</td>
<td>47.75 ± 1.54</td>
<td>48.25 ± 1.75</td>
</tr>
<tr>
<td><strong>Accuracy (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>85.63 ± 8.42</td>
<td>93.98 ± 2.90</td>
<td>93.24 ± 2.68</td>
<td>91.70 ± 3.70</td>
</tr>
<tr>
<td><strong>% Omission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.09 ± 3.35</td>
<td>22.73 ± 3.46</td>
<td>21.73 ± 2.62</td>
<td>26.44 ± 4.09</td>
</tr>
<tr>
<td><strong>Correct latency (go/no-go)</strong></td>
<td>0.82 ± 0.04</td>
<td>0.82 ± 0.02</td>
<td>0.81 ± 0.02</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td><strong>Incorrect latency (go)</strong></td>
<td>0.84 ± 0.23</td>
<td>0.75 ± 0.23</td>
<td>0.79 ± 0.19</td>
<td>0.96 ± 0.21</td>
</tr>
<tr>
<td><strong>Magazine latency (go)</strong></td>
<td>1.21 ± 0.06</td>
<td>1.20 ± 0.07</td>
<td>1.13 ± 0.08</td>
<td>1.15 ± 0.05</td>
</tr>
<tr>
<td><strong>Premature responses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.75 ± 5.23</td>
<td>24.13 ± 6.72</td>
<td>31.13 ± 5.96</td>
<td>30.50 ± 6.73</td>
</tr>
<tr>
<td><strong>p[HR]</strong></td>
<td>0.44 ± 0.09</td>
<td>0.43 ± 0.09</td>
<td>0.44 ± 0.05</td>
<td>0.33 ± 0.09</td>
</tr>
<tr>
<td><strong>p[FA]</strong></td>
<td>0.41 ± 0.08</td>
<td>0.46 ± 0.11</td>
<td>0.44 ± 0.07</td>
<td>0.36 ± 0.09</td>
</tr>
<tr>
<td><strong>Incorrect latency (no-go)</strong></td>
<td>0.79 ± 0.04</td>
<td>0.78 ± 0.09</td>
<td>0.66 ± 0.04</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td><strong>Magazine latency (no-go)</strong></td>
<td>1.63 ± 0.56</td>
<td>2.44 ± 0.58</td>
<td>2.57 ± 1.11</td>
<td>3.12 ± 1.23</td>
</tr>
<tr>
<td><strong>SI</strong></td>
<td>0.01 ± 0.07</td>
<td>-0.08 ± 0.10</td>
<td>0.01 ± 0.05</td>
<td>0.02 ± 0.08</td>
</tr>
<tr>
<td><strong>RI</strong></td>
<td>-0.16 ± 0.14</td>
<td>-0.11 ± 0.16</td>
<td>-0.12 ± 0.11</td>
<td>-0.31 ± 0.15</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ± SEM. Significant values are in bold *p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 20 s. Target trials are represented as (go) and non-target trials are represented as (no-go).
Table 9: Effect of atomoxetine in the HI subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>41.13 ± 2.41</td>
<td>48.25 ± 2.41</td>
<td>51.25 ± 2.74</td>
<td>48.88 ± 3.95</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>94.51 ± 1.76</td>
<td>93.71 ± 1.41</td>
<td>95.67 ± 1.73</td>
<td>95.20 ± 0.83</td>
</tr>
<tr>
<td>% Omission</td>
<td>18.44 ± 2.41</td>
<td>15.34 ± 2.35</td>
<td>17.08 ± 2.55</td>
<td>15.13 ± 2.70</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.79 ± 0.04</td>
<td>0.82 ± 0.03</td>
<td>0.74 ± 0.03</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.59 ± 0.09</td>
<td>0.66 ± 0.21</td>
<td>0.92 ± 0.20</td>
<td>0.88 ± 0.16</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.27 ± 0.13</td>
<td>1.46 ± 0.14</td>
<td>1.44 ± 0.15</td>
<td>1.71 ± 0.22*</td>
</tr>
<tr>
<td>Premature responses</td>
<td>61.13 ± 2.99</td>
<td><strong>29.74 ± 4.34</strong>*</td>
<td><strong>24.38 ± 4.03</strong>*</td>
<td><strong>21.50 ± 3.62</strong>*</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.53 ± 0.07</td>
<td>0.52 ± 0.08</td>
<td>0.32 ± 0.06</td>
<td>61.00 ± 0.07</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.56 ± 0.07</td>
<td>0.52 ± 0.08</td>
<td><strong>0.32 ± 0.06</strong></td>
<td><strong>0.43 ± 0.10</strong></td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.72 ± 0.04</td>
<td>0.91 ± 0.16</td>
<td>0.67 ± 0.06</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.37 ± 0.26</td>
<td>1.54 ± 0.28</td>
<td>1.42 ± 0.26</td>
<td>2.29 ± 0.56</td>
</tr>
<tr>
<td>SI</td>
<td>-0.04 ± 0.06</td>
<td>0.10 ± 0.07</td>
<td><strong>0.28 ± 0.08</strong></td>
<td><strong>0.18 ± 0.10</strong></td>
</tr>
<tr>
<td>RI</td>
<td>0.09 ± 0.11</td>
<td>0.10 ± 0.14</td>
<td>-0.13 ± 0.11</td>
<td>0.04 ± 0.15</td>
</tr>
</tbody>
</table>

Measures are shown as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001. Shown in bold compared with vehicle treated animals. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 20 s.

Table 10: Effect of atomoxetine in the LI subgroup (0.5, 1.0, 2.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Vehicle</th>
<th>0.5 mg/kg</th>
<th>1.0 mg/kg</th>
<th>2.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>52.38 ± 3.23</td>
<td>48.50 ± 3.43</td>
<td>46.63 ± 4.05</td>
<td>51.25 ± 3.17</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>89.86 ± 3.70</td>
<td>88.65 ± 3.03</td>
<td>93.72 ± 1.88</td>
<td>96.73 ± 0.82</td>
</tr>
<tr>
<td>% Omission</td>
<td>13.21 ± 1.80</td>
<td><strong>19.09 ± 2.64</strong>*</td>
<td>13.64 ± 2.97</td>
<td>15.75 ± 2.33</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.75 ± 0.03</td>
<td>0.77 ± 0.05</td>
<td>0.73 ± 0.04</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>1.21 ± 0.14</td>
<td>0.85 ± 0.11</td>
<td>0.93 ± 0.14</td>
<td>1.04 ± 0.22</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.30 ± 0.15</td>
<td>1.09 ± 0.08</td>
<td>1.25 ± 0.14</td>
<td>1.35 ± 0.09</td>
</tr>
<tr>
<td>Premature responses</td>
<td>23.50 ± 4.91</td>
<td>28.25 ± 6.14</td>
<td>33.50 ± 5.43</td>
<td>24.38 ± 4.20</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.63 ± 0.03</td>
<td><strong>0.49 ± 0.06</strong></td>
<td>0.63 ± 0.07</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.55 ± 0.06</td>
<td>0.56 ± 0.08</td>
<td>0.53 ± 0.08</td>
<td><strong>0.44 ± 0.06</strong></td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.83 ± 0.02</td>
<td>0.76 ± 0.06</td>
<td>0.74 ± 0.07</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.18 ± 0.16</td>
<td><strong>2.44 ± 0.67</strong></td>
<td><strong>2.05 ± 0.60</strong></td>
<td><strong>2.16 ± 0.39</strong></td>
</tr>
<tr>
<td>SI</td>
<td>0.04 ± 0.07</td>
<td>-0.09 ± 0.08</td>
<td>0.12 ± 0.07</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>RI</td>
<td>0.21 ± 0.08</td>
<td>0.05 ± 0.12</td>
<td>0.17 ± 0.13</td>
<td>0.05 ± 0.07</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold *p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 20 s. Target trials are represented as (go) and non-target trials are represented as (no-go).
8. PUTATIVE THERAPEUTIC TARGETS FOR SYMPTOM SUBTYPES OF ADULT ADHD: D4 RECEPTOR AGONISM AND COMT INHIBITION IMPROVE ATTENTION AND RESPONSE INHIBITION IN A NOVEL TRANSLATIONAL ANIMAL MODEL.

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*Manchester Pharmacy School, University of Manchester, Oxford Rd, Manchester, M13 9PT, UK

[Submitted for publication in European Neuropsychopharmacology]
Abstract

Prefrontal cortical dopamine plays an important role in cognitive control, specifically in attention and response inhibition; the core deficits in ADHD. We have previously shown that methylphenidate and atomoxetine differentially improve these deficits dependent on baseline performance. The present study extends this work to investigate the effects of putative therapeutic targets in our model. A selective dopamine D4 receptor agonist (A-412997) and the catechol-O-methyl-transferase (COMT) inhibitor; tolcapone, were investigated in the combined subtype of adult ADHD (ADHD-C). Adult female rats were trained to criterion in the 5C-CPT (5-Choice Continuous Performance Task) and then separated into subgroups according to baseline levels of sustained attention, vigilance, and response disinhibition. The subgroups included: high-attentive (HA) and low-attentive with high response disinhibition (ADHD-C). The ADHD-C subgroup was selected to represent the combined subtype of adult ADHD. Effects of tolcapone (3.0, 10.0, 15.0 mg/kg) and A-412997 (0.1, 0.3, 1.0 umol/kg) were tested by increasing the variable inter-trial-interval (ITI) duration in the 5C-CPT. Tolcapone (15 mg/kg) significantly increased sustained attention, vigilance and response inhibition in ADHD-C animals, and impaired attention in HA animals. A-412997 (1.0 umol/kg) significantly increased vigilance and response inhibition in ADHD-C animals only, with no effects in HA animals. This is the first study to use the translational 5C-CPT to model the adult ADHD-C subtype in rats and to study new targets in this model. Both tolcapone and A-412997 increased vigilance and response inhibition in the ADHD-C subgroup. D4 and COMT are important potential therapeutic targets in adult ADHD that warrant further investigation.
8.1 Introduction

The development of an improved animal model of adult ADHD is essential to improve our understanding of the neurobiological underpinnings of this chronic, heterogeneous, debilitating disorder. Many animal models of ADHD have been proposed, predominantly focusing on childhood ADHD. However, the evidence clearly shows that ADHD is not limited to childhood and often persists into adulthood with an adult prevalence of 3-5% (Kessler et al., 2006; Kooij et al., 2010).

Recently, we have developed a translational rat model of the inattentive subtype (ADHD-I) and impulsive symptoms in adult ADHD (Tomlinson et al., 2014). Using the five-choice continuous performance task (5C-CPT) we selected animals with high levels of impulsivity (HI subgroup) modeling the impulsive traits in the combined (ADHD-C) subtype of adult ADHD. The inattentive subgroup (LA animals) were selected based on impairments in sustained attention and reduced vigilance. Thus, mimicking the deficits in sustained attention and vigilance observed in ADHD-I in the clinic (Faraone et al., 2006a). Our work with this model has demonstrated that the effects of standard ADHD medication, (methylphenidate and atomoxetine) varies according to the subtype selected (Tomlinson et al., 2014). This is a finding of critical importance for selection of the most appropriate treatments for adult subtypes of this disorder.

The most widely used medications in adult ADHD; the psychostimulants, increase extrasynaptic dopamine levels in a non-selective manner (Volkow et al., 2002; Volkow et al., 2003) and are associated with a large side effect burden including insomnia, appetite suppression, headache, gastric irritation and abuse liability (Pliszka, 2007). We have also shown that the effects of both methylphenidate and atomoxetine are dependent on the level of symptom expression (Tomlinson et al., 2014). The generation of a more selective compound, with an improved side effect profile requires the identification of a specific target that improves the cortical cognitive processes (attention and impulsivity) and the striatal motor processes (hyperactivity).

Our work also highlights the importance of using an appropriate translational cognitive task (5C-CPT) and a relevant animal model. By using the 5C-CPT we are able to differentiate between these two types of impulsivity. The 5C-CPT (established in our laboratory for rats, Barnes et al., 2012a; b, used in Tomlinson et al., 2014 and in the current study), adapted from the 5-CSRTT, is more consistent with the human CPT. CPTs are the most widely utilised tests to measure attention in ADHD patients. The
key difference with the 5C-CPT from the 5-CSRTT is the inclusion of non-target trials (together with the target trials) allowing for measurements of response inhibition and vigilance. The 5C-CPT has been widely validated in mice (Young et al., 2009), rats (Barnes et al., 2012a, b), and importantly, has recently shown cross-species validity to humans (McKenna et al., 2013; Young et al., 2013). By using the 5C-CPT therefore, this model may provide a useful tool in finding novel targets for improved pharmacotherapy for adult ADHD.

One aim of the present study is to extend our findings and ensure that response inhibition deficits are not due to just an increase in motor impulsivity (increased premature responding). Our previous work selected a group of animals with both increased response disinhibition and motor impulsivity. The work separates response inhibition from motor impulsivity. Previously, groups using the 5-CSRTT have reported that high attentive rats tend to be less impulsive and low attentive rats more impulsive (as measured by increased premature responding) (Cole and Robbins, 1987; Puumala et al., 1996). Others have shown a spectrum of impulsivity independent on the level of attention (Blondeau and Della-Hagedom, 2007). Using the 5C-CPT to discriminate between different forms of impulsivity allows us to examine inattention and response inhibition, in addition to premature responding. Only measuring premature responding, a measure of motor impulsivity and used in the 5-CSRTT, does not enable assessment of the spectrum of impulsive behaviours which can be achieved using the 5C-CPT.

In the current study we firstly: aim to extend our work by combining symptoms of inattention and deficits in response inhibition to form, for the first time, a model of the combined adult ADHD subtype (ADHD-C). We aim to provide a means by which improved understanding of the neurobiology of adult ADHD may be achieved. This in turn will provide a mechanism for identifying novel targets for this debilitating illness and so improve therapeutic strategies. Our second aim is to evaluate novel targets for ADHD pharmacotherapy using a dopamine receptor D4 (DRD4) agonist and a catecholamine-O-methyl-transferase (COMT) inhibitor.

Conforming to the dopamine theory of ADHD, both COMT and DRD4 have been widely associated with ADHD. The DRD4 has been suggested as a specific target to improve symptoms of ADHD. The procognitive effects of DRD4 agonists have been demonstrated in unimpaired rats (Woolley et al., 2008) and in the spontaneously hypertensive rat (SHR) (Browman et al., 2005). The SHR is the result of genetic
manipulations, and has been widely used as a model of childhood ADHD (Sagvolden, 2000). However, this approach fails to model adult ADHD, and utilises a manipulation with little aetiological relevance (induced hypertension) and therefore lacks translational validity for adult ADHD. When exploring potential new targets for adult ADHD it is essential that the model exhibits a high level of translation, so that the findings can be readily applied to the clinical population. We present such a model in this paper.

The DRD4 has been implicated in cognitive impairments associated with ADHD including deficits in response inhibition, novelty seeking behaviours and hyperactivity in animals and humans (Avale et al., 2004; Benjamin et al., 1996; Ebstein et al., 1996; Falzone et al., 2002; Young et al., 2011). DRD4 receptors are highly expressed in brain regions known to be affected in the illness including frontal cortex, and hypothalamus (Ariano et al., 1997; Tarazi and Baldessarini, 1999) with low expression in the cerebellum (Barili et al., 2000) in humans and rodents (Ariano et al., 1997). D4 knockout (KO) mice show lower basal extracellular striatal DA and reduced overflow of DA in the striatum and nucleus accumbens core (Thomas et al., 2007). Young and colleagues (2011) have elegantly demonstrated that D4 KO mice have attenuated response inhibition, which in turn produces abnormal attentional performance.

Taken together, these findings support the hypothesis that the DRD4 is a target for ADHD pharmacotherapy worth exploring in some detail. A-412997, a highly selective, DRD4 full agonist, with high affinity for the rat and human dopamine D4 receptor has been identified (Moreland et al., 2005). The present experiments assess the effects of A-412997 in our rat model of ADHD in order to investigate the role of DRD4 in ADHD symptoms of inattention, response disinhibition and impaired vigilance, which can be readily assessed using the 5C-CPT.

For comparative purposes, we have explored a second putative target for adult ADHD in our model. The main enzyme responsible for dopamine metabolism; COMT, performs approximately 60% of the DA degradation in the prefrontal cortex (PFC) (Diaz-Asper et al., 2006). The important role of COMT in the PFC on dopamine metabolism, has led to interest in the putative role of COMT in the aetiology of polygenic ADHD (Beiderman et al., 2004; Salatino-Oliveira et al., 2011; Tekin and Cummings, 2002). The single nucleotide polymorphism (SNP: known as Val158Met or rs4680) in COMT is one of the most widely associated SNPs in ADHD (Zhang et al., 2012). Due to the important involvement of COMT in inactivating dopamine in the
PFC, demonstrated by neurochemical, behavioural and neuroimaging studies, these studies will investigate the role of the selective COMT inhibitor tolcapone in our rodent model of adult ADHD. Tolcapone is a selective COMT inhibitor that readily crosses the blood brain barrier and is currently used as an adjunct treatment in Parkinson’s disease (Ceravolo et al., 2002). Reduced COMT activity either via genetic (Papeleo et al., 2008) or pharmacological (Lapish et al., 2009; Turnbridge et al., 2004) manipulation improves cognitive function, such as working memory, attention, executive function and emotional processing in animal studies.

To our knowledge only one study has examined the effects of tolcapone in the widely used test of attention and impulsivity – the 5-choice serial reaction time task (5-CSRTT) in rodents finding no effects on performance (Paterson et al., 2011). This could be due to inability of the 5-CSRTT to robustly detect changes in vigilance and response inhibition in a manner consistent with human continuous performance tests (CPTs). Further research is clearly required to clarify the mechanism by which it produces its effects.

This study is the first to examine the role of these two putative targets in a novel animal model of the symptoms of the ADHD-C subtype of adult ADHD using the 5C-CPT. This model allows extensive investigation of the behavioural traits observed in the adult illness and enables investigation of novel pharmacotherapeutic strategies.

8.2 Experimental Procedures

8.2.1. Subjects and housing conditions

Subjects were 40 adult female Lister-hooded rats (Charles River, UK; weighing 240 ± 10g at the start of training) housed in groups of five on a reversed 12 hr light: dark cycle (lights on at 19:00 h). We use female rats because the 5C-CPT has been carefully validated in female rats in our laboratory (Barnes et al., 2012a; 2012b) and they show enhanced cognitive performance in certain tests (Sutcliffe et al., 2007). All animals were housed in a temperature (21 ± 2°C) and humidity (55 ± 5%) controlled environment. Animals had free access to food (Special Diet Services, UK) and water until one week prior to training when food restriction was initiated. Thereafter rats were maintained at approx. 90% of their free-feeding body weight (10g rat chow/rat/day). Water was available for the duration of this study ad libitum. All experiments took place in the dark phase of the light: dark cycle under red light, between 0900 hr and
1600 hr, illumination of the light in the chamber was a punishment following an omission, incorrect or premature response. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and University ethical guidelines.

8.2.2. Apparatus

The 5C-CPT apparatus consisted of eight 25 cm x 25 cm aluminium chambers, each enclosed within a wooden sound attenuating box. Within each box there was a low-level fan to provide ventilation and mask extraneous background noise. The rear wall of the testing chamber contained nine individual apertures, four of which were occluded, leaving apertures 1, 3, 5, 7 and 9 free for presentation of light stimuli. All eight chambers were connected to a PC and data collection and initial analysis was controlled by K-limbic software (Conclusive Solutions), which generated an Excel spreadsheet (Microsoft) containing the raw data for statistical analysis.

8.2.3. 5C-CPT training procedure

The 5C-CPT procedure is similar to the standard 5-CSRTT procedure; both methods include target (go) trials in which the animal must respond to a light stimulus by nose-poking in the illuminated aperture. However, the 5C-CPT procedure also includes non-target (no-go) trials, when the animal must withhold from responding to a five-light (all apertures are illuminated) stimulus in order to receive a food reward. For a detailed description of the standard 5C-SRTT training and testing procedure see Eagle and Robbins (2003a).

Figure 1: Schematic of the 5C-CPT trial types

![Figure 1: Schematic of the 5C-CPT trial types](image)
**Figure 1**: Example of the two trial types in the 5C-CPT. During target trials the rat must respond to the stimulus by nose-poking beyond the infra-red (IR) beam in the location of the cue stimulus (aperture). Cue stimuli can appear in any one of the five locations. Non-target trials occur when all five cue lights come on at once, and the rat must inhibit from responding in any of the five locations (apertures) adapted from Young et al., (2013).

The training procedure used was based on the mouse 5C-CPT procedure (Young et al., 2009), with one adaptation – initially placing greater emphasis on non-target trials by adjusting the proportion of target and non-target trials. The training procedure is well established in our laboratory and has been described in detail previously see Barnes et al., 2012a; 2012b and most recently in Tomlinson et al., 2014. See table 1 for a full description of 5C-CPT measures.

Training sessions consisted of 120 trials or lasted 30 min. Training began with stimulus duration (SD) and limited hold duration (LH) set at 10 s. The proportion of target and non-target trials per session was 77 target trials and 43 non-target trials, later increased to 84 target trials and reduced to 36 non-target trials. The inter-trial interval (ITI) and time out (TO) remained constant throughout training (TO - 5 s and ITI – variable mean 5 s). However, as animals’ performance improved, the SD was reduced to 2 s, and the LH reduced to 2 s. The SD was reduced for individual rats in stages (10, 8, 4 and 2 s). Animals had to satisfy set criteria to progress to the next stage of training until reaching the target criterion of >70% accuracy, <25% omissions, >65% correct rejections for two consecutive days, previously described in Tomlinson et al., (2014). Animals were fully trained when they reached the target parameters under the standard training conditions (2 s SD, 5 s ITI, 2 s LH) for two consecutive days; this took approximately 24 weeks. After this, all animals were then trained once - three times per week Monday to Friday. Two animals were excluded at this point, as they failed to reach the set criteria.
Table 1: Description of the measurements from target and non-target trials in the 5C-CPT

<table>
<thead>
<tr>
<th>Trial Type</th>
<th>Measurement</th>
<th>Definition</th>
<th>Correlated Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Trial Measures</td>
<td>Accuracy (%)</td>
<td>The percentage of correct responses not including omissions</td>
<td>Selective Attention</td>
</tr>
<tr>
<td></td>
<td>Correct (%)</td>
<td>Similar to Accuracy, but including omitted trials</td>
<td>General Task</td>
</tr>
<tr>
<td></td>
<td>Omissions (%)</td>
<td>The percentage of go trials not responded to</td>
<td>Sustained Attention/Motivation</td>
</tr>
<tr>
<td></td>
<td>Perseverative Response</td>
<td>An incorrect response in the same hole as a previous correct response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Correct Latency (s)</td>
<td>Time from trial presentation to correct response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incorrect Latency (s)</td>
<td>Time from trial presentation to incorrect response</td>
<td></td>
</tr>
<tr>
<td>Non-Target Measures</td>
<td>Correct Rejections (%)</td>
<td>Percentage of non-target trials not responded to</td>
<td>Response Inhibition</td>
</tr>
<tr>
<td>Non Trial Measures</td>
<td>Premature Response</td>
<td>Response during inter-trial interval</td>
<td>Motor Impulsivity</td>
</tr>
<tr>
<td></td>
<td>Magazine Latency (s)</td>
<td>Time from reward dispense to collection</td>
<td>Motivational State</td>
</tr>
<tr>
<td></td>
<td>Total Trials</td>
<td>Number of target and non-target trials completed</td>
<td></td>
</tr>
<tr>
<td>Signal Detection Theory</td>
<td>Hit Rate, p[HR]</td>
<td>Percent correct expressed as a proportion</td>
<td>General Task</td>
</tr>
<tr>
<td></td>
<td>False Alarm Rate, p[FA]</td>
<td>Proportion of non-target trials responded to</td>
<td>Response</td>
</tr>
<tr>
<td></td>
<td>Sensitivity Index, SI</td>
<td>A non-parametric calculation based on signal detection theory</td>
<td>Vigilance</td>
</tr>
<tr>
<td></td>
<td>Responsivity Index, RI</td>
<td>A non-parametric calculation based on signal detection theory</td>
<td>Response Strategy</td>
</tr>
</tbody>
</table>

8.2.4. 5C-CPT testing procedure - selection of ADHD-C and HA subgroups

The procedure used for subgroup selection is described in detail in Tomlinson et al., (2014). Briefly, following acquisition of the 5C-CPT all rats were screened for 5 consecutive days under standard training (baseline) conditions. The means of the attentional measures (accuracy and SI) and response inhibition (p[FA]) were calculated for the 5 days for individual animals. Using the calculated attentional means and response inhibition scores, the rats were then assigned to either a high-attentive (HA) or low attentive with response inhibition deficits (ADHD-C) subgroup, according to the following parameters: HA (accuracy >90%, SI >0.3, p[FA] <0.3) and ADHD-C (<90% accuracy, SI <0.3, p[FA] >0.3). The 6 animals that showed significant fluctuations in
attentional performance over the 5 days (i.e. means meeting highest and lowest quartiles) were excluded, leaving 16 rats per subgroup (ADHD-C and HA) remaining.

8.2.5. Drugs

A-412997 dihydrochloride (2-(30,40,50,60-tetrahydro-20H-[2,40]bipyridinyl-10-yl)-N-m-tolyl-acetamide) (Moreland et al., 2005), (Tocris Cookson, Bristol, UK) was dissolved in acetic acid; pH=6.0 with 1 M NaOH, and made-up to a final volume with 0.9% NaCl. A-412997 was administered intraperitoneally (IP) in a volume of 1.0 ml/kg 30 min before behavioural testing, doses are in µmol/kg. Tolcapone (Kemprotec Limited, Middlesbrough, UK) was dissolved in 0.9% NaCl with a few drops of tween (Tunbridge et al., 2004) and administered IP in a volume of 1 ml/kg 30 min prior to testing. Each test day was separated by a three-day washout period (no drug administration).

8.2.5.2 5C-CPT drug testing in ADHD-C and HA

A-412997 and tolcapone were tested in HA and ADHD-C rats using the 5C-CPT. Drugs were administered according to a fully randomised Latin-square within subjects design with a minimum of 72 h between drug challenge sessions. Experiments were separated by a 1-week washout period. Animals were tested on a Tuesday and Friday and trained under standard conditions in between test days for one day (Wednesday). On the remaining days (Monday and Thursday) the animals were not trained or tested.

Following separation into subgroups – HA (n=16) and ADHD-C (n=16) animals were then randomised to either receive A-412997 or tolcapone (half of each subgroup received each drug, i.e. n=8). A-412997 (0.1, 0.3, 1.0 µmol/kg) or vehicle (0.9% NaCl) was administered to HA (n=8) and LA (n=8) subgroups. Tolcapone (3.0, 10, 15 mg/kg) or vehicle (0.9% NaCl) was administered to HA (n=8) and ADHD-C (n=8) subgroups. Doses of both compounds were selected based on previous studies in male rats assessing attention, impulsivity and hyperactivity in similar behavioural paradigms (Browman et al., 2005; Paterson et al., 2011). Animals were tested in the 5C-CPT at an increased variable ITI (10 s), 2 s SD and 2 s LH, as previously described (Tomlinson et al., 2014).
8.2.6. Data and statistical analysis

Data are expressed as mean ± SEM and analysed using SPSS version 16.0. The data were checked for normality and where appropriate (normally distributed) were analysed using parametric measures. Overall subgroup performance using individual measures (e.g. accuracy, premature responses etc.) were normally distributed and analysed by one-way between-subjects ANOVA. Where appropriate; when the overall ANOVA was significant the LSD post-hoc Fisher’s Least Significant Difference test was applied.

Analysis of performance (individual performance measures) following drug treatment during challenge sessions was carried out by using a repeated measure two-way ANOVA analysis with treatment as the repeated measure and the subgroup as the between-subjects measure followed by LSD planned comparisons analysis. This allowed reduction of variability by utilising the within-subjects analysis to compare each animal to itself in each treatment group.

8.3 Results

8.3.1. ADHD-C and HA subgroup comparison

One-way ANOVA revealed that ADHD-C animals performed significantly worse compared with HA animals under standard conditions. The attentional measures of accuracy (sustained attention) $[F(1,38)= 6.47, p<0.001; \text{fig 2a}]$ and SI (vigilance) $[F(1,38)= 6.47, p<0.001; \text{fig 2b}]$ were significantly reduced in ADHD-C compared with HA animals. Response inhibition was significantly reduced in ADHD-C animals, shown as significantly increased p[FA] $[F(1,38)= 6.47, p<0.001; \text{fig 2c}]$. 
Figure 2: ADHD-C and high-attentive subgroups

Figure 2: Subgroup differences in attention. (A) Subgroup differences in target go-trial accuracy in the 5C-CPT under standard conditions, (B) Subgroup differences in vigilance (SI). (C) Subgroups differences in false alarm responding (p[FA]). The subgroups are HA and ADHD-C. Data are expressed as the mean ± SEM over 5 days, n=16 per sub-group. One-way ANOVA followed by post-hoc analysis showed a significant reduction in accuracy, SI and p[FA] (***p<0.001) ADHD-C compared with HA.

8.3.2. Effects of A-412997 in ADHD-C and HA animals

Treatment of the ADHD-C subgroup with A-412997 significantly improved vigilance in the 5C-CPT compared with vehicle. Repeated measures two-way ANOVA showed a significant Dose X Group interaction for vigilance as measured by SI [F(3,42)= 3.54, p<0.05; fig 3b]. Planned comparisons analysis showed a significant increase in the HA vehicle group compared with vehicle treated ADHD-C animals (p<0.05). In the ADHD-C subgroup, SI was significantly increased at the highest doses; 0.3 and 1.0 µmol/kg (p<0.05; p<0.01 respectively; fig 3b), and increased to a level that closely approached significance at the lowest dose; 0.1 µmol/kg (p=0.06; NS) compared with vehicle. No effects of A-412997 were observed in HA animals. The measure of RI showed a significant Dose X Group interaction following repeated measures two-way ANOVA [F(3,42)= 5.19, p<0.01]. RI is the nonparametric measure used to assess the response bias of the animal; the ‘tendency to respond’, and therefore changes in RI
suggest a change in response bias. Post-hoc analysis revealed a difference between the two vehicle groups that did not reach statistical significance ($p=0.09$; NS). However in ADHD-C animals, following planned analysis, 1.0 μmol/kg significantly reduced RI when compared to the vehicle group ($p<0.05$; fig 3d). Unexpectedly, RI was reduced to a level even lower than that in HA rats although this effect was not significant ($p=0.19$; NS).

A significant Dose X Group interaction was observed for response inhibition (false alarm responding) as revealed by repeated measures two-way ANOVA [$F_{(3,42)}= 6.95$, $p<0.01$]. HA vehicle animals made significantly less false alarm responses to non-target stimuli compared to the ADHD-C vehicle group ($p<0.01$, fig 3c). Planned comparisons analysis also showed that, in ADHD-C animals, A-412997 at 0.3 and 1.0 μmol/kg significantly reduced false alarm responding ($p<0.01$; $p<0.001$ respectively compared with the vehicle control; fig 3c) to a level equivalent to that of HA animals.

*Figure 3: Effect of A-412997 in ADHD-C and HA subgroups*

(a) (b) (c) (d)

**Figure 3**: Effects of A-412997 (0.1, 0.3 and 1.0 mg/kg, i.p. 30 min prior to testing) on overall performance in the 5C-CPT with an enhanced attentional load (variable ITI 10 s) in HA and ADHD-C subgroups. (A) Attentional measures in target-trials (percent accuracy). (B) Vigilance in the two subgroups; SI denotes sensitivity index; the non-parametric measure of vigilance and the ability to discriminate between go and non-target trials. (C) Response inhibition (false alarm responding). (D) Response bias of the animals, RI denotes responsivity index, in the two subgroups. All data are expressed as mean ± SEM, asterisks (**$p<0.05$, $p<0.01$, $p<0.001$) indicate significant differences
compared to vehicle, crosses (+p<0.05, ++p<0.01) indicate significant differences between vehicle HA and vehicle ADHD-C animals. All data were analysed using repeated measures two-way ANOVA followed by planned post-hoc comparisons.

8.3.3. Effects of tolcapone in ADHD-C and HA animals

Treatment with tolcapone increased attention in ADHD-C animals only. Repeated measures two-way ANOVA revealed a significant Dose X Group interaction for the total number of trials [F(3,42)= 4.19, p<0.01]. Planned comparisons revealed that ADHD-C and HA vehicle animals completed a similar number of trials (p=0.144; NS). Tolcapone at 15 mg/kg significantly (p<0.05) decreased the total number of trials completed in HA, but not ADHD-C animals. Repeated measures two-way ANOVA also showed a significant Dose X Group interaction for accuracy in the go-trials [F(3,42)= 3.81, p<0.05]. Planned comparisons showed a significant increase in accuracy in HA compared with ADHD-C vehicle treated animals (p<0.001; fig 4a). In the ADHD-C group, accuracy was significantly increased at the highest doses; 10 and 15 mg/kg (p<0.01; p<0.05 respectively), to a level comparable to the vehicle HA group. A significant Dose X Group interaction was observed for hit rate [F(3,42)= 3.47, p<0.05]. At 15 mg/kg, tolcapone significantly increased hit rate (p<0.01) in ADHD-C animals, but decreased hit rate in HA animals at all 3 doses (p<0.05; p<0.01; p<0.01). A significant Dose X Group interaction was also observed for premature responses [F(3,42)= 3.78, p<0.05]. At 15 mg/kg, tolcapone significantly decreased hit rate (p<0.01) in ADHD-C animals and in HA animals (p<0.05). Repeated measures two-way ANOVA also showed a significant Dose X Group interaction for vigilance as measured by SI [F(3,42)= 5.57, p<0.01; fig 4b]. Planned comparisons showed a significant increase in HA vehicle animals compared with ADHD-C animals for this measure (p<0.001, fig 4b). SI was significantly increased at the highest dose of 15 mg/kg (p<0.05) in ADHD-C animals and significantly reduced in HA animals compared with the respective vehicle groups (p<0.01, fig 4b). The effect of 10 mg/kg in the ADHD-C group to increase SI closely approached statistical significance (p=0.06; NS). There were no significant effects of tolcapone on RI [F(3,42)= 2.19, p=0.13; NS] fig 4d.

Repeated measures two-way ANOVA revealed that impulsivity measures were also affected by tolcapone; false alarm responding showed a significant Dose X Group interaction [F(3,42)= 3.40, p<0.05; fig 4c]. Planned comparisons showed that vehicle treated ADHD-C animals had significantly increased p[FA] compared with HA vehicle
treated animals ($p<0.01$), and at the highest dose (15 mg/kg) $p[FA]$ was significantly reduced in ADHD-C animals only ($p<0.01$, fig 4c).

**Figure 4:** Effect of tolcapone in ADHD-C and HA subgroups

Figure 4: Effects of tolcapone (3.0, 10 and 15 mg/kg, i.p. 30 min prior to testing) on overall performance in the 5C-CPT with an enhanced attentional load (variable ITI 10 s) in HA and ADHD-C subgroups. (A) Attentional measures in go-trials (percent accuracy). (B) Vigilance in the two subgroups; SI denotes sensitivity index; the non-parametric measure of vigilance and the ability to discriminate between target and non-target trials. (C) Response inhibition (false alarm responding). (D) Response bias of the animals, RI denotes responsivity index, in the two subgroups. All data are expressed as mean ± SEM, asterisks (*$p<0.05$, **$p<0.01$) indicate significant differences compared to vehicle, crosses (++$p<0.1$; +++$p<0.001$) indicate significant differences between vehicle HA and vehicle ADHD-C animals. All data were analysed using repeated measures two-way ANOVA followed by planned post-hoc comparisons.

**8.4 Discussion**

The present study demonstrates that rats selected with deficits in sustained attention, vigilance and response inhibition in the 5C-CPT, represent a translational animal model of symptoms in the combined subtype of adult ADHD. These deficits in attention and vigilance are shown by decreased accuracy in responding to target stimuli and decreased SI (a non-parametric measure of vigilance taking into account target and non-target stimulus responses). The increased false alarm responding of ADHD-C rats to
non-target stimuli is indicative of a deficit in response inhibition. Taken together; deficits in response inhibition and attention in the 5C-CPT mimic the deficits in clinical adult ADHD, as enhanced false alarm responding often accompanies impaired CPT performance (Groman et al., 2009).

These findings extend our earlier work in rats modeling the inattentive subtype (ADHD-I) of adult ADHD, and the impulsive traits in the combined (ADHD-C) and predominantly hyperactive-impulsive (ADHD-HI) subtypes of adult ADHD (Tomlinson et al., 2014). The first aim of the present study was to combine symptoms of inattention and deficits in response inhibition to form a model of the symptoms of ADHD-C. We have expanded our previous work as we have now characterised a combined model of inattention and impulsivity. This model can be utilised to investigate the mechanisms underlying attention and impulsivity (specifically response inhibition) and how they are interconnected. Impulsivity is suggested to be a multifaceted construct with motor impulsivity mediated by the serotonergic system, and response inhibition via the dopaminergic system. We have further demonstrated the value of the 5C-CPT in dissociating between the two forms of impulsivity, motor impulsivity and response inhibition.

The second aim of the present study was to investigate the effects of putative therapeutic targets, to enhance attention and response inhibition in ADHD-C animals. Our findings extend previous studies and demonstrate the cognitive enhancing properties of tolcapone and A-412997. In the present study, both compounds increased vigilance and reduced false alarm responding at the highest doses in ADHD-C animals. A-412997 did not enhance sustained attention but increased the SI at the two highest doses in ADHD-C, indicative of an increase in vigilance in this subgroup. However, at the highest dose there was a reduction in responsivity index (RI), suggesting that the change in SI may not be due to increased vigilance but may be due to a change in strategy or response bias of the animal. Thus, we cannot conclude that the change in SI at 1.0 umol/kg is due to an increase in vigilance. In contrast, however at the lower dose of 0.3umol/kg there was no accompanying change in RI indicating that, at this dose, there was indeed an increase in vigilance in ADHD-C animals produced by D4 receptor stimulation.

For the first time we have shown that A-412997 at 0.3 and 1.0 umol/kg reduced false alarm responding in ADHD-C adult rats. This reduction was to a level comparable to that of vehicle HA animals, normalising the response inhibition deficits. Importantly,
the decrease in false alarm responding was not due to an overall decrease in responding, as the hit rate did not change. However, at 1.0umol/kg there was a change in RI indicating a possible change in strategy or response bias of the animals. These findings support the role of dopamine and specifically the role of DRD4 in response inhibition. Our findings are consistent with previous findings in DRD4 knock-out mice that show response inhibition deficits in the 5C-CPT (Young et al., 2011). To date previous studies investigating the role of the D4 receptor in ADHD have focused on hyperactivity, using tests of the locomotor hyperactivity to mimic that observed in childhood ADHD. Administration of CP-293019, u-101958, L-745870 and S-18126 (DRD4 antagonists) attenuate the hyperactivity present in the neurotoxin 6-hydroxydopamine neonatal lesion model of ADHD in rats (Zhang et al., 2002a; Zhang et al., 2001) and mice (Avale et al., 2004), suggesting a role of the DRD4 in hyperactivity symptoms of childhood ADHD. We did not measure hyperactivity between subgroups in adult rats and this is a limitation. Also as our results suggest, we would predict different levels of activity between the subtypes. A-412997 has also been shown to restore delay dependent deficits in the novel object recognition task in rats (Woolley et al., 2008). Others have also shown A-412997 to improve cognitive performance in SHR rat pups (proposed as a model of childhood ADHD) in the 5-trial inhibitory avoidance test (Browman et al., 2005). We have extended these studies by using a selection of adult rats from within a normal population, based on extremes of normal behaviours and a translational task; the 5C-CPT. Our findings show that the DRD4 mediates response inhibition and vigilance in adult ADHD with no effects on motor impulsivity or sustained attention in the ADHD-C subtype. These data suggest that the DRD4 may denote a new target for treatment of improving symptoms in the adult ADHD-C subtype. Woolley and colleagues (2008) have also shown that A-412997 does not have the potential drug abuse liability associated with psychostimulant treatment making DRD4 a desirable target in ADHD.

The second agent used; tolcapone exerts its effects by COMT inhibition, it increases extracellular levels of dopamine in the rat PFC (Tunbridge et al., 2004) and has little or no effect on extracellular dopamine levels in the striatum (Budygin et al., 1999). The PFC plays an important role in cognitive function, including attentional and inhibitory control processes. Here, we have shown that tolcapone at the two highest doses (10 and 15 mg/kg) increased sustained attention while 15 mg/kg also increased vigilance in ADHD-C animals only. The increase in SI in ADHD-C at 15 mg/kg was not accompanied by a significant effect on RI suggesting that the SI increase was due to a
real increase in vigilance in ADHD-C animals. However, despite the increase in SI in ADHD-C animals, they did fail to reach the vigilance level of the HA vehicle animals, suggesting that the level of increase was not sufficient to completely overcome the deficit. Tolcapone at the highest dose (15 mg/kg) also reduced false alarm responding in ADHD-C animals, indicative of an increase in response inhibition.

Interestingly, 15 mg/kg of tolcapone decreased vigilance and sustained attention in HA animals to the same level as that observed following 15 mg/kg in ADHD-C animals. However, at 15 mg/kg in HA animals, the number of trials completed decreased. This suggests a reduction in motivational state, not due to a change in motor activity as response latencies did not differ across groups. Another possibility could be genetic differences between the groups as COMT activity is genetically influenced, with the highest level of variance resulting from the Val158Met polymorphism. The difference between homozygotes Val-COMT and homozygotes Met-COMT is estimated to be 35% COMT enzyme activity in the human brain (Chen et al., 2004). Given that dopamine in the PFC and cognitive performance have an inverted U-relationship, i.e. with too much or too little dopamine leading to impairments in cognition e.g. in working memory (Cools and D'Esposito, 2011; Robbins and Arnsten, 2009; Tunbridge, 2010; Williams and Goldman-Rakic, 1995), genetic differences in COMT could affect this shift. Farrell and colleagues (2012) have elegantly shown how these genetic differences can effect the direction of cognitive consequences produced by tolcapone. In humans pharmacological COMT inhibition has been shown to impair performance in Met-COMT subjects, and improve performance in Val-COMT (higher COMT activity) subjects (Farrell et al., 2012; Giakoumaki et al., 2008). While not suggesting that the ADHD-C rats had this polymorphism, given the strong genetic influence on COMT, these animals may have discrete genetic or epigenetic differences. This genetic difference may be leading to a differential response to tolcapone in both rats and humans. This possibility requires further exploration.

Using the translational 5C-CPT we have selected subgroups of rats with opposite extremes of behaviour (Tomlinson et al., 2014). By utilising the ADHD-C rat subgroup displaying severe impairments in attention and response inhibition we have now demonstrated an animal model of the symptoms of the ADHD-C subtype. The majority of animal models of ADHD focus on the hyperactivity component in ADHD. Hyperactivity diminishes in adult ADHD; by using our rat model of the symptoms of ADHD-C (inattention and response disinhibition) we have been able to examine the
precognitive effects of two compounds targeting the dopaminergic system. For the first time we have shown that tolcapone improves attention and response inhibition in a rat model of the symptoms of the ADHD-C subtype. We have also shown that A-412997 improves response inhibition and vigilance in ADHD-C animals. These findings highlight the role of D4 agonism, and COMT inhibition, in the processes of attention and response inhibition. Our findings emphasise the importance of focusing on the endophenotypes of adult ADHD and selecting appropriate treatments dependent on symptom expression. We have also highlighted important novel therapeutic targets for treating inattention and response inhibition.
1a  
ADHD-C and HA

1b  
ADHD-C and HA

1c  
ADHD-C and HA

**Figure legends**

Figure 1a, 1b and 1c: Histograms showing the distribution of ADHD-C and HA animals’ accuracy scores (1a), sensitivity index (1b), and false alarm rate (1c) (ADHD-C; n=8, HA; n=8). The darker bars represent the HA animals, and the grey bars the ADHD-C animals.
Table 1: 5C-CPT measures in the HA and ADHD-C subgroups

<table>
<thead>
<tr>
<th></th>
<th>HA</th>
<th>ADHD-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>117.00 ± 2.45</td>
<td>116.89 ± 2.58</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>94.50 ± 0.77</td>
<td>82.30 ± 1.52***</td>
</tr>
<tr>
<td>% Omission</td>
<td>13.00 ± 3.14</td>
<td>28.00 ± 4.60***</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.73 ± 0.05</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.74 ± 0.20</td>
<td>0.89 ± 0.14</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.12 ± 0.15</td>
<td>1.62 ± 0.31</td>
</tr>
<tr>
<td>Premature responses</td>
<td>15.13 ± 4.49</td>
<td>23.44 ± 3.46</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.59 ± 0.08</td>
<td>0.31 ± 0.09***</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.29 ± 0.02</td>
<td>0.49 ± 0.03***</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.63 ± 0.03</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>1.38 ± 0.06</td>
<td>1.43 ± 0.12</td>
</tr>
<tr>
<td>SI</td>
<td>0.32 ± 0.04</td>
<td>-0.14 ± 0.07***</td>
</tr>
<tr>
<td>RI</td>
<td>-0.14 ± 0.09</td>
<td>-0.18 ± 0.07</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold
*p<0.05, **p<0.01, ***p<0.001. Training parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 5 s. Target trials are represented as (go) and non-target trials are represented as (no-go).

Table 2: Effect of A-412997 in the HA subgroup (0.1, 0.3, 1.0 umol/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th></th>
<th>A-412997</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>Processed trials</td>
<td>92.00 ± 8.00</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>97.21 ± 1.50</td>
</tr>
<tr>
<td>% Omission</td>
<td>18.45 ± 3.14</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>0.74 ± 0.20</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.41 ± 0.15</td>
</tr>
<tr>
<td>Premature responses</td>
<td>14.13 ± 5.26</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>4.09 ± 1.91</td>
</tr>
<tr>
<td>SI</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td>RI</td>
<td>-0.15 ± 0.10</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold
*p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 10 s. Target trials are represented as (go) and non-target trials are represented as (no-go).
Table 3: Effect of A-412997 in the ADHD-C subgroup (0.1, 0.3, 1.0 umol/kg) following a reduced event-rate challenge in the 5C-CPT.

<table>
<thead>
<tr>
<th>A-412997</th>
<th>Vehicle</th>
<th>0.1umol/kg</th>
<th>0.3umol/kg</th>
<th>1.0umol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>83.75 ± 3.70</td>
<td>86.13 ± 5.15</td>
<td>78.50 ± 7.30</td>
<td>76.63 ± 3.49</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>91.41 ± 1.55</td>
<td>93.22 ± 1.70</td>
<td>90.94 ± 2.21</td>
<td>89.86 ± 4.37</td>
</tr>
<tr>
<td>% Omission</td>
<td>19.61 ± 1.11</td>
<td>17.32 ± 1.40</td>
<td>20.75 ± 2.65</td>
<td>23.62 ± 2.45</td>
</tr>
<tr>
<td>Correct latency</td>
<td>0.76 ± 0.02</td>
<td>0.79 ± 0.04</td>
<td>0.75 ± 0.02</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency</td>
<td>0.92 ± 0.07</td>
<td>0.76 ± 0.10</td>
<td>1.04 ± 0.17</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>Magazine latency</td>
<td>1.23 ± 0.05</td>
<td>1.25 ± 0.08</td>
<td>1.50 ± 0.12</td>
<td>1.33 ± 0.09</td>
</tr>
<tr>
<td>Premature responses</td>
<td>23.88 ± 4.02</td>
<td>15.38 ± 2.58</td>
<td>18.25 ± 6.25</td>
<td>20.25 ± 5.54</td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.48 ± 0.02</td>
<td>0.55 ± 0.03</td>
<td>0.46 ± 0.06</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.57 ± 0.01</td>
<td>0.51 ± 0.04</td>
<td><strong>0.27 ± 0.06</strong></td>
<td><strong>0.20 ± 0.03</strong> ***</td>
</tr>
<tr>
<td>Incorrect latency</td>
<td>0.69 ± 0.02</td>
<td>0.67 ± 0.03</td>
<td>0.61 ± 0.09</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>Magazine latency</td>
<td>2.17 ± 0.56</td>
<td>2.55 ± 1.06</td>
<td>1.64 ± 0.31</td>
<td>2.12 ± 0.54</td>
</tr>
<tr>
<td>SI</td>
<td>-0.08 ± 0.03</td>
<td>0.03 ± 0.05</td>
<td><strong>0.15 ± 0.04</strong></td>
<td><strong>0.20 ± 0.03</strong> **</td>
</tr>
<tr>
<td>RI</td>
<td>0.04 ± 0.02</td>
<td>0.07 ± 0.05</td>
<td>-0.20 ± 0.11</td>
<td><strong>-0.38 ± 0.08</strong> *</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold *p<0.05, ** p<0.01, ***p<0.001, compared with vehicle. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 10 s.

Table 4: Effect of tolcapone in the HA subgroup (3.0, 10.0, 15.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT measures.

<table>
<thead>
<tr>
<th>Tolcapone</th>
<th>Vehicle</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
<th>15.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>94.63 ± 4.78</td>
<td>83.00 ± 8.61</td>
<td>81.63 ± 7.90</td>
<td><strong>80.38 ± 5.96</strong></td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>96.36 ± 1.68</td>
<td>92.57 ± 2.28</td>
<td>91.99 ± 1.82</td>
<td><strong>86.49 ± 3.98</strong></td>
</tr>
<tr>
<td>% Omission</td>
<td>13.59 ± 1.64</td>
<td>19.99 ± 2.88</td>
<td>20.26 ± 1.35</td>
<td>19.63 ± 3.65</td>
</tr>
<tr>
<td>Correct latency (go/no-go)</td>
<td>0.70 ± 0.03</td>
<td>0.71 ± 0.04</td>
<td>0.72 ± 0.03</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>Incorrect latency (go)</td>
<td>1.05 ± 0.23</td>
<td>0.98 ± 0.18</td>
<td>0.94 ± 0.16</td>
<td>0.66 ± 0.19</td>
</tr>
<tr>
<td>Magazine latency (go)</td>
<td>1.55 ± 0.16</td>
<td>1.50 ± 0.15</td>
<td>1.28 ± 0.08</td>
<td>1.35 ± 0.07</td>
</tr>
<tr>
<td>Premature responses</td>
<td>27.13 ± 1.17</td>
<td>29.38 ± 3.86</td>
<td>23.38 ± 3.20</td>
<td><strong>14.88 ± 2.45</strong></td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.65 ± 0.04</td>
<td><strong>0.48 ± 0.06</strong> *</td>
<td><strong>0.48 ± 0.03</strong> **</td>
<td><strong>0.47 ± 0.08</strong> **</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.34 ± 0.04</td>
<td>0.27 ± 0.03</td>
<td>0.29 ± 0.05</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Incorrect latency (no-go)</td>
<td>0.69 ± 0.04</td>
<td>0.78 ± 0.04</td>
<td>0.79 ± 0.05</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td>Magazine latency (no-go)</td>
<td>2.12 ± 0.38</td>
<td>3.21 ± 1.75</td>
<td>4.90 ± 2.57</td>
<td>3.30 ± 1.78</td>
</tr>
<tr>
<td>SI</td>
<td>0.33 ± 0.04</td>
<td>0.22 ± 0.07</td>
<td>0.21 ± 0.09</td>
<td><strong>0.08 ± 0.08</strong> **</td>
</tr>
<tr>
<td>RI</td>
<td>-0.01 ± 0.08</td>
<td>-0.26 ± 0.06</td>
<td>-0.25 ± 0.05</td>
<td><strong>-0.13 ± 0.13</strong></td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold *p<0.05, **p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 2 s SD, 5 s TO, 2 s LH, a variable ITI averaging 10 s. Target trials are represented as (go) and non-target trials are represented as (no-go).
Table 5: Effect of tolcapone in the ADHD-C subgroup (3.0, 10.0, 15.0 mg/kg) following a reduced event-rate challenge in the 5C-CPT measures.

<table>
<thead>
<tr>
<th>Measures</th>
<th>Vehicle</th>
<th>3.0 mg/kg</th>
<th>10.0 mg/kg</th>
<th>15.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed trials</td>
<td>79.75 ± 5.12</td>
<td>79.13 ± 3.75</td>
<td>76.50 ± 5.75</td>
<td>80.13 ± 3.87</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>85.21 ± 2.49</td>
<td>90.48 ± 3.67</td>
<td><strong>94.17 ± 1.69</strong></td>
<td><strong>91.46 ± 2.56</strong></td>
</tr>
<tr>
<td>% Omission</td>
<td>13.58 ± 1.06</td>
<td>17.36 ± 2.00</td>
<td>12.91 ± 1.70</td>
<td>16.88 ± 1.95</td>
</tr>
<tr>
<td>Correct latency</td>
<td>0.71 ± 0.03</td>
<td>0.72 ± 0.03</td>
<td>0.71 ± 0.03</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Incorrect latency</td>
<td>0.90 ± 0.15</td>
<td>0.89 ± 0.17</td>
<td>0.87 ± 0.12</td>
<td>0.94 ± 0.09</td>
</tr>
<tr>
<td>Magazine latency</td>
<td>1.28 ± 0.13</td>
<td>1.49 ± 0.23</td>
<td>1.28 ± 0.14</td>
<td>1.74 ± 0.29</td>
</tr>
<tr>
<td>Premature responses</td>
<td>46.50 ± 10.08</td>
<td>26.88 ± 3.19</td>
<td>23.63 ± 7.92</td>
<td><strong>20.75 ± 2.33</strong></td>
</tr>
<tr>
<td>p[HR]</td>
<td>0.37 ± 0.07</td>
<td>0.42 ± 0.05</td>
<td>0.42 ± 0.06</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>p[FA]</td>
<td>0.66 ± 0.04</td>
<td>0.72 ± 0.08</td>
<td>0.71 ± 0.04</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Incorrect latency</td>
<td>0.72 ± 0.06</td>
<td>0.72 ± 0.06</td>
<td>0.77 ± 0.07</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>Magazine latency</td>
<td>1.59 ± 0.26</td>
<td>1.66 ± 0.42</td>
<td>1.39 ± 0.18</td>
<td>1.62 ± 0.27</td>
</tr>
<tr>
<td>SI</td>
<td>-0.14 ± 0.06</td>
<td>-0.05 ± 0.06</td>
<td>0.08 ± 0.07</td>
<td><strong>0.07 ± 0.04</strong></td>
</tr>
<tr>
<td>RI</td>
<td>0.30 ± 0.08</td>
<td>0.13 ± 0.10</td>
<td>0.25 ± 0.09</td>
<td>0.05 ± 0.08</td>
</tr>
</tbody>
</table>

Measures are shown as observed mean ±SEM. Significant values are in bold * represents p<0.05, ** p<0.01, ***p<0.001. Reduced event-rate challenge parameters consisted of 1.0 s stimulus duration, 5 s Time Out Period, 2 s Limited Hold, a variable inter-trial interval (ITI) averaging 10 s.
9. NEUROCOGNITIVE DEFICITS ARE CORRELATED WITH SYMPTOM EXPRESSION IN ADULTS WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

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³ 5 Boroughs Partnership NHS Foundation Trust

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Abstract

Aims: Adults with ADHD show cognitive deficits across varying domains, including; sustained attention, response inhibition and executive function. The literature to date is inconsistent and requires further clarification of the core cognitive deficits in adult ADHD. Little is known about the relationship between performance in cognitive tasks and the level of symptom expression in patients with ADHD. In this study we aimed to investigate firstly, whether adults with ADHD (unmedicated) showed deficits in cognition compared with controls. Secondly, to investigate whether medicated ADHD patients showed a lower level of impairment compared with unmedicated patients. Finally, to explore the association between specific ADHD symptoms and cognitive deficits.

Methods: Two groups of adult ADHD patients (n=79) and a group of healthy controls (n=31) with no history of ADHD were recruited. The ADHD group included patients not yet taking medication (unmedicated, n=41) and patients stable on treatment (medicated, n=38). Each participant completed several cognitive tasks including the rapid visual information processing task (RVIP), intra-extra dimensional shift task (IED), stop-signal task (SST) and the spatial working memory task (SWM) from the Cambridge Automated Neuropsychological Test Battery (CANTAB). Finally, participants completed the Conners’ Adult ADHD Rating Scale, long version (CAARS:LV) which was used to measure the level of symptom expression.

Results: Unmedicated adult ADHD patients showed deficits across all of the cognitive domains measured (sustained attention, executive function, response inhibition, and spatial working memory) compared with controls. Interestingly no deficits were observed in the medicated group of ADHD patients compared with controls. However in the medicated group, poor performance in the RVIP and SWM was correlated with high levels of hyperactive-impulsive symptoms. Poor performance in the RVIP in unmedicated patients was correlated with higher inattentive symptoms. Poor SWM performance in the unmedicated group was associated with total ADHD symptoms and the poor IED task performance was correlated with high levels of hyperactivity only. However poor SWM performance was associated with high levels of impulsivity and hyperactivity. Gender differences were observed in the unmedicated group; males were more impaired in the RVIP and SWM compared to females, and females more impaired in the IED task compared with males.
Conclusions: Unmedicated adults with ADHD have deficits in sustained attention, executive function, working memory and response inhibition. These deficits do not appear to be present in patients stabilised on medication. In the medicated group, higher levels of impulsivity and hyperactivity appear to be related to impairments in sustained attention and spatial working memory on CANTAB tasks. However in unmedicated ADHD patients, poor performance in the sustained attention task was associated with higher levels of overall inattentive symptoms. In unmedicated ADHD patients deficits in attentional set-shifting was associated with increased hyperactivity.
9.1 Introduction

Attention-deficit hyperactivity disorder (ADHD) is a common and debilitating, lifelong neuropsychiatric disorder (Faraone and Biederman, 2005) associated with cognitive dysfunction in both childhood and adulthood. (Nigg et al., 2005; Seidman, 2006). Adult ADHD is associated with academic and social disability caused partly by cognitive impairments, specifically in executive function (Diamantopoulou et al., 2007; Ni et al., 2013).

The clinical manifestation of ADHD is a complex phenotype. It is plausible that alongside, or within, the typical ADHD subtypes, cognitive subgroups (endophenotypes) within ADHD exist. Indeed, in a recent study using the rapid visual processing (RVP) CANTAB task, in a large sample of children with ADHD (n=438), the researchers report that a specific RVIP endophenotype exists for ADHD (Gau and Huang, 2014). The search and exploration of endophenotypes in ADHD are vital to enable a targeted treatment approach, as different cognitive endophenotypes may respond differently to treatment. We have recently shown in animal models of the subtypes of adult ADHD, that responses to methylphenidate and atomoxetine differ dependent on the baseline level of inattention or impulsivity (Tomlinson et al., 2014).

Studies have suggested that deficits in executive function should be an independent target for ADHD therapy (Arnsten and Pliszka, 2011; Faraone et al., 2005a). However, before treatment can be targeted to one or more cognitive symptoms, more research is required in adults with ADHD in order to identify the core deficits and validate new neuropsychological instruments to assess these deficits. Gau et al., (2014) argue that sustained attention assessed by the CANTAB RVIP may be an endophenotype of ADHD, and the measurements generated by the RVIP may be used as state markers of ADHD. Studies comparing adults with ADHD and healthy controls using CANTAB tasks are limited and have modest to small sample sizes (n<20) (Aron et al., 2003b; Clark et al., 2007; Dowson et al., 2004; McLean et al., 2004; Pironti et al., 2013). Additionally to date, samples have been limited to predominately males, and therefore results cannot be directly applied to females. More research in larger samples (including males and females) is clearly required to evaluate the CANTAB tasks as valid tests to assess cognition in adult ADHD.

Our primary aim in the present study is to comprehensively investigate the specific cognitive deficits in a large sample of unmedicated adult ADHD patients. Also, we aim to compare the cognitive deficits between males and females in healthy population and
in an unmedicated ADHD group. The chosen neuropsychological test system is the objective, computerised language independent system, CANTAB. The use of a number of CANTAB tasks in a large ADHD sample will enable the evaluation of these cognitive tasks in assessing and detecting cognitive impairments in adult ADHD.

We also aim to identify areas of cognition that are not improved by treatment with medication. This will be achieved by comparing the cognitive deficits in two groups (a group stabilised on ADHD medication and a group as yet unmedicated) with each other, and with a healthy control group. This may enable the identification of deficits to be used as targets for new therapeutics.

ADHD patients with differential predominant symptom expression i.e. symptom subtypes, may have altered underlying neuropsychological disturbances (Nikolas and Nigg, 2014). These may potentially be sensitive to different pharmacological and non-pharmacological interventions, i.e. studies such as ours may enable stratification of patients into different treatment groups. Very few studies in adults have examined the relationship between the different symptoms (or subtypes) of ADHD and associated cognitive deficits. This is of particular importance considering the complex, dimensional structure of ADHD. Many researchers believe that ADHD is best conceptualised as dimensional, that is ADHD symptoms exist on a continuum (Kolokotroni et al., 2014). Recent studies have focused on children, assessing the association between ADHD subtypes and various neuropsychological domains, however these studies have mainly focused on executive function (Guerts et al., 2005; Loo et al., 2007; Martel et al., 2007; Nikolas and Nigg, 2014; Riccio et al., 2006; Scheres et al., 2004; Solanto et al., 2007; Wodka et al., 2008). Our study aims to enhance this work and expand it into adult ADHD by focusing on the correlation between deficits in specific cognitive domains and symptom expression.

Furthermore, most studies in this area have focused on the inattentive and combined subtypes with little focus on the impulsive-hyperactive subtype. Research to date has produced mixed findings, mainly using modest sample sizes. Results overall suggest that the combined subtype are impaired in more cognitive domains compared with the inattentive subtype (Hinshaw et al., 2002; Klorman et al., 1999; Nigg et al., 2002). In agreement with this, a recent study in a large sample of ADHD patients (n=498) aged between 6-17 years demonstrated large neurocognitive differences between ADHD patients and age-matched controls (Mendez et al., 2010). The differences were greatest in the combined subtype compared with the inattentive subtype, across all of the
neurocognitive domains tested. The authors conclude that the DSM-IV ADHD subtypes match the severity of behavioural and neurocognitive impairments among ADHD children rather than independent configurations each representing one distinct etiology.

It remains unclear which neurocognitive impairments make unique contributions to ADHD symptom expression in adults and importantly the impact of treatment on cognitive function in the various subtypes. The inclusion of multiple domains of cognitive function via specific tasks is important for a more comprehensive exploration of the deficits occurring and correlating with the different symptom subtypes of ADHD. Thus the final aim of this study was to explore the relationship between the various symptoms of ADHD (inattention, impulsivity and hyperactivity) and deficits in specific cognitive domains. We hypothesised that impaired performance in the CANTAB attention tasks (RVIP and IED) will correlate with higher levels of self-reported inattention. We also predicted that response inhibition impairments in the stop-signal task would be associated with higher self-reported scores of impulsivity in the ADHD group. We finally expected there to be larger deficits across all cognitive domains, in patients scoring higher for total ADHD symptoms.

9.2 Methods

9.2.1 Participants

The study design is shown in appendix 1. The final sample consisted of a total of 110 participants (controls and ADHD patients). The ADHD sample included 79 patients between the ages of 18-65 (22 females, 57 males, mean age = 28.5 years) with a diagnosis of ADHD according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed; DSM-IVTR; American Psychiatric Association, 1994) criteria, see Table 2 for demographic data. The control sample consisted of 31 healthy participants without a diagnosis of ADHD (16 females, 15 males, mean age = 26.10 years). Participants with ADHD were recruited from two UK-based specialist adult ADHD clinics. To be included in the study, participants must have been diagnosed with ADHD by a specialist adult ADHD consultant psychiatrist. In the two clinics the diagnosis of ADHD was based on DSM-IVTR criteria. The diagnostic process involved completion of the Wender Utah Rating Scale, adult ADHD current behaviour scale and a semi-structured interview based on the DSM-IV criteria for ADHD (Barkley and Murphy, 2005). A semi-structured psychiatric interview adapted from the mini-SCID for DSM-IV; (First et al., 1996) was also conducted to screen for other psychiatric conditions.
The diagnostic process was conducted over 2-4 consultations with the clinical team (including a specialist adult ADHD consultant psychiatrist and specialist adult ADHD nurse). Informants (relatives, partners) were also asked to complete informant-rating scales and attend appointments with the patient. All participants with ADHD in adulthood, also had a diagnosis of ADHD, or symptoms of ADHD in childhood. Upon entry into the study the ADHD sample was split into two groups; Group 1 - unmedicated patients (n = 41); Group 2 - patients stable on medication (n = 38).

9.2.2 Recruitment and Screening

Control participants were recruited via advertisements in the University and recruitment emails sent to local businesses. In addition to the criteria below, control participants were excluded if they screened positive for ADHD or if they had a history of childhood ADHD. This was determined by the Conners’ Adult ADHD Rating Scales, with a T-score >70 on CAARS sections-E, F, G and H the threshold for exclusion. Two participants screened positive for ADHD and were excluded at this point.

Exclusion criteria included: intellectual disability (WTAR estimated verbal IQ score <80), clinically unstable psychiatric disorders (psychosis, suicidal behaviours), bipolar disorder, current major depressive episode, current drug or alcohol abuse or dependence, current use of a mood stabilizer or antipsychotic, currently pregnant, epilepsy and/or head trauma.

9.2.3 Materials

9.2.3.1 Rating scales and IQ testing

ADHD symptoms: Conner’s Adult ADHD Rating Scales (CAARS) were developed based on manifestations of adult ADHD and DSM criteria (Conners et al., 1999). CAARS have been well validated, the Internal consistency coefficients range from 0.77 to 0.97, with 2- to 4-week test-retest reliability coefficients (Cronbach’s alpha) ranging from 0.71 to 0.98 (all correlations significant, \( p<0.001 \)) (Erhardt et al., 1999). CAARS consists of eight clinical scales, both self-report or observer-report measures of current ADHD symptoms and behaviours. The long version of the self-report CAARS was used (CAARS-SL) in this study, which consists of 66 items separated into 8 subsections (A-H). These subsections include: CAARS-A Inattentive/Cognitive problems, CAARS-B Hyperactivity/Restlessness, CAARS-C Impulsivity/Emotional Lability, CAARS-D Problems with Self-Concept, CAARS-E DSM IV Inattentive Symptoms, CAARS-F DSM-IV Hyperactive-Impulsive Symptoms, CAARS-G DSM-IV Total
ADHD Symptoms and CAARS-H ADHD Index. The T-scores for each subsection were calculated and used in this study to minimize the effects of age and gender on ADHD behavioral symptoms. All participants also completed the psychometrically validated WEISS Functional Impairment Rating Scale (WFIRS-S) a self-report measure (Weiss et al., 2007; Weiss et al., 2005). The WFIRS-S is specific to impairments in ADHD and consists of a 69-item survey in seven different areas (family, work, school, life skills, self-concept, social, risk).

The Wechsler Test of Adult Reading (WTAR) was completed by all participants and provided an estimated verbal IQ.

9.2.3.2 Neuropsychological testing (CANTAB tasks)

The CANTAB battery has been validated in many studies including various neuroimaging studies in patients with various cognitive impairments and in healthy participants. The following five CANTAB tasks were administered to all participants.

Rapid Visual Information Processing (RVIP)

The RVIP is a visual continuous performance test adapted and simplified from Wesnes and Warburton’s task (Wesnes and Warburton, 1984) that assesses sustained attention (Sahakian et al., 1989). Numbers (1-9) were presented pseudo-randomly one at a time at a speed of 100 digits/min. The participant was required to detect three target sequences (3-5-7, 2-4-6, and 4-6-8) and respond by pressing a press-pad button when they had seen the last number of the sequence (within 1800 ms). The participant was instructed to try and detect as many sequences as they could during the task lasting 4 min. The various measures calculated during this task include: total hit score (the number of times the target sequence was detected); total misses (the number of times the target sequence was missed); total false alarms (the number of responses made outside of the target sequence); total correct rejections (the number of stimuli inappropriately responded to). These measures were used to generate 1. Probability of hits ($h$) = total hits/(hits + misses); 2. Probability of false alarms ($f$) = total false alarms/(false alarms + correct rejections); 3. $A' = (0.5+[h-f]+(h-f)^2)/(4*hf^*(1-f))$: a signal detection theory measure of sensitivity to respond to the target (Sahgal, 1987); 4. Correct response mean latency.
Spatial Working Memory (SWM)

The SWM task is based on a self-ordered test (Petrides and Milner, 1982) and is adapted from Olton’s radial arm maze (Olton, 1987). It is a task involving working memory processing of spatial locations. On each trial, the participant was asked to search for a blue token hidden in one of an array of coloured boxes. The participant has to touch a box (one at a time) on the touch-screen to reveal the blue token in one of the boxes. When the blue-token is revealed, the participant must remember the box they had opened. Once a token is found in a particular box it will never occur in that box again (during that trial). When each token is revealed, it is then moved to a column at the side of the screen. At any one time, one token is hidden under one box and the participant must search until it is found. The trial is completed when each token has been found in every box on the screen, the number of boxes gradually increases after each trial. The task involves four 4-box, four 6-box and four 8-box trials (16 trials in total). There are various actions that are measured during the task including 1. ‘Between-search’ error, which occurs when the participant returns to a box in which a token has already been located in that particular trial. 2. Total errors, or within-search errors occur when the participant selects the same empty box twice; Previous research has shown that adopting a particular strategy can improve performance, therefore; 3. A strategy score was also generated for the task (for further details of the precise strategy score calculation see: (Dowson et al., 2004)), the lower the strategy score value indicates a more consistent use of the core effective strategy.

Intra/Extra Dimensional Set Shift (IED)

This task assesses set-shifting by the ability to maintain selective attention on specific attributes of compound stimuli across different examples (intradimensional shift) and then shift attention to a previously irrelevant attribute of stimuli or extradimensional shift (EDS; (Downes et al., 1989)). The task involved nine stages, with the initial part of the stage requiring the participant to acquire the rule through trial and error. Once the rule has been discovered and correctly adhered to on six consecutive occasions, the rule then changes to a new rule. Three target indices were included 1. Pre-EDS (errors made before the EDS stage); 2. EDS errors (errors made in the EDS stage); 3. Completed stages (total number of stages completed successfully).
Stop-Signal Task (SST)

Stop-signal tasks are classic paradigms used to measure pre-potent response inhibition (Logan, 1994). Participants were required to make a fast response in ‘go-trials’ (press the left button on a keypad when a left arrow is displayed and right when the right arrow is displayed), and stop themselves from responding during a ‘no-go trial’ (signaled by a 300-Hz tone). The no-go trials were made more difficult by ¾ of the trials being go-trials (75%). The tasks included 5 blocks of 64 trials each, and at the end of each block participants were given visual feedback for their average ‘go-trial’ reaction time and errors made during go-trials. During this time, participants were urged to do their best to stop at the beep while continuing to respond as quickly as possible during go-trials. The beep (auditory stop-signal) occurrence changes throughout the test, thus it could not be predicted to occur at a specific time in a particular order. The four key outcome variables include: 1. SSRT (time in ms to suppress prepotent motor responses, indicative of inhibitory control); 2. Mean ‘go-trial’ reaction time (time to press the button when an arrow is displayed); 3. Directional errors (number of incorrect presses of the button); 4. Stop signal detection (SSD).

9.2.4 Data Analysis

Data were analysed using SPSS (Version 20) and are expressed as mean ± SD. The unmedicated group and the medicated group were compared to the control group for all measures (CANTAB measures and CAARS scores), and with each other. Data were checked for normality; the CAARS data were not normally distributed and were analysed using the non-parametric Kruskal-Wallis test. The remaining measures were normally distributed and analysed by one-way ANOVA followed by Bonferroni post-hoc analysis. The effects of gender were compared using the independent samples t-test. Spearman’s rho was used to determine whether there were any associations between CAARS scores and CANTAB measures.

9.3 Results

One way ANOVA revealed no significant differences for verbal IQ ($F_{(2,107)} = 1.44$, $p=0.24$), and age ($F_{(2,107)} = 1.84$, $p=0.17$) between unmedicated, medicated and control groups.
9.3.1 CANTAB results:

9.3.1.1 Rapid Visual Information Processing (RVIP)

One-way ANOVA showed a significant group effect for correct rejections ($F_{(2,104)} = 7.6$, $p<0.001$; table 1), post-hoc analysis revealed that unmedicated patients scored significantly lower than controls ($p<0.001$, Cohen’s $d=0.87$). Unmedicated patients also scored significantly lower than medicated patients ($p<0.01$, Cohen’s $d=0.67$). One-way ANOVA revealed a significant difference for RVIP A’ ($F_{(2,104)} = 6.04$, $p<0.01$; table 1). Post-hoc testing showed that A’ was significantly lower in unmedicated patients compared with both controls and medicated patients ($p<0.01$, Cohen’s $d=0.67$, 0.66; respectively). There was a significant group effect for RVIP misses ($F_{(2,104)} = 7.81$, $p<0.001$; table 1), post-hoc analysis showed that unmedicated patients scored significantly higher compared to controls ($p<0.001$, Cohen’s $d=0.86$) and medicated patients ($p<0.01$, Cohen’s $d=0.61$).

9.3.1.2 Spatial Working Memory (SWM)

One-way ANOVA revealed group differences for both measures of errors: total errors ($F_{(2,104)} = 5.38$, $p<0.01$; table 1); and between errors ($F_{(2,104)} = 5.00$, $p<0.01$; table 1). Bonferroni post-hoc analysis showed that unmedicated patients scored significantly higher between errors ($p<0.01$, Cohen’s $d=0.78$) and total search errors ($p<0.01$, Cohen’s $d=0.81$) compared with controls. One-way ANOVA also showed that strategy was significantly affected ($F_{(2,104)} = 5.62$, $p<0.01$; table 1), post-hoc analysis showed that unmedicated patients had a poorer score (higher value) in strategy compared with medicated patients ($p<0.05$, Cohen’s $d=0.49$) and controls ($p<0.001$, Cohen’s $d=0.85$). No significant differences between the medicated and the control group were found.

9.3.1.3 Intra-extra dimensional shift (IED)

One-way ANOVA revealed a significant group effect for IED stages ($F_{(2,104)} = 3.12$, $p<0.05$; table 1), post-hoc analysis revealed that unmedicated patients completed significantly less stages compared with medicated patients and controls ($p<0.05$, Cohen’s $d=0.43$, 0.49; respectively). ANOVA revealed that there was a significant effect of group on EDS errors ($F_{(2,104)} = 3.00$, $p<0.05$; table 1), post-hoc analysis revealed that unmedicated patients made more errors compared with both medicated patients and controls ($p<0.05$, Cohen’s $d=0.45$, 0.49; respectively).
9.3.1.4 Stop-Signal Task (SST)

One-way ANOVA showed a group effect for directional errors ($F_{(2,104)} = 4.78$, $p<0.01$; table 1), with post-hoc analysis revealing that unmedicated patients made more errors compared with medicated patients ($p<0.05$, Cohen’s $d=0.40$) and controls ($p<0.01$, Cohen’s $d=0.80$). Stop signal detection (SSD) was also significantly affected ($F_{(2,104)} = 3.47$, $p<0.05$; table 1), post-hoc analysis showed that unmedicated patients had significantly decreased detection scores compared with medicated patients and controls ($p<0.05$, Cohen’s $d=0.46, 0.63$; respectively).

<table>
<thead>
<tr>
<th>Table 1: CANTAB measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD Medicated Group (n=38)</td>
</tr>
<tr>
<td>ADHD Unmedicated Group (n=41)</td>
</tr>
<tr>
<td>Control Group (n=31)</td>
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<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td><strong>F value</strong></td>
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<tr>
<td><strong>P value</strong></td>
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<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>RVP</td>
</tr>
<tr>
<td>Correct rejections</td>
</tr>
<tr>
<td>250.41 ± 12.18</td>
</tr>
<tr>
<td>242.23 ± 12.20***</td>
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<tr>
<td>253.13 ± 12.86</td>
</tr>
<tr>
<td>7.62</td>
</tr>
<tr>
<td>0.001</td>
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<tr>
<td>False alarms</td>
</tr>
<tr>
<td>1.51 ± 1.30</td>
</tr>
<tr>
<td>2.45 ± 3.37</td>
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<tr>
<td>2.10 ± 2.64</td>
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<tr>
<td>1.26</td>
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<tr>
<td>0.29</td>
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<tr>
<td>Total misses</td>
</tr>
<tr>
<td>9.95 ± 5.69</td>
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<tr>
<td>13.55 ± 5.60***</td>
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<tr>
<td>8.47 ± 5.54</td>
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<tr>
<td>7.81</td>
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<tr>
<td>0.001</td>
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<tr>
<td>$A'$</td>
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<tr>
<td>0.90 ± 0.05</td>
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<tr>
<td>0.86 ± 0.07**</td>
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<tr>
<td>0.91 ± 0.08</td>
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<tr>
<td>6.04</td>
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<tr>
<td>0.01</td>
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<tr>
<td>SWM</td>
</tr>
<tr>
<td>Strategy</td>
</tr>
<tr>
<td>28.97 ± 7.62</td>
</tr>
<tr>
<td>32.33 ± 5.92***</td>
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<tr>
<td>27.10 ± 6.33</td>
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<tr>
<td>6.52</td>
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<tr>
<td>0.01</td>
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<tr>
<td>Total search errors</td>
</tr>
<tr>
<td>20.84 ± 17.60</td>
</tr>
<tr>
<td>28.46 ± 18.90**</td>
</tr>
<tr>
<td>15.13 ± 13.68</td>
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<tr>
<td>5.38</td>
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<tr>
<td>0.01</td>
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<tr>
<td>Between errors</td>
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<tr>
<td>20.65 ± 17.27</td>
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<tr>
<td>28.00 ± 18.78**</td>
</tr>
<tr>
<td>15.32 ± 13.50</td>
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<tr>
<td>5.00</td>
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<td>0.01</td>
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<tr>
<td>IED</td>
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<tr>
<td>Pre ED errors</td>
</tr>
<tr>
<td>7.49 ± 3.52</td>
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<tr>
<td>7.63 ± 6.56</td>
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<td>5.87 ± 1.71</td>
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<tr>
<td>1.50</td>
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<td>EDS errors</td>
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<tr>
<td>6.54 ± 8.18</td>
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<tr>
<td>10.98 ± 9.78*</td>
</tr>
<tr>
<td>6.87 ± 8.35</td>
</tr>
<tr>
<td>3.00</td>
</tr>
<tr>
<td>0.05</td>
</tr>
<tr>
<td>Stages completed</td>
</tr>
<tr>
<td>8.76 ± 0.64</td>
</tr>
<tr>
<td>8.29 ± 1.19*</td>
</tr>
<tr>
<td>8.71 ± 0.69</td>
</tr>
<tr>
<td>3.12</td>
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<tr>
<td>0.05</td>
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<tr>
<td>SST</td>
</tr>
<tr>
<td>Directional errors</td>
</tr>
<tr>
<td>4.76 ± 7.34</td>
</tr>
<tr>
<td>7.90 ± 8.35**</td>
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<tr>
<td>2.90 ± 3.05</td>
</tr>
<tr>
<td>4.78</td>
</tr>
<tr>
<td>0.01</td>
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<tr>
<td>Mean go-reaction time</td>
</tr>
<tr>
<td>441.25 ± 85.44</td>
</tr>
<tr>
<td>419.96 ± 50.96</td>
</tr>
<tr>
<td>432.59 ± 84.11</td>
</tr>
<tr>
<td>0.79</td>
</tr>
<tr>
<td>0.46</td>
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<tr>
<td>Detection (SSD)</td>
</tr>
<tr>
<td>236.35 ± 121.16</td>
</tr>
<tr>
<td>184.66 ± 102.66*</td>
</tr>
<tr>
<td>243.93 ± 84.78</td>
</tr>
<tr>
<td>3.47</td>
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<tr>
<td>0.04</td>
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<tr>
<td>SSRT</td>
</tr>
<tr>
<td>175.92 ± 80.27</td>
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<tr>
<td>200.84 ± 86.10</td>
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<tr>
<td>160.52 ± 43.08</td>
</tr>
<tr>
<td>2.68</td>
</tr>
<tr>
<td>0.07</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (SD).
Significant differences between unmedicated and medicated compared with controls are shown as ***p<0.001, **p<0.01, *p<0.05.
Significant differences between ADHD groups are shown as "p<0.01, "p<0.05.

9.3.1.5 Gender differences

The independent t-test revealed a significant difference between males and females in the control group for SWM total errors ($t_{(29)}=2.14$, $p<0.05$, Cohen’s $d=0.76$; table 2) and SWM between errors ($t_{(29)}=2.05$, $p<0.05$, Cohen’s $d=0.73$; table 2), showing females made more errors. Also in the control group the independent t-test revealed a significant difference between male and females for IED pre-ED errors ($t_{(29)}=-3.46$, $p<0.01$, Cohen’s $d=1.26$; table 2), males made more errors.

In the unmedicated ADHD group the independent t-test revealed a significant difference between males and females for SWM total search time ($t_{(37)}=-1.59$, $p<0.05$, Cohen’s
d=0.59; table 2), males had increased times. Also in the unmedicated group males made significantly more false alarm response in the RVIP (t\(_{(37)}\)=-2.10, p<0.05, Cohen’s d=0.41; table 2), compared with females. Independent t-test analysis also revealed that females made significantly more errors in total in the IED (t\(_{(37)}\)=1.38, p<0.05, Cohen’s d=0.41; table 2) compared with males. No other measures were significantly different between males and females, table 2.

<table>
<thead>
<tr>
<th>Table 2: CANTAB measurements: Gender differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD Unmedicated Group (n = 41)</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td><strong>RVP</strong></td>
</tr>
<tr>
<td>Correct rejections</td>
</tr>
<tr>
<td>False alarms</td>
</tr>
<tr>
<td>Total misses</td>
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<tr>
<td><strong>SWM</strong></td>
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<tr>
<td>Strategy</td>
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<tr>
<td>Total search errors</td>
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<td>Between errors</td>
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<td>Stages completed</td>
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<tr>
<td><strong>SST</strong></td>
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<tr>
<td>Directional errors</td>
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<tr>
<td>Mean go-reaction time</td>
</tr>
<tr>
<td>Detection (SSD)</td>
</tr>
<tr>
<td>SSRT</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (SD)
Significant differences between male and female are shown as ***p<0.001, **p<0.01, *p<0.05.

9.3.2 ADHD rating scale scores

Kruskal-Wallis analysis showed that CAARS-D (chi\(^2\)=41.62, df=2, p<0.001) scores differed significantly between groups. Pairwise comparisons revealed that the unmedicated ADHD groups scored significantly higher than the control group (p<0.001; table 3). There were no significant differences between the two ADHD groups (medicated and unmedicated) for this measures (CAARS-D).

Kruskal-Wallis analysis showed that scores were significantly different between groups for all CAARS scales chi\(^2\)>51.78, df=2, p<0.001; table 3. Pairwise comparisons revealed that both medicated and unmedicated ADHD groups scored significantly higher than controls (p<0.001) on all measures. The unmedicated ADHD group scored significantly higher than the medicated ADHD group on CAARS-A, E, (p<0.01) and B, C, F, G, H (p<0.05).
9.3.3 Associations between symptoms and CANTAB measures

Spearman’s rho was used to determine whether there were any associations between subsection CAARS scores and CANTAB measures in medicated and unmedicated patients and controls.

CAARS-F was significantly negatively correlated with RVIP A’ (rho=-0.40, n=38, p<0.01), correct rejections (rho=-0.32, n=38, p<0.05; table 4) in the ADHD medicated group. CAARS-F was also significantly positively correlated with RVIP misses (rho=0.39, n=38, p<0.05) in medicated patients. CAARS-F in medicated patients was also positively correlated with SWM errors (rho=0.34, n=38, p<0.05), between errors (rho=0.34, n=38, p<0.05) and strategy (rho=0.36, n=38, p<0.05). In medicated patients, there was a significant negative correlation between SST go-reaction time and CAARS-
A (rho=-0.37, n=38, p<0.05) and SST signal detection and CAARS-H (rho=-0.35, n=38, p<0.05; table 4).

There was a significant negative correlation in the ADHD unmedicated group for RVIP A' (rho=-0.41, n=40, p<0.05) and correct rejections (rho=-0.41, n=40, p<0.01; table 5) with CAARS-E. There was a significant positive correlation in the ADHD unmedicated group for RVIP misses and CAARS-A (rho=0.31, n=40, p<0.05) and CAARS-E (rho=0.34, n=40 p<0.03). There were significant positive correlations in the ADHD unmedicated group for SWM strategy with CAARS-B (rho=0.41, n=40, p<0.01), CAARS-C (rho=0.41, n=40, p<0.01), CAARS-E (rho=0.36, n=40, p<0.03) and CAARS-H (rho=0.35, n=40, p<0.05). There were also significant positive correlations in the ADHD unmedicated group for SWM errors with CAARS-B (rho=0.37, n=40, p<0.05), CAARS-C (rho=0.33, n=40, p<0.05), CAARS-E (rho=0.42, n=40, p<0.01) and CAARS-H (rho=0.32, n=40, p<0.05; table 2). There were also significant positive correlations in the ADHD unmedicated group for SWM between errors with CAARS-B

<table>
<thead>
<tr>
<th>Table 4: CANTAB and symptoms correlations – medicated group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAARS</strong> – Conner’s Adult ADHD Rating Scale</td>
</tr>
<tr>
<td><strong>RVIP</strong> – Rapid visual information processing; <strong>IED</strong> – intra-extra dimensional shift; <strong>SWM</strong> – spatial working memory; <strong>SST</strong> – stop-signal task.</td>
</tr>
<tr>
<td><strong>Misses</strong></td>
</tr>
<tr>
<td><strong>A</strong></td>
</tr>
<tr>
<td><strong>B</strong></td>
</tr>
<tr>
<td><strong>C</strong></td>
</tr>
<tr>
<td><strong>D</strong></td>
</tr>
<tr>
<td><strong>E</strong></td>
</tr>
<tr>
<td><strong>F</strong></td>
</tr>
<tr>
<td><strong>G</strong></td>
</tr>
<tr>
<td><strong>H</strong></td>
</tr>
</tbody>
</table>

The values represent the number per group (e) mean (M) and standard deviation (SD)

CAARS – Conner’s Adult ADHD Rating Scale
RVIP – Rapid visual information processing; IED – intra-extra dimensional shift; SWM – spatial working memory; SST- stop-signal task.

*** denotes p<0.001 ** p<0.01, * p<0.05
(rho=0.37, n=40, p<0.05), CAARS-C (rho=0.34, n=40, p<0.05), and CAARS-E (rho=0.41, n=40, p<0.01). In the ADHD unmedicated group there was also a significant negative correlations for IED stages with CAARS-B (rho=-0.37, n=41, p<0.05), and a positive correlation for IED EDS errors with CAARS-B (rho=0.36, n=41, p<0.05; table 5).

**Table 5: CANTAB and symptoms correlations – unmedicated group**

<table>
<thead>
<tr>
<th>CAARS</th>
<th>RVIP</th>
<th>IED</th>
<th>SWM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Misses</td>
<td>A'</td>
<td>Correct Rejections</td>
</tr>
<tr>
<td>A</td>
<td>0.31</td>
<td>-0.16</td>
<td>-0.30</td>
</tr>
<tr>
<td></td>
<td>p=0.05*</td>
<td>p=0.34</td>
<td>p=0.06</td>
</tr>
<tr>
<td>B</td>
<td>-0.03</td>
<td>-0.01</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>p=0.86</td>
<td>p=0.95</td>
<td>p=0.73</td>
</tr>
<tr>
<td>C</td>
<td>0.17</td>
<td>-0.05</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>p=0.29</td>
<td>p=0.76</td>
<td>p=0.20</td>
</tr>
<tr>
<td>D</td>
<td>0.01</td>
<td>-0.11</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>p=0.99</td>
<td>p=0.50</td>
<td>p=0.67</td>
</tr>
<tr>
<td>E</td>
<td>0.34</td>
<td>-0.41</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>p=0.03*</td>
<td>p=0.01**</td>
<td>p=0.66</td>
</tr>
<tr>
<td>F</td>
<td>-0.24</td>
<td>0.13</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>p=0.14</td>
<td>p=0.43</td>
<td>p=0.18</td>
</tr>
<tr>
<td>G</td>
<td>0.20</td>
<td>-0.05</td>
<td>-0.24</td>
</tr>
<tr>
<td></td>
<td>p=0.23</td>
<td>p=0.77</td>
<td>p=0.14</td>
</tr>
<tr>
<td>H</td>
<td>0.15</td>
<td>-0.09</td>
<td>-0.20</td>
</tr>
<tr>
<td></td>
<td>p=0.37</td>
<td>p=0.57</td>
<td>p=0.22</td>
</tr>
</tbody>
</table>

The values represent the number per group (n) mean (M) and standard deviation (SD)
CAARS= Conner's Adult ADHD Rating Scale
RVIP = Rapid visual information processing; IED = intra-extra dimensional shift; SWM = spatial working memory; SST- stop-signal task.
*** denotes p<0.001 ** p<0.01, * p<0.05

Spearman’s rho revealed a significant negative correlation in the control group for SST errors and CAARS-B (rho=-0.54, n=31, p<0.01), CAARS-C (rho=-0.40, n=31, p<0.05), CAARS-E (rho=-0.37, n=31, p<0.04), CAARS-F (rho=-0.36, n=31, p<0.05), CAARS-G (rho=-0.42, n=31, p<0.05), and CAARS-H (rho=-0.40, n=31, p<0.05; table 6). No other correlations were detected.
Discussion

Previous studies in adult ADHD using CANTAB tasks have yielded conflicting findings; possibly due to small sample sizes, the use of predominately male individuals and differences in medication status, meta-analysis: Chamberlain et al., 2010. In the present study we have used a larger, more heterogeneous sample of adults with ADHD (males and females) recruited from two geographically distinct clinics. There are a limited number of studies examining gender differences using CANTAB in ADHD, therefore we have extended these studies in a larger sample and shown gender differences in cognition in adults with ADHD. This study has also shown correlations between the individual level of symptom expression in ADHD and specific areas of cognition as assessed by CANTAB.

We have shown that unmedicated patients with ADHD have deficits in all four of the cognitive domains assessed by CANTAB tasks. The cognitive domains impaired in this group include; sustained attention, spatial working memory, executive function and response inhibition. In this study we were able to show that adults with ADHD

### Table 6: CANTAB and symptoms correlations – control group

<table>
<thead>
<tr>
<th>CAARS</th>
<th>Neuropsychological Task</th>
<th>SST</th>
<th>Direction: Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>-0.17</td>
<td>p=0.38</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-0.54</td>
<td>p=0.01**</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>-0.40</td>
<td>p=0.03*</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>-0.23</td>
<td>p=0.21</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>-0.37</td>
<td>p=0.04**</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>-0.26</td>
<td>p=0.05*</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>-0.42</td>
<td>p=0.02*</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>-0.40</td>
<td>p=0.03*</td>
</tr>
</tbody>
</table>

The values represent the number per group (n), mean (M) and standard deviation (SD)
CAARS – Conner’s Adult ADHD Rating Scale
RVIP – Rapid visual information processing; IID – intra-extra dimensional shift; SWM – spatial working memory; SST- stop-signal task.
*** denotes p<0.001 ** p<0.01, * p<0.05
currently stable of medication showed no significant deficits in the cognitive domains assessed compared with healthy controls.

Unmedicated ADHD patients showed significant impairments in the RVIP indicating deficits in sustained attention compared with healthy participants and patients currently taking ADHD stimulant medication. The sensitivity of patients to respond to target stimuli (A’ measure) was significantly lower in unmedicated ADHD patients who also failed to correctly reject non-target sequences (correct rejections). Unmedicated patients did not appear to be more impulsive (as measured by false alarm responding) compared to controls, however interesting there was a clear gender difference. Males were more impulsive than females in the RVIP making more false alarm responses compared to females. This is the first time this finding has been shown in this population, and warrants further investigation in a larger sample. This finding also highlights the need to take into account gender differences when using the RVIP in the adult ADHD population.

These various measures in the RVIP task (more misses, lower A’ score and less correct rejections) were associated with higher self-reported symptoms of inattention (CAARS-E) in unmedicated patients. Thus, for the first time in adults, these data support the RVIP task as a valid measure of sustained attention in patients with ADHD not currently taking medication. Our findings are consistent with the childhood ADHD literature, showing deficits in RVIP performance, however there is a clear need for more research in larger samples of adults.

Interestingly, poor performance on the RVIP task was associated with higher self-reported symptoms of hyperactivity-impulsivity (CAARS-F) in the medicated group of patients. There is a marked lack of studies using the RVIP to assess sustained attention in adult ADHD compared with healthy controls. To our knowledge only one study exists in a small sample (n=20) and only reports a minor impairment in the hit rate in the ADHD group (Pironiti et al., 2013). To our knowledge the only two studies examining RVIP performance in adult ADHD following treatment have reported conflicting results. Turner et al (2005) showed that methylphenidate improved RVIP accuracy in a sample of adults (n=18) with ADHD. Others have reported no effects of atomoxetine on RVIP performance in a sample of adults (n=20) with ADHD (Chamberlain et al., 2007). Extending previous findings we were also able to compare the medicated group with a large sample of unmedicated patients (n=41). Medicated patients performed at the same performance level as controls and interestingly at a
significantly higher level compared to unmedicated patients. The majority of our medicated sample was stable on methylphenidate (methylphenidate; n=33, atomoxetine; n=5), thus we can broadly conclude that treatment with methylphenidate resulted in an improvement in RVIP performance.

Our study revealed that spatial working memory was also significantly impaired in unmedicated patients with ADHD, as indicated by a poor strategy score, and more errors compared with controls. These results are consistent with previous reports in adults with ADHD (Clark et al., 2007; Dowson et al., 2004; McLean et al., 2004). Studies in children with ADHD assert that the deficits observed in SWM have the largest effect size of all neuropsychological tests (Diamantopoulou et al., 2007; Faraone et al., 2005a). Extending the current literature we have also shown gender differences in SWM performance in healthy controls and in unmedicated patients. In the healthy control group, females made more between errors and total errors compared with males. In the unmedicated group - male patients took longer to searching for the token in the task, suggesting a delay in processing speed.

Adding to previous reports, we have shown that poor performance in the SWM task was associated with high self-report scores of total ADHD symptoms (CAARS-H) including inattention (CAARS-E), impulsivity (CAARS-C) and hyperactivity (CAARS-B) symptoms. Interestingly, although medicated patients did not show impairments in SWM performance compared with controls, there were still significant correlations between poorer performance and increased self-reported symptoms of hyperactivity and impulsivity (CAARS-F). This extends findings from a previous study showing that only impulsive symptoms are associated with SWM impairments (Dowson et al., 2007). This finding also supports the conceptualization of ADHD as a dimensional disorder, and that the symptoms of ADHD are not simple distinct categories. Many experts support the notion that ADHD is best understood dimensionally (Barkley, 2006; Sonuga-Barke, 2005), and this finding in medicated patients suggests the importance of using a top-down approach during the diagnostic process. Correlations between working memory and self-reported levels of hyperactivity and impulsivity, in this study show how these cognitive domains are linked and not distinct categories in ADHD.

A large literature base supports the role of dopamine in the modulation of aspects of SWM (Cools and D'Esposito, 2011; Guerts et al., 2005; Loo et al., 2007; Martel et al., 2007; Nikolas and Nigg, 2014; Riccio et al., 2006; Scheres et al., 2004; Solanto et al., 2007), but the role of noradrenaline is less well understood. Methylphenidate (a
dopamine reuptake inhibitor, which also affects prefrontal cortical noradrenaline levels) (Hinshaw et al., 2002) enhances working memory deficits in younger adults with ADHD (Mehta et al., 2000; Turner et al., 2005), but not in older (elderly) adults with ADHD (Turner et al., 2003); perhaps due to reductions in synaptic dopamine levels with ageing (Wodka et al., 2008). Evidence supporting the role of dopamine and noradrenergic corticostriatal pathways in working memory deficits comes from studies showing the effectiveness of methylphenidate and guanfacine (alpha 2a receptor agonist) in improving spatial working memory (Klorman et al., 1999). However neuroimaging studies are needed to identify specific regions and circuitry involved.

Unmedicated patients also showed impairments in attentional-set-shifting in our study, indicating executive function deficits compared with healthy controls. This group completed less stages and made more errors in the extra-dimensional stages of the IED task, correlated with higher levels of hyperactive symptoms (CAARS-B). The deficits indicated difficulties in making transitions, problem-solving flexibility, switch or alternate attention, and changing focus from one topic to another. To date studies showing deficits in set-shifting in adults with ADHD have proven rather inconclusive (Barkley et al., 2008; Piek et al., 2007; Rohlf et al., 2012). However recently, and consistent with our findings, a study in a large sample of adults with ADHD (n=60) has demonstrated deficits in set-shifting (Halleland et al., 2012). Performance on the SST (used to assess response inhibition) was also impaired in the unmedicated group; i.e. they made more directional errors and failed to successfully detect the stop-signal compared to controls. There were no associations with the level of specific symptom expression in patients with ADHD. However in the control group, higher scores of self-reported ADHD symptoms/behaviours (inattention, impulsivity and inattention) were correlated with greater errors in the SST. Previous studies have shown that impairments in the SST are seen in individuals with ADHD, mainly increased SSRT (Chamberlain et al., 2007). A recent meta-analysis also support our findings of response inhibition impairments in adults with ADHD (Chamberlain et al., 2010). The SST is proposed to be under the control of noradrenergic networks, thought to be dysfunctional in ADHD. Response inhibition is coordinated via a neural network consisting of the right inferior frontal gyrus, bilateral anterior cingulate cortices, and supplementary motor area (Aron et al., 2007; Hampshire et al., 2010; Rubia et al., 2001). Preclinical studies showing enhanced response inhibition following treatment with methylphenidate and atomoxetine (Nigg et al., 2002) (Tomlinson et al., 2014; Winstanley et al., 2006a)
support our findings showing that ADHD patients treated with methylphenidate show less deficits compared to untreated patients.

The results from both ADHD groups (medicated and unmedicated) suggest that patients treated with ADHD medication have less severe cognitive deficits compared with untreated adult ADHD patients; possibly indicating that treatment may reduce some of the cognitive impairments seen in adult ADHD. This is by no means an absolute conclusion because the adult ADHD groups used in our study were two separate groups (medicated and unmedicated not pre and post treatment) and this is an important limitation of our study. Although IQ and age were not significantly different between groups, the results may have been affected by a number of other differences between the groups including genotype. The ideal study design would be a longitudinal one examining cognitive function pre and post treatment in the same cohort of patients. Previous research does generally support the finding that stimulant medication improves specific cognitive deficits in adults with ADHD. However, there is a clear need for more research in this area using larger sample sizes of both males and females (to date the gender tested in the majority of studies is males). We have shown in a sample of male and females that there are clear gender differences in cognitive impairments. Our sample was limited to only Caucasian adults; therefore the results may not generalise to other ethnic or racial groups. While we only included adults with a diagnosis of ADHD in childhood and adulthood we did not control for the use of ADHD medication during childhood.

We have extended previous research by studying adults with ADHD. We have shown that unmedicated patients with ADHD continue to suffer cognitive impairments in adulthood. Results from the present study show that the unmedicated group had more impulsive and hyperactive ADHD symptoms compared with controls, which correlate with poorer SWM and RVP performance. However this was not the case in medicated patients. We speculate that there may be common underlying neural-correlates in ADHD affecting these specific symptoms and cognitive deficits. Deficits in SWM in unmedicated patients appear to be related to the severity of total ADHD symptom expression. These results suggest that treatment may improve inattention, which in turn improves SWM or vice versa, with correlations still apparent between poor SWM performance and hyperactivity following treatment.

The utility of neuropsychological tests in assessing drug responses should not be overlooked, especially in ADHD a dimensional disorder. We have also confirmed that
CANTAB tasks are sensitive to measuring cognitive dysfunction in adult ADHD, consistent with a recent meta-analysis (Chamberlain et al., 2010). CANTAB has proven a useful tool both in terms of assessments (as in this study) and to differentiate between specific brain regions involved in different aspects of cognition, by using functional neuroimaging techniques. Our work highlights the need to focus on utilising CANTAB tests in ADHD neuroimaging studies, examining the effects of different baseline levels of ADHD symptoms on dysfunction in the cognitive domains and the relationship with medication-induced improvements.

In conclusion, we have clarified inconsistencies in the current literature with regards to the specific cognitive deficits in adult ADHD. The present study adds to current understanding by showing the correlations between explicit symptom levels and impairments in specific cognitive domains. We have demonstrated that these cognitive deficits differ between two groups of patients – medicated and unmedicated. Importantly, this study reveals that medicated patients still continue to have deficits in sustained attention and also self-report higher levels of inattentive symptoms compared with healthy controls. This finding raises the question, as to whether or not current treatment is effective in improving the attention deficits in ADHD to a level comparable to controls. If this is indeed the case, improved or alternative treatments must be developed. Furthermore our results support the idea of stratifying patients into groups according to symptom levels and the nature of the cognitive impairments observed and seeking appropriate therapeutic strategies.
10. METHYLPHENIDATE NORMALISES DEFICIENT FACIAL EMOTION RECOGNITION IN ADULTS WITH ADHD

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Abstract

Aims: Adults with ADHD often have difficulties in recognizing emotions from facial expressions. However, it is not known whether medication treatment can normalize these deficits. In this study we aimed to investigate firstly, whether treatment with methylphenidate improves emotion recognition in adult ADHD patients. Secondly, investigate emotion recognition abilities of adult ADHD patients compared with a healthy control group. Finally we aim to explore if emotion recognition impairments are as a result of a general cognitive dysfunction or are a specific impairment in social perception.

Methods: Two groups of adult ADHD participants (n=79) and a group of healthy control participants (n=31) with no history of ADHD were recruited. The ADHD group included patients not yet taking medication (group 1, n=41) and patients stable on medication (group 2, n=38). Each participant completed the Emotion Recognition Task (ERT) and four further neuropsychological tasks from the Cambridge Automated Neuropsychological Test Battery (CANTAB). Finally, 15 participants from group 1 were followed up after commencing treatment on methylphenidate (approximately 8-12 weeks later) and the emotion recognition and sustained attention tasks were repeated.

Results: Adult ADHD patients not currently taking medication showed deficits in recognising sadness, anger, fear and disgust compared with controls. Anger recognition proved to be a specific deficit in social perception whereas sadness, disgust and fear were influenced by deficits in attention and working memory. Patients currently stable on medication made less recognition errors but still showed deficits recognising disgust and anger compared with controls. Methylphenidate normalised the recognition of the negative emotions (sadness, anger, fear, disgust), improvements in attention accounted for the improvements in sadness, fear and disgust recognition but not anger recognition.

Conclusions: Unmedicated adults with ADHD have deficits in recognising negative emotions. Adults stable on ADHD medication have reduced deficits compared with unmedicated patients. Methylphenidate improves emotion recognition deficits in adults with ADHD.
10.1 Introduction

Facial emotion recognition is fundamental for successful social interaction, essential for well-being in humans and other species (Uekermann et al., 2010). Impairments in the ability to correctly recognize different emotions from facial expressions is associated with low social competence, low popularity in peer groups and increased antisocial behavior such as aggression (Marsh and Blair, 2008). Studies have shown that children with ADHD have deficits in emotion-processing (Da Fonseca et al., 2009), and specifically in facial emotion recognition (Boakes et al., 2008; Collin et al., 2013; Kagan et al., 1964; Passarotti et al., 2010b; Pelc et al., 2006; Schwenck et al., 2013; Shin et al., 2009; Sinzig et al., 2008). In relation to these deficits children with ADHD show reduced social competence (Lee et al., 2012), and are less accepted by their peers (DuPaul et al., 2004; Hoza et al., 2005). Research has shown that children with ADHD have particular difficulty recognizing negative emotions (Cadesky et al., 2000; Singh et al., 1998; Williams et al., 2008). However, others groups have failed to find such impairments in negative emotion recognition in ADHD (Downs and Smith, 2004; Greenbaum et al., 2009). This could be due to sample characteristics and/or methodological differences. Clearly there is an urgent need for more research in this area.

Indeed, studies investigating the accuracy of facial (non-verbal) emotion recognition in unmedicated adults with ADHD are particularly limited with mixed findings (Friedman et al., 2003; Miller et al., 2011; Rapport et al., 2002). These studies used small ADHD sample sizes (n<35), highlighting the requirement for larger sized studies in adults. Rapport et al. (2002) using black and white photographs depicting different emotions, found that adults with ADHD (n=28) had specific deficits in the recognition of happiness, fear and anger compared with controls. Freidman et al (2003) showed adults with ADHD had diminished focus on emotion when viewing emotional scenes and emotional impairments as rated by self-report and peer-performance based measures. Furthermore, Ibanez et al. (2011) have shown using event related potential (ERP) cortical deficits in emotion modulation for faces in a sample of 10 adult ADHD patients compared with 10 controls, specifically in response to positive emotion stimuli. Others have also shown similar reductions in reactivity to positive stimuli evaluation adults with ADHD (Herrmann et al., 2009). Further research is required to clarify the precise deficits in facial emotion recognition.
Additionally, previous research has not examined associations between facial emotion recognition deficits and specific ADHD symptoms.

An important consideration for a targeted treatment approach is whether these deficits in emotion recognition reflect a primary social cognitive deficit or are secondary to a more general cognitive dysfunction (e.g. inattention or impulsiveness). The majority of studies use children and adolescents, predominantly males; and to date the findings of these studies are mixed. Several studies support the notion that emotion recognition deficits are explained by a specific impairment in processing information about emotions (Da Fonseca et al., 2009; Yuill and Lyon, 2007). In adults with ADHD, Rapport et al (2002) reported that the emotion recognition deficits observed in this group were not associated with gross visuoperceptual processes or attentional abilities indicating emotion-specific deficits in this population. In contrast to the findings above are the results from studies concluding emotion recognition deficits are as a result of a general cognitive dysfunction (e.g. attention and impulsivity). A visual attention deficit has been suggested to be a moderator of emotion recognition in children with ADHD. Again, the majority of studies are in children and adolescents with ADHD, with only one study in adults. Contrary to the results of Rapport et al (2002), Miller and colleagues (2011) in a study of 33 adults with ADHD and 18 control participants, have shown impaired facial nonverbal affect recognition in ADHD, with a specific link to inattentive symptoms, however this has yet to be replicated. This suggests that the deficits are largely related, predominately to inattentive symptoms and not a specific underlying deficit in emotion processing (Miller et al., 2011). Identifying the link between the cognitive dysfunction observed in ADHD and specific emotion recognition abilities will enable a more targeted treatment approach.

Few studies have examined the effect of ADHD medication on facial emotion recognition. Imaging studies (fMRI) in methylphenidate treated children and adolescents with ADHD have demonstrated differences in emotion processing mechanisms. Posner and colleagues (2011) demonstrated that methylphenidate attenuated hyperactivity in the medial prefrontal cortex (mPFC) to levels comparable to healthy controls during an emotional processing task (Posner et al., 2011a). They further demonstrated, during the same task, atypical connectivity between the amygdala and lateral prefrontal cortex (LPFC) and increased right amygdala activity, which were also normalized following treatment with methylphenidate (Posner et al., 2011b). Methylphenidate-naïve adults, with a history of ADHD during childhood showed decreased activation in the subgenual cingulate and ventral striatum during an emotion
perception task (Schlochtermeier et al., 2011) while adults treated with methylphenidate during childhood showed no differences compared to controls (Schlochtermeier et al., 2011). These findings suggest that the differences in neural responses to emotional stimuli are dependent on previous methylphenidate exposure. Williams et al (2008) demonstrated impaired anger and fear emotion recognition in a sample of 51 unmedicated adolescents with ADHD compared with a group of matched controls n=51. The ADHD group then commenced treatment with immediate-release methylphenidate, the mean dose was 24.1mg/day, and were followed-up after 4 weeks. For the first time the authors showed that methylphenidate improved the accuracy of facial emotion expression recognition. The authors also showed, using event related potentials, that methylphenidate normalized neural activity in occipitotemporal brain systems in adolescents with ADHD (Williams et al., 2008). Methylphenidate has also been shown to improve emotional motivational impairments in a small sample of adults with ADHD (Conzelmann et al., 2011) and overall emotional symptoms (Rosler et al., 2010). However, despite some emotion recognition studies in children and adolescents, there is a paucity of research in adult ADHD. The present study provides new evidence for the effects of medication on facial emotion recognition deficits in adult ADHD.

The primary aim of this study was to examine whether medication (methylphenidate) would normalize facial emotion recognition deficits in adults with ADHD. We hypothesize, based on previous findings in children and adolescents, that treatment with methylphenidate would improve negative emotion recognition in adults with ADHD. A secondary aim of our study was to investigate associations between facial emotion recognition and specific ADHD symptoms and cognitive dysfunctions. Based on previous studies, we hypothesized that facial emotion recognition deficits would be most strongly associated with inattentive symptoms.

10.2. Methods

10.2.1 Participants

The sample used for primary analysis consisted of a total of 110 participants. The sample included 79 participants between the ages of 18 and 65 (22 females, mean age = 28.5 years) with a diagnosis of ADHD according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed; DSM-IVTR; American Psychiatric Association, 1994) criteria. The sample also consisted of 31 age and IQ matched healthy controls. Participants with ADHD were recruited from two UK-based specialist adult ADHD clinics. Only patients diagnosed with ADHD by a specialist adult ADHD consultant
psychiatrist were included in the study. In both clinics the diagnosis of ADHD was based on DSM-IVTR criteria. The diagnostic process involved completion of the Wender Utah Rating Scale, adult ADHD current behavior scale and a semi-structured interview based on the DSM-IV criteria for ADHD (Barkley and Murphy, 2005). A semi-structured psychiatric interview (based on the mini-SCID for DSM-IV; (First et al., 1996) was also conducted to screen for other psychiatric conditions. The diagnostic process was conducted over 2-4 consultations with the clinical team (comprising a specialist adult ADHD consultant psychiatrist and specialist adult ADHD nurse). Informants (relatives, partners) were also asked to complete informant-rating scales and attend appointments with the patient. All participants with adult ADHD in order to meet diagnostic criteria must also have had a diagnosis of ADHD, or reported symptoms of ADHD in childhood. Of those ADHD patients participating in the study, 41 were unmedicated (group 1) and 38 were stable on medication (group 2).

Group 1, although unmedicated upon entry into the study, was commenced on medication after completion of part one of this study. Briefly, part one consisted of neuropsychological testing, including emotion recognition testing and completion of an IQ test and ADHD symptom rating scale. A sample of 15 participants (6 females, mean age = 31.6 years) from group 1 were followed-up after starting treatment (methylphenidate) and formed group 3. Group 1 were recruited during the period between being diagnosed with ADHD and receiving their first dose of medication at the follow-up assessment meeting. This timescale for recruitment was approximately 1-2 weeks. A number of participants did not start treatment with ADHD medication (n=6), some participants commenced treatment on atomoxetine (n=4) and some methylphenidate (n=31). The methylphenidate group were followed-up (n=31), some dropped-out (n=11), some had a depressive episode (n=1), some were not stable on methylphenidate at 8 weeks (n=3) and n=1 switched to atomoxetine. The remaining 15 participants stable on methylphenidate were followed-up and included in part 2 (repeated emotion recognition testing) of this study. The second group of patients who entered the study stable on medication were not followed-up. Control participants consisted of 31 adults without a diagnosis of ADHD (16 females, mean age = 26.10 years). Refer to appendix 1 for a schematic diagram of the study protocol.

10.2.2 Recruitment and Screening

Control participants were recruited via advertisements in the University and emails sent to local businesses. In addition to the criteria below control participants were excluded
if they screened positive for ADHD or if they had a history of childhood ADHD. This was determined by the Conner’s Adult ADHD Rating Scales (CAARS), with a T-score >70 on CAARS (see section 2.3) the threshold for exclusion. Two participants screened positive for ADHD and were excluded at this point.

Exclusion criteria included: intellectual disability (WTAR estimated verbal IQ score <80), clinically unstable psychiatric disorders (psychosis, suicidal behaviours), bipolar disorder, current major depressive episode, current drug or alcohol abuse or dependence, current use of a mood stabilizer or antipsychotic medication, currently pregnant, suffering from epilepsy and/or head trauma.

10.2.3 Materials

**ADHD symptoms:** CAARS were developed based on manifestations of adult ADHD and DSM criteria (Conners et al., 1999). CAARS have been well validated, the internal consistency coefficients range from 0.77 to 0.97, and 2- to 4-week test-retest reliability coefficients (Cronbach’s alpha) range from 0.71 to 0.98 (Erhardt et al., 1999). CAARS consists of eight clinical scales, both self-report or observer-report measures of current ADHD symptoms and behaviours. The long version of the self-report CAARS was used (CAARS-SL) in this study, which consisted of 66 items separated into 8 subsections (A-H). These subsections included: CAARS-A Inattentive/Cognitive problems, CAARS-B Hyperactivity/Restlessness, CAARS-C Impulsivity/Emotional Labiality, CAARS-D Problems with Self-Concept, CAARS-E DSM IV Inattentive Symptoms, CAARS-F DSM-IV Hyperactive-Impulsive Symptoms, CAARS-G DSM-IV Total ADHD Symptoms, CAARS-H ADHD Index. The T-scores for each subsection were calculated and used in this study to minimise the effects of age and gender on ADHD behavioural symptoms. All participants also completed the psychometrically validated self-report measure of functional impairment across several domains including; social, work, risk, life-skills, the Functional Impairment Rating Scale, (WFIRS-S; (Weiss et al., 2007; Weiss et al., 2005). The WFIRS-S is specific to impairments in ADHD and consists of 69-item survey in seven different areas (family, work, school, life skills, self-concept, social, risk).

The Wechsler Test of Adult Reading (WTAR) was completed by all participants and provided an estimated verbal IQ.
Neuropsychological Tasks

Four neuropsychological tests were administered on a colour monitor with a touch sensitive screen. These tasks were selected from the Cambridge Automated Neuropsychological Test Battery (CANTAB). The tasks included: 1. The rapid visual information processing (RVIP) task, which is a visual continuous performance test adapted and simplified from Wesnes and Warburton’s task (Wesnes and Warburton, 1984) that assesses sustained attention (Sahakian et al., 1989). 2. The spatial working memory (SWM) task is based on a self-ordered test (Petrides and Milner, 1982) and is adapted from Olton’s radial arm maze (Olton, 1987). It is a task involving working memory processing of spatial locations. 3. The intra/extra dimensional set shift (IED) task assesses set-shifting by the ability to maintain selective attention on specific attributes of compound stimuli across different examples (intradimensional shift) and then shift attention to a previously irrelevant attribute of stimuli or extradimensional shift (EDS; (Downes et al., 1989)). 4. The stop-signal task (SST) is a classic paradigm used to measure pre-potent response inhibition (Logan, 1994). For detailed methods see chapter 9.

Emotion Recognition

The final task included in this study was the CANTAB Emotion Recognition Task (ERT; Ltd, 2012 (Penton-Voak et al., 2012; Penton-Voak et al., 2013). The ERT assesses the participant’s ability to recognise emotions in facial expressions. Participants were shown a series of stimuli on computer-morphed images compiled from facial features of male individuals expressing explicit emotions. There are 15 stimuli for each of the 6 emotions (happiness, sadness, fear, anger, disgust, surprise). The task starts with a cross appearing in the centre of the black touchscreen for 1.5 – 2.5 s, then the morphed male face appears depicting one of the 6 emotions. The image is displayed for 200 ms at full intensity and is then digitally covered by a speckled grey rectangle (the mask) for 250 ms. After the masked image has disappeared, the participant must select the emotion from 6 boxes on the touchscreen that they consider most closely corresponds to the emotion depicted in the face. Each box contains the word of one emotion (6 boxes in total), which are assigned at random at the beginning of the task. The forced choice format in recognizing and identifying emotional expressions has been validated by Rosenberg and Ekman (1995) in a similar task. The task takes approximately 10 – 20 minutes to complete and consists of two blocks of 90
trials each, which run consecutively with no break in-between. The correct (%) recognition of each emotion was calculated for each emotion.

10.2.4 Procedure

10.2.4.1 Part One

The study was approved by the National Health Service (NHS) Research Ethics committee (REC) (Greater Manchester West REC). All participants provided informed written consent to participate in the study. Subsequently, participants completed the CAAR:SL, WTAR and CANTAB tasks including the ERT. Group 1 completed these tasks and questionnaires before starting treatment, and were unmedicated at this stage. Group 2 were stabilised on treatment when they completed this procedure. Control participants also completed all of the same tasks and questionnaires. This took place during a meeting with a trained researcher under the supervision of a specialist adult ADHD consultant psychiatrist. Participants received £20.00 to compensate for any inconvenience incurred. Control participants and those in group 2 completed part one and did not enter part two (follow-up).

10.2.4.2 Follow-up

Group 1 were the only group followed-up. All the participants in group 1 (n = 41) were not taking ADHD medication at the point of entry into the study and during completion of part one of the study. The 15 participants who were followed-up were deemed stable when they had been titrated to a therapeutic dose of methylphenidate and regularly taking that dose for >14 days. At the follow-up, all of group 3 completed the CAAR:SL, the ERT and RVIP for the second time.

10.2.5 Data Analysis

Data were analysed with SPSS (Version 20) and are expressed as mean ± SD. Group 1 and group 2 were compared to the control group for all part one measures (emotion recognition and CAARS scores). Group 1 and group 2 were compared to the control group and to each other for all part one measures (emotion recognition and CAARS scores). When data were checked for normality; the CAARS data were not normally distributed (possibly due to ceiling effects) and were subsequently analysed using the non-parametric Kruskal-Wallis test. The remaining measures were normally distributed and analysed by one-way ANOVA followed by Bonferroni post-hoc analysis. Spearman’s rho was used to determine whether there were any associations between
subsection CAARS scores and emotion recognition (% correct) in each group. Data were also analysed using the cognitive measures and IQ as covariates.

For group 1 - the part one (before treatment) and part two (8 weeks after being on methylphenidate) emotion recognition scores were compared using the Wilcoxon test. This was used to assess the measures after treatment; Mann-Whitney U analysis was used to compare Group 1 to the control group (both before and after treatment). Spearman’s rho was used to determine whether there were any associations between subsection CAARS scores and emotion recognition (% correct) before and after follow-up for group 1.

10.3. Results

10.3.1.1 Demographic and CAARS Scores

One way ANOVA revealed no significant difference in IQ scores between group 1 and 2 and controls ($F_{(2,107)}=1.44, p=0.24$); and no difference in age ($F_{(2,107)}=1.84, p=0.17$) see table 1. Kruskal-Wallis analysis showed differences between groups for each of the subsection CAARS scores (see Table 1).
Table 1: Demographic data and CAARS T-scores – Part One

<table>
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<tr>
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<th>ADHD Medicated Group</th>
<th>ADHD Unmedicated Group</th>
<th>Control Group</th>
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<td>WTAR</td>
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<td>+70.00***</td>
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<td>CAARS B – Hyperactivity/ restlessness</td>
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<td>11.29</td>
<td>70.00***</td>
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<td>CAARS C – Impulsivity/emotional lability</td>
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<td>77.00***</td>
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<td>11.57</td>
<td>70.04***</td>
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<td>11.39</td>
<td>90.00***</td>
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<tr>
<td>CAARS F – DSM-IV Hyperactive-impulsive symptoms</td>
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<td>14.22</td>
<td>84.00***</td>
<td>9.33</td>
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<td>CAARS G – DSM-IV ADHD symptoms total</td>
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<td>11.33</td>
<td>90.00***</td>
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<td>CAARS H – ADHD Index</td>
<td>41.50</td>
<td>15.30</td>
<td>76.00***</td>
<td>10.14</td>
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</table>

Age and WTAR data are expressed as mean ± standard deviation (SD)
CAARS A-H data are expressed as median ± standard deviation (SD)
Significant differences between unmedicated and medicated compared with controls are shown as ***p<0.001, **p<0.01, *p<0.05.
Significant differences between ADHD groups are shown as †p<0.01, ‡p<0.05

10.3.1.2 Emotion Recognition

We examined group differences in emotion recognition. The part-one emotion recognition scores were compared for group 1 (unmedicated), group 2 (stable on treatment) and control group. One-way ANOVA revealed a significant effect in the (%) correct recognition of sadness (F(2,107)=3.75, p=0.03, fig 1a) between groups. Post-hoc analysis revealed that the unmedicated ADHD group (group 1, part one) scored significantly lower in % correct recognition of sadness compared with controls (p<0.05, fig 1a), Cohen’s d=0.64, but not the medicated group 2. There was a significant main effect of group on the ability to correctly recognise anger (F(2,107)=7.33, p=0.001, fig 1b).
Further post-hoc analysis showed that both groups (medicated; group 2, Cohen’s d=0.56 and unmedicated group 1, Cohen’s d=0.86), had a significant reduction in (%) anger recognition compared with controls (p<0.05; fig 1b). The medicated group 2 had significantly enhanced anger recognition compared with the unmedicated group 1, fig 1b, Cohen’s d=0.41. One-way ANOVA showed a significant difference between groups in the (%) correct recognition of disgust (F(2,107)=4.11, p=0.02, fig 1c), with post-hoc analysis revealing both medicated (Cohen’s d=0.54) and unmedicated (Cohen’s d=0.61) groups scored significantly worse than controls (p<0.05; p<0.01, respectively, fig 1c), but were no different from each other (group 1 vs. 2). A group difference for correct recognition of fear also reached statistical significance (F(2,107)=3.54, p=0.03, fig 1d). Post hoc analysis showed that the unmedicated group 1 scored significantly lower compared with both groups (medicated group 2, Cohen’s d=0.44, and the control group, Cohen’s d=0.65) (p<0.05, fig 1d). The medicated group 2 did not significantly differ from controls in the correct recognition of fear or sadness. One way ANOVA revealed no effect of condition or group differences in correctly recognizing happiness (F(2,107)=2.85, p=0.06, fig 1e), and surprise (F(2,107)=1.00, p=0.37, fig 1f) for all groups.

After repeating the analyses using IQ as a covariate, the results showed that unmedicated ADHD patients still significantly differed in the recognition of sadness (p<0.05), anger (p<0.001), disgust (p<0.01) and fear (p<0.05) compared with controls. However after repeating the analyses using the sustained attention measures (RVIP measures) as covariates, unmedicated patients only differed in the recognition ability of anger (p<0.001), compared with controls. Medicated ADHD patients also significantly differed in the recognition of anger (p<0.05) compared with controls using RVIP measures as covariates. When using attentional set-shifting (IED) and response inhibition (SST) measures as covariates unmedicated patients significantly differed compared with controls in the recognition of sadness (p<0.05), anger (p<0.001), disgust (p<0.05) and fear (p<0.05). Spatial working memory measures when used as covariates also had an effect on the results; unmedicated patients only significantly differed in the recognition of anger (p<0.001) compared with controls. When accounting for all influencing factors; using IQ, RVIP and SWM measures as covariates the only emotion recognition difference between groups was observed in the recognition of anger between controls and unmedicated patients (p<0.001).

We also examined associations between emotion recognition and the level of ADHD symptoms. Recognition of disgust was significantly negatively associated with inattentive, hyperactive, impulsive, and total ADHD symptoms in the treated group.
Recognition of surprise was significantly positively associated with self-concept and total ADHD symptoms in the treated group (group 2). Fear recognition was also significantly positively correlated with impulsive symptoms (Table 2). Recognition for fear and sadness was significantly negatively associated with total ADHD symptoms in the untreated group 1, Table 2. Recognition of fear was significantly positively associated with inattentive ADHD symptoms and recognition of disgust was significantly negatively associated with impulsive symptoms in the control group. A positive correlation was also revealed for recognising sadness and inattentive, impulsive, hyperactive and total ADHD symptoms in the control group. No other correlations reached significance (Table 2).

<table>
<thead>
<tr>
<th>Group 1 (unmedicated)</th>
<th>Group 2 (ADHD medicated)</th>
<th>Control Group n=31</th>
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<tbody>
<tr>
<td>ADHD n=41</td>
<td></td>
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<tr>
<td>Sadness</td>
<td>Fear</td>
<td>Disgust</td>
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<td></td>
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<td>Fear</td>
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<tr>
<td>CAARS A</td>
<td>0.17</td>
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<tr>
<td></td>
<td>p=0.29</td>
<td>p=0.91</td>
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<tr>
<td>CAARS B</td>
<td>-0.23</td>
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<td></td>
<td>p=0.14</td>
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<td>CAARS C</td>
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<td>-0.18</td>
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<td></td>
<td>p=0.44</td>
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<td>CAARS D</td>
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<td></td>
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The values represent the number per group (n) mean (M) and standard deviation (SD). CAARS = Conner's Adult ADHD Rating Scale. *** denotes p<0.001, ** p<0.01, * p<0.05.
Figure 1: Correct (%) emotion recognition in medicated, unmedicated ADHD patients and controls.

Figure 1: Emotion recognition (% correct). Group differences in the correct recognition of (A) sadness, (B) anger, (C) disgust, (D) fear, (E) happiness, (F) surprise. The groups are: controls (n=31), medicated ADHD patients, upon entry into the study (n=38) and unmedicated ADHD patients (n=41). Data are expressed as the mean ± SEM. Asterisks (**p<0.001; **p<0.01; *p<0.05) indicate significant differences compared to the control group, crosses (+p<0.05) indicate significant differences between medicated and unmedicated ADHD patients. All data were analysed using one-way ANOVA followed by planned post-hoc pairwise comparisons.
We also examined associations between emotion recognition and the level of ADHD symptoms. Recognition of disgust was significantly negatively associated with inattentive, hyperactive, impulsive, and total ADHD symptoms in the treated group (Group 2; Table 2). Recognition of surprise was significantly positively associated with self-concept and total ADHD symptoms in the treated group (Group 2). Fear recognition was also significantly positively correlated with impulsive symptoms (table 2). Recognition for fear and sadness was significantly negatively associated with total ADHD symptoms in the untreated Group 1, Table 2. Recognition of fear was significantly positively associated with inattentive ADHD symptoms and recognition of disgust was significantly negatively associated with impulsive symptoms in the control group. A positive correlation was also revealed for recognising sadness and inattentive, impulsive, hyperactive and total ADHD symptoms in the control group. No other correlations reached significance (table 2).

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10.3.2.1 Group 3 – Treatment effects

10.3.2.1.1 Emotion recognition

The group of patients started on methylphenidate were followed-up (group 3) and retested. The results of the emotion recognition task before and after treatment were compared (approximately 8 weeks apart). Wilcoxon analysis revealed a significant increase in the correct recognition of anger ($t=2.70$, $n=15$, $p=0.007$, Cohen’s $d=0.82$, fig 2b), disgust ($t=1.93$, $n=15$, $p=0.05$, Cohen’s $d=0.84$, fig 2c) and fear ($t=2.67$, $n=15$, $p=0.008$, Cohen’s $d=1.18$, fig 2d) following treatment with methylphenidate. The improvement of correct recognition of sadness following methylphenidate treatment closely approached significance; ($t=1.74$, $n=15$, $p=0.08$, fig 2a). There were no significant effects of treatment on correct recognition of happiness ($t=0.34$, $n=15$, $p=0.73$, fig 2e) or surprise ($t=0.28$, $n=15$, $p=0.78$, fig 2f) before treatment compared with the control group. When accounting for all influencing factors; using IQ and RVIP measures as covariates the only emotion recognition improvement was observed in the recognition of anger before and after treatment in ADHD patients ($p<0.001$).

Group 3 was compared with controls at both time points – before and after treatment on methylphenidate (approximately 8 weeks apart) using the Mann-Whitney U test. Before treatment, group 3 scored significantly worse than controls in the recognition of negative emotions. Mann-Whitney U analysis revealed that there were significant impairments in the recognition of sadness ($u=369.00$, $n=46$, $p=0.001$, Cohen’s $d=1.28$, fig 2a), anger ($u=370.00$, $n=46$, $p=0.001$, Cohen’s $d=2.09$, fig 2b), disgust ($u=351.00$, $n=46$, $p=0.005$, Cohen’s $d=1.00$, fig 2c) and fear ($u=353.50$, $n=46$, $p=0.004$, Cohen’s $d=0.76$, fig 2d) in group 3 before treatment compared with controls all of which were normalized following treatment with methylphenidate. When accounting for all influencing factors; using IQ and RVIP measures as covariates the only emotion recognition difference between groups was observed in the recognition of anger between controls and ADHD patients before treatment ($p<0.001$).

Group 3 at follow-up (after treatment) did not significantly differ from the medicated group (Group 2) in part 1 of the study.

ADHD symptom expression (CAARS scores) for group 3 at two time points (before and after treatment) were tested for correlations with emotion recognition scores. Recognition of sadness was significantly negatively associated with inattentive symptoms; ($\rho=-0.55$, $n=15$, $p=0.03$), self-concept; ($\rho=-0.64$, $n=15$, $p=0.01$) and total...
ADHD index score; CAARS-H (rho=-0.52, n=15, p=0.05) in group 3 before treatment. Following treatment, Spearman’s rho showed that there was a negative correlation between hyperactivity; CAARS-B and disgust recognition (rho=-0.53, n=15, p=0.04). No other correlations reached significance.

Figure 2: Correct (%) emotion recognition before and after treatment with methylphenidate compared to controls

Figure 2: Emotion recognition (% correct) before and after methylphenidate treatment. Differences in the correct recognition of (A) sadness, (B) anger, (C) disgust, (D) fear, (E) happiness, (F) surprise. The groups are; controls (n=31), ADHD patients followed-up after treatment (n=15). Data are expressed as the mean ± SEM. Asterisks (***p<0.001; **p<0.01; *p<0.05) indicate significant differences after treatment; crosses (+p<0.05) indicate significant differences between the control group and ADHD patients. Data were analysed using the Wilcoxon test; this was used to assess the measures after treatment. Mann-Whitney U analysis was used to compare Group 1 to the control group (both before and after treatment).
10.4. Discussion

10.4.1.1 Emotion recognition and treatment effects

This study provides new evidence showing that methylphenidate normalised deficient emotion processing in adults with ADHD. We have also confirmed previous findings showing that adults with ADHD (unmedicated; group 1) have significant impairments in recognising negative emotions from facial expressions compared with healthy controls. This group (unmedicated) displayed clear deficits recognising sadness, anger, disgust and fear. The ADHD group stable on medication also showed deficits in the recognition of disgust compared with controls. This group also showed deficits in the recognition of anger compared with controls but not to the extent of the unmedicated group and in fact had significantly enhanced anger recognition compared with unmedicated patients. All groups were able to successfully recognise happiness and surprise to a level comparable to healthy controls.

Although research in adults is limited in this area, our findings are in agreement with the most consistent findings in the adult ADHD literature. Adults with ADHD have difficulties in recognising the negative emotions - anger and fear in faces (Rapport et al., 2002; Uekermann et al., 2010) a finding also produced from studies in children (Pelc et al., 2006; Singh et al., 1998; Williams et al., 2008).

We followed-up 15 patients (group 3) after methylphenidate treatment and assessed their emotion recognition ability for the second time. There is a potential for practice effects, however tests were at least 2 months apart, no feedback or reinforcement (negative or positive) was given during or after the test. The emotion recognition assessments were carried out before starting treatment and after a stable therapeutic dose had been determined (approximately 8-12 weeks after starting treatment). Before treatment, patients showed impairments in recognising sadness, anger, fear and disgust compared with controls. This is in agreement with results from the unmedicated group who also showed these deficits in negative emotion recognition. This is the first time that facial emotion recognition has been reassessed following treatment with methylphenidate in adults with ADHD. Interestingly, following treatment, this group showed significant improvements in recognising anger, disgust and fear but not sadness, although there was some improvement this failed to achieve statistical significance. The recognition of all four negative emotions improved to a level comparable to controls, i.e. medication with methylphenidate normalised the dysfunction in emotion recognition in previously unmedicated adults with ADHD.
Our results are in agreement with findings showing that adults, children and adolescents with ADHD have deficits in emotional processing, and that methylphenidate normalises these deficits. Posner et al (2011) demonstrated that right amygdala over-activation, and enhanced connectivity between the amygdala and LPFC was associated with fearful face presentations in adolescents with ADHD. This amygdala hyperactivation and amygdala-LPFC connectivity was normalised by stimulant medication (Posner et al., 2011b). It is recognised that the amygdala plays an important role in the recognition of fear and sadness (Murphy et al., 2003), suggesting that the adults in our study may have deficits in amygdalar-mediated neuronal signaling that are restored by treatment with methylphenidate, normalising behaviour. Posner et al (2011a) have also shown that adolescents with ADHD have increased reactivity in the mPFC during an emotional processing task, which was reduced by methylphenidate to a level similar to healthy controls. In an elegant study, Williams et al (2008) assessed adolescents with ADHD before and after treatment with methylphenidate and reassessed emotion recognition. Before treatment, male adolescents showed impairments in recognising threat related emotions (fear, anger), and after treatment showed mild but still significant emotion recognition improvements (Williams et al., 2008). In addition, methylphenidate normalised atypical event-related brain potentials in response to angry faces. We have extended these findings into an adult population of both males and females with ADHD and shown improvements in four negative emotion recognition deficits. Williams et al (2008) used a sample of ADHD patients with self-reported mood and/or anxiety disturbances; we have further extended these findings showing improvements in negative emotion recognition, in a sample of adults not currently experiencing episodes of anxiety or depression.

Whilst the current adult ADHD literature is limited, a small number of studies have investigated the effects of methylphenidate on emotional dysregulation (as measured by the affect-modulated startle paradigm; (Schlochtermeier et al., 2011) and emotional-motivational dysfunctions (Conzelmann et al., 2011). Our findings are in support of these studies; methylphenidate treatment during childhood ADHD has been shown to improve the response the emotional stimuli in adulthood. Adults with ADHD in childhood not treated with methylphenidate showed decreased activation in the ventral-striatum and subgenual cingulate in response to emotional stimuli, compared to adults treated with methylphenidate in childhood (Schlochtermeier et al., 2011). We have added to these findings by showing that treatment in adulthood with methylphenidate continues to improve emotion recognition deficits in ADHD. Conzelmann et al. (2011)
tested the effect of methylphenidate in adults with ADHD, and showed improvements in the affect-modulation startle paradigm in response to pleasant stimuli. These findings suggest improvements in emotional processing by acute methylphenidate, broadly in agreement with our findings. We have shown that longer-term methylphenidate treatment (approximately 8-12 weeks) improves, specifically, negative emotion recognition deficits in adults with ADHD. We were able to compare these results to two different groups of patients, one taking medication and one not yet taking medication.

10.4.1.2 Emotion recognition and ADHD symptom expression

In unmedicated ADHD patients, we have also shown that the higher the score of total ADHD symptoms, the worse the correct recognition of sadness. Interestingly, inattention scores and total ADHD index were also correlated with the correct recognition of sadness in the follow-up group. Thus, the higher the level of inattention, or the more severe the ADHD symptoms, the more impaired the ability to recognise sadness. The recognition of disgust in medicated patients was correlated with scores of impulsivity, inattention, hyperactivity and total ADHD index scores. Thus, the more ADHD symptoms across domains; the worse the ability to recognise disgust from facial expressions. The recognition of fear was also significantly associated with total ADHD symptoms in unmedicated patients. These findings indicate that the more severe the ADHD symptomatology, the higher the impairments in emotion recognition, suggesting involvement of a common neural pathway. Further studies using imaging techniques are required to identify this pathway.

To our knowledge this is the first study to examine correlations in ADHD symptom expression with facial emotion recognition in adults before and after medication. Others have examined the associations between emotion regulation processing and inattention and impulsivity in healthy university students (Shalom et al., 2013) and concluded that impulsive symptoms may play a role in emotion regulation. Miller et al. (2011) examined differences in affect recognition between subtypes in adults with ADHD, and found that the inattentive group made more errors when identifying fearful emotions. However Miller and colleagues failed to show any differences between ADHD subtypes and controls, possibly due to low sample size and reduced statistical power. We have used a larger sample in the current study, and extended the findings of Miller et al (2011).
In using additional objective neuropsychological tasks of attention, attentional set-shifting, impulsivity and working memory we were able to measure behaviour and control for the effects of cognition on emotion recognition. We have demonstrated that specific aspects of cognition can directly influence the recognition of a number of emotions in ADHD. We have also shown that some emotion recognition deficits cannot be attributed to general dysfunctions in various aspects of cognition. IQ did not show to have an effect the recognition of any emotions. Interestingly, inattention as measured by the RVIP accounted for, or contributed to, the emotion recognition deficits in fear, disgust and sadness in the unmedicated and medicated ADHD groups. These findings are in line with findings in children and adolescents with ADHD (Shin et al., 2009; Sinzig et al., 2008) and adults with ADHD (Miller et al., 2011). Working memory was controlled for by using SWM measures, and showed to influence emotion recognition in a similar manner to attention; thus the impairments in sadness, fear and disgust recognition could be attributed to difficulties in SWM.

However RVIP and SWM measures did not influence the recognition of anger in both ADHD groups, suggesting a specific deficit in the social perception of anger. Groups that suggest a specific emotion-processing deficit in ADHD support this finding e.g. Rapport et al (2002) concluded that the emotion recognition deficits observed in adults with ADHD when processing affective stimuli are not due to fundamental deficits in attention. The results from work in adolescent boys with ADHD also suggest a specific emotion recognition pattern, which is not due to attention problems. This conclusion was drawn, as overall emotion recognition did not alter during the test, indicating that changes in selective attention toward the emotion stimuli were unaffected (Aspan et al., 2014). Our work extends these findings by using a larger sample of adults with ADHD, including males and females currently taking medication or unmedicated at the time of testing.

Interestingly when we controlled for response inhibition and attentional-set shifting using measures from the SST and IED task unmedicated patients still showed impairments in the recognition of the negative emotions (fear, disgust, sadness and anger). These findings suggest that response inhibition and attentional set-shifting do not influence emotion recognition, this is consistent with the work by Yuill and Lyon (2007) who included an ‘inhibitory scaffolding’ during a face-processing task to control for response inhibition. The authors showed that ADHD boys had a selective difficulty in facial expression processing compared with controls. Interestingly the emotion matching was not markedly improved by the ‘inhibitory scaffolding’; suggesting
specific socio-cognitive problems in ADHD (Yuill and Lyon, 2007). Da Fonseca et al. (2009) have also shown that deficits in emotion recognition processing was not due to a general cognitive dysfunction (e.g. impulsivity) supporting a specific emotion-processing deficit (Da Fonseca et al., 2009). When combining all of the cognitive measures, (to assess the a combined effect of inattention, response disinhibition and deficits in working memory and attentional set-shifting), anger recognition was still impaired in unmediated adults with ADHD. This is the first time that a single study has examined the effects of all four cognitive domains on emotion recognition in adults with ADHD. Also for the first time, we have shown that methylphenidate improves the recognition of fear, disgust and sadness possibly by improving attention deficits, response inhibition and working memory. However the improvement in anger recognition following treatment with methylphenidate does not appear to be due to parallel improvements in overall cognition. It would appear that methylphenidate is also effective at treatment specific emotion processing deficits in ADHD. This finding warrants further investigation in a larger sample using functional neuroimaging.

Determining the origin of emotion recognition deficits in ADHD, whether they be a specific perceptive deficit or due to a wider more ‘general cognitive dysfunction’ are imperative for the clinical implications. These results highlight the importance of treating the core symptoms of ADHD, to enable the subsequent improvement in negative emotion recognition (apart from anger recognition). Although we have indicated that methylphenidate improves anger recognition, it is not yet known if other ADHD treatments will have the same effect. We have suggested that the deficit in anger recognition is a separate specific deficit in social perception; therefore improvements in ADHD core symptoms (inattention and impulsivity) alone are not adequate enough to improve anger recognition in ADHD. The use of a social cognitive therapy, in unmedicated patients with ADHD may improve the social impairments, but does require further research.

Limitations

Several limitations of the study warrant consideration. Our sample was only Caucasian and therefore our findings may not generalize to other ethnic groups. In addition, we did not account for lifetime psychiatric comorbidity. Furthermore, self-report measures have several limitations, in order to improve this; future studies should be combined with more objective and other-informant measures. The findings from the follow-up of group 3 (after medication) are different to the findings from the medicated group (group
2) with ADHD. In the same group of patients (group 3), treatment with methylphenidate normalized negative emotion recognition deficits. These differences in results from group 2 and 3 could be due to the effects of different medication, as some of the participants in group 2 were taking atomoxetine or d-amphetamine. The differences could have also been due to several other factors, including genetic or other differences between groups. Future work should focus on adults with ADHD and combine imaging techniques with emotion recognition and processing tasks, examining the effects of stimulant and non-stimulant medication. The role of social skills training and other psychological approaches needs to be explored in this group, and compared with the effects of medication.

Conclusions

Our findings show that unmedicated adults with ADHD show impairments in correctly recognising negative emotions. We have shown correlations with symptom expression and specific emotion recognition deficits. Impairments in the recognition of sadness were correlated with higher levels of inattentive and total ADHD symptoms, and fear with total ADHD symptoms. Importantly, we have shown that treatment with methylphenidate for approximately 8 weeks produced a significant improvement in negative emotion recognition in adults. These findings highlight the importance of emotion recognition impairments in the symptomatology of ADHD and the potential role of methylphenidate treatment in improving social and emotional competence in adults with ADHD.
DISCUSSION
11. DISCUSSION

During the course of this thesis, the neurocognitive effects of current ADHD agents and novel agents have been evaluated in a novel animal model of adult ADHD. The neurocognitive effects, including emotion recognition, of current ADHD agents have also been assessed in a clinical population with adult ADHD. The studies in this thesis were undertaken to fulfill six main aims:

i. To establish an animal model of the core symptoms of adult ADHD using a translational behavioural paradigm.

ii. To assess the selective effects of standard ADHD medication on the different behavioural phenotypes using this animal model.

iii. To investigate potential novel therapeutic targets for treating adult ADHD using this animal model.

iv. To evaluate the core cognitive deficits in treated and untreated adult patients with ADHD.

v. To study specific emotion recognition deficits in adults with ADHD, and examine if these result from a general cognitive impairment.

vi. To investigate the effects of short-term treatment with a stimulant medication on emotion recognition in adults with ADHD.

Whilst the search for novel therapeutic targets in adult ADHD continues, these results highlight encouraging new potential targets, and the importance of utilising a translational animal model and behavioural tasks of relevance to the disorder. These results also reveal that individual responses to current treatments may be, in part, due to baseline levels of symptom expression. This highlights the importance of targeting specific neurocognitive domains; taking an individual approach to treatment defined by baseline levels of neurocognitive deficits. The clinical results presented in this thesis confirm the core neurocognitive deficits in adult ADHD and emphasise the need to consider emotion recognition deficits as a core feature of adult ADHD.

The work outlined in the thesis highlights potential treatment targets, and the importance of utilising effective therapeutic strategies. These results also emphasise the areas of future work still required to improve the approach currently taken in ADHD treatment practice. The exact therapeutic effects in different ADHD patient populations need to be explored and implemented into clinical practice. It remains apparent that novel therapeutics are still required for treating ADHD in adults, focusing on targeting...
social cognition alongside the core symptoms of ADHD. The following sections present the contributions of this work to the existing adult ADHD literature, and the research still required.

11.1 Main Findings: Preclinical studies

To date, animal models of ADHD have focused on childhood ADHD, no model of adult ADHD has been developed so far and clearly there is an urgent need for this in order to develop better medication. It has been suggested that ADHD manifests differently in adulthood, with a reduction occurrence of motor hyperactivity in adults compared with children with the disorder. A translational animal model must incorporate the core symptoms of ADHD using adult animals and use an appropriate behavioural task. There is a clear requirement for improved therapeutics in adult ADHD to overcome the current disadvantages of the stimulants (i.e. dependency) and non-stimulants (unwanted side-effects, i.e. cardiovascular events). Currently methylphenidate is NICE-recommended as a first-line treatment in all adults with ADHD, with the exception of patients at risk of cardiac events and/or substance abuse history. This is a broad therapeutic approach, and does not account for individual symptom expression (i.e. ADHD subtype) and associated cognitive and emotional recognition deficits. The individual effects of the stimulants and non-stimulants on different symptom domains; attention (vigilance and sustained attention), impulsivity (motor impulsivity and response inhibition), and cognition (executive function and social cognition), require thorough evaluation both in a clinical population and in animal models. This would enable a more personalised approach to treatment strategy, and potentially greater benefits for patients.

Behavioural testing in rodents using the 5C-CPT enabled the characterisation of a novel animal model of the core symptoms of adult ADHD. It is evident from previous preclinical work, that rats exhibiting varying-levels of behaviour in other operant tasks (e.g. 5-CSRTT and stop-signal tasks) can be used to evaluate the underlying neurobiology of the symptom subtypes and the efficacy of novel pharmacological agents. The 5C-CPT model outlined in this thesis utilised the extreme natural levels of sustained attention, vigilance, motor impulsivity and response inhibition (the core deficits in adult ADHD) to model ADHD symptoms in adult female rats. Furthermore the translational 5C-CPT has allowed for detailed analysis of the effectiveness of the currently used ADHD medication in the separate symptom domains of inattention and impulsivity. In addition, the animal model developed through work in this thesis
enabled exploration of novel ADHD therapeutic targets, specifically the DRD4 receptor and COMT inhibition.

11.1.1 ADHD animal model

In the work reported here, animals with high and low levels of impulsivity and/or attention have been identified. The low-attentive (LA) group may represent the inattentive subtype of ADHD and the high-impulsive (HI) group, the impulsive symptoms seen in the other subtypes of ADHD. Previous groups have shown that rats can be selected based on impairments in sustained attention in the 5-CSRTT thought to model the inattentive symptoms in ADHD (Puumala et al., 1996). The work in this thesis extends these findings by using the highly translational task; the 5C-CPT, and selecting animals with deficits in sustained attention and vigilance. Deficits in both sustained attention and vigilance in the CPT are observed in ADHD (Huang-Pollock et al., 2012), making it imperative to include these both in a translational animal model of ADHD. Thus, the LA animals potentially provide a translational model of the inattentive subtype of ADHD.

Previous work has shown that high and low impulsive rats can be identified based on premature responding (impulsive behaviour) in the 5-CSRTT (Dalley et al., 2007). High-impulsive rats have been shown to have greater vulnerability to the reinforcing effects of psychostimulants (Dalley et al., 2007; Robinson et al., 2009), and a higher ascension of cocaine and nicotine self-administration (Dalley et al., 2007; Diergaarde et al., 2008). The work in this thesis extends these findings by selecting rats with high levels of both motor impulsivity and response disinhibition in the same task. Others have used a number of behavioural tasks and selected rats with high motor impulsivity and response inhibition, however by using the 5C-CPT these rats can be selected using one task only. The 5C-CPT’s consistency with the human CPT allows for the same measurements of motor impulsivity (premature responding) and response inhibition (false alarm responding) greatly enhancing the translational value of the model.

Extending the current literature, the work in this thesis outlines a new approach to select animals with a combination of both attention deficits and impulsive behaviour. For the first time, a group of animals showing impairments in baseline levels of sustained attention, vigilance, and response inhibition were selected using the 5C-CPT. These animals have the potential to be used to model the combined subtype of ADHD.
All animals used in the work outlined in this thesis were adult rats, thus the results can be translated specifically to adult ADHD. Though animals selected according to performance in this way are potential models of ADHD subtypes, they did not display high levels of motor hyperactivity. Further research is needed to evaluate whether adult animals with hyperactivity can be selected using the 5C-CPT. It is important to note however, that hyperactivity in ADHD is generally seen not to persist from childhood into adulthood (Biederman et al., 2000). Future research should also focus on assessing the low attentive, high impulsive and ADHD-C rats in different tasks of attention and impulsivity. It would be particularly interesting to assess the impulsive animals in a delayed-discounting paradigm, and in a gambling task such as the Iowa gambling task.

11.1.2 Effects of methylphenidate and atomoxetine

Previous research has shown that baseline levels of impulsivity can alter the effect that particular drugs have on impulsivity. These drugs include, not only the ADHD agents, but cocaine and nicotine; which are shown to increase impulsivity in low-impulsive animals and reduce impulsivity in high-impulsive animals (Caprioli et al., 2013; Dalley et al., 2007; Kayir et al., 2014; Kolokotroni et al., 2014; Mendez et al., 2010; Paine et al., 2003; Roesch et al., 2007; Winstanley et al., 2009). Jupp and Dalley (2014) have elegantly summarised the differential effects of various psychopharmacological agents in various animal models of impulsivity, including rats with high levels of trait-like impulsivity in the 5-CSRTT.

Pharmacological agents used to treat ADHD produce different effects on impulsivity. Consistent with the findings reported in this thesis, acute methylphenidate has been shown to increase impulsivity in the 5-CSRTT in low-impulsive and/or in ‘normal rats’ (Dalley et al., 2007; Fernando et al., 2012; Jupp et al., 2013). However in high-impulsive rats, acute administration of methylphenidate reduced premature responding, a form of impulsive action, in the 5C-CPT, consistent with limited previous studies in the 5-CSRTT (Puumala et al., 1996). In line with these results are the findings in various discounting paradigms. Acute administration of methylphenidate has been shown by some groups to increase impulsive choice in discounting tasks, however others have shown methylphenidate to decrease impulsive choice (reviewed in Jupp and Dalley, 2014). Thus, baseline levels of impulsivity, the strain and sex of the animal, and the paradigm used can impact on the effect of methylphenidate. The results in this thesis also demonstrate that the non-stimulant agent atomoxetine reduced premature responding in the 5C-CPT in both high and low impulsive rats. These results are
consistent with the findings in the 5-CSRTT-literature (Blondeau and Dellu-Hagedom, 2007; Fernando et al., 2012; Navarra et al., 2008; Robinson et al., 2008). The unique aspect of the results outlined in this thesis relate to the ability of the 5C-CPT to measure response inhibition in a manner consistent with human CPTs (Young et al., 2009). For the first time the results reported in this thesis demonstrate that atomoxetine improves response inhibition in high impulsive animals, and to a lesser extent also in low-impulsive animals. Whilst this result has not been previously demonstrated in the 5C-CPT, atomoxetine has been shown to reduce impulsivity in the SSRTT in ‘normal’ rats (Bari et al., 2009; Robinson et al., 2008).

The majority of research examining attention in rodents utilises the 5-CSRTT and male rats. The work in this thesis extends the current literature by using the 5C-CPT and female rats. The unique aspect of the 5C-CPT is the inclusion of non-target stimuli, which allows for signal detection theory measurements and provides improved translation to human CPTs. In using the 5C-CPT, this thesis reports for the first time that atomoxetine increased vigilance in a baseline-dependent manner, alleviating the deficits in low-attentive animals. Interestingly, atomoxetine also enhanced vigilance in high-impulsive animals. Extending previous findings from the 5-CSRTT (Puumala et al., 1996; Robinson et al., 2008), this thesis has shown that both methylphenidate and atomoxetine also increased sustained attention in low-attentive animals only. Further work is now required to assess the effects of chronic administration of methylphenidate and atomoxetine (as used in the clinical setting) in these subgroups of animals.

It is clear from the results outlined in this thesis, that enhancements in behavioural performance are dependent on baseline level of performance. Thus, when modeling disorders and assessing the effects of pharmacological agents, the importance of natural variations in behaviour must not be under-estimated. It is most likely that previous research, not accounting for these differences between animals, may have missed important effects specific to one or more behavioural phenotype (subgroup). It is also plausible that these specific behavioural phenotypes have different underlying neurobiological substrates. The clinical implications of this finding suggest the implementation of a strategic approach when treating the ADHD subtypes. There is most likely to be a sub-section of patients that will respond preferentially to one type of medication over another. It has long been acknowledged that some patients may respond well to a particular treatment or intervention, not just in psychiatric illness but also across all disciplines of medicine, whereas other patients may receive little or no benefit. There is a clear need to sub-categorise disorders beyond their simple phenotype.
Disorders need to be categorised according to the specific mechanisms or genotype contributing to the phenotype. This specific categorisation will reduce the obscuring of benefit in a subgroup of responders.

The subgroups identified in the animal studies-LA, HI, ADHD-C, could have a potential future use for establishing predictive biomarkers, which is essential in preclinical stages for improved drug-development. The identification of biomarkers is critical to enable the selection of a patient subpopulation most likely to respond to the drug and therefore improving success in phase II and phase III clinical trials and ultimately for patient benefit.

Future research needs to focus on a head-to-head comparison of atomoxetine and methylphenidate, assessing the effects on different aspects of impulsivity and inattention in both children and adults. Natural variation in baseline levels of inattention and impulsivity need to be accounted for when evaluating the effectiveness of such medication.

11.1.3 Novel therapeutic targets for inattention and impulsivity

The validity of the 5C-CPT in evaluating the behavioural effects of ADHD pharmacological agents and novel agents has been established for the first time in this thesis. The D4 agonist A-412997 and the COMT inhibitor tolcapone, increased vigilance and response disinhibition in animals showing deficits in sustained attention, vigilance and response inhibition. These findings support the role of dopamine in the core deficits in ADHD, and suggest DRD4 as a potential target for ADHD-C subtype treatment. In line with these findings are clinical studies implicating the DRD4 in response inhibition, novelty seeking and hyperactivity (Benjamin et al., 1996; Ebstein et al., 1996). Previous animal studies have supported the involvement of the DRD4 in ADHD (Avale et al., 2004; Falzone et al., 2002; Michaelides et al., 2010; Thomas et al., 2007; Young et al., 2011; Zhang et al., 2002a), and also DRD4 agonist studies in animals have shown the precognitive effects of these agents in rodents (Browman et al., 2005; Paine et al., 2003; Woolley et al., 2008). The work presented in this thesis illustrates for the first time that tolcapone improved deficits in sustained attention and vigilance, but that it also further impaired performance in ‘normal’ animals supporting the postulated inverted-U relationship of dopamine in the PFC (Levy, 2009). These findings are of particular importance in ADHD in view of hypotheses concerning the role of COMT in the aetiology of ADHD (Beiderman et al., 2004; Salatino-Oliveira et al., 2011; Tekin and Cummings, 2002). Polymorphisms in the COMT gene have
received considerable interest in ADHD literature and have been implicated in the aetiology of ADHD (Zhang et al., 2012). The findings of this thesis support the role of COMT in ADHD. However, further research is required in both humans and animals to confirm these assertions. To date, one clinical proof-of-concept study reports no effects of a D4 antagonist in the treatment of adult ADHD (Rivkin et al., 2012), effects of D4 agonism have yet to be evaluated. To date, no RCTs exist examining the potential role of COMT inhibitors in the treatment of adult ADHD. COMT and A-412997 were only tested in ADHD-C and HA animals, further work is required to test these agents in low-attentive and high impulsive animals to examine their effects in the different behavioural phenotypes (subgroups). Gillies et al., (2014) have elegantly presented the extensive sex differences in the mesolimbic system, highlighting the need to study both males and females both clinically and preclinically. Thus, further work is also needed in male rats and in different strains to assess the effects of both drugs on performance. A chronic treatment regime (as used in humans) warrants additional investigation to increase the translational validity of the findings. Functional imaging studies in adults with ADHD (and animal models) given acute doses of these agents would also be extremely useful in identifying brain regions of interest in ADHD. Electrophysiological and in-vivo microdialysis studies in combination with the 5C-CPT would also be very useful to improve the understanding of the precise effect of these compounds on PFC neurons and neurotransmission.

11.2 Main Findings: Clinical Studies

There is a lack of studies using CANTAB tasks to evaluate the cognitive deficits in adults with ADHD. To date and to the knowledge of the author, only five CANTAB studies exist which compare adults with ADHD to healthy controls. These studies have limited sample sizes (n=13-20), use predominantly males and have yielded conflicting findings; this is possibly due to the modest sample sizes and gender bias (Aron et al., 2003a; Clark et al., 2007; Dowson et al., 2004; McLean et al., 2004; Pironti et al., 2013). A principal aim of the current work was to use a number of CANTAB tasks to identify the core neurocognitive deficits, including emotion recognition deficits, in a larger sample of medicated (n=38) and unmedicated (n=41) patients compared with healthy controls (n=31). The second principal aim was to investigate if the emotion recognition impairments were as a result of a general cognitive dysfunction or were specific deficits unaffected by the core cognitive deficits in ADHD. The third principal aim of this work was to assess the effects of ADHD medication (methylphenidate) on emotion
recognition performance in ADHD. A final aim was to examine any gender differences in cognitive performance within these groups of adults with ADHD.

The clinical neuropsychological testing (CANTAB) outlined in this thesis has confirmed the core cognitive deficits in adult ADHD. The data presented show that deficits in attentional set-shifting, response inhibition and spatial working memory are observed in unmedicated patients but are not apparent in ADHD patients stabilised on medication (methylphenidate or atomoxetine). Performance in rapid visual processing (an indicator of sustained inattention), spatial working memory and attentional set-shifting differed between males and females in unmedicated patients. The importance of the RVIP is its translational relevance to the 5C-CPT, as both tasks allow for calculation of the vigilance measure – A’ or sensitivity index. This work highlights the potential beneficial effects of quantifying cognitive deficits during the ADHD treatment process. The results also show differences in impairments across cognitive domains in males and females, highlighting the importance of including female participants in future research.

Deficits in social cognition have been widely recognised in children (Nixon, 2001) and adults (Schutte and Petermann, 2006) with ADHD; the aspect of social cognition examined in this thesis included facial emotion recognition. It is less well understood in adults if emotion recognition deficits stem from a general cognitive dysfunction in ADHD or are a specific impairment in social perception in ADHD. Results from the current studies confirm previous findings of the presence of emotion recognition deficits in ADHD. Importantly, the results reported in this thesis illustrate for the first time that improvements in emotion recognition persist even following stabilisation on methylphenidate. Impairments in social cognition have been reported to be the main challenge in patients’ daily lives impairing quality of life (McQuade and Hoza, 2008; Nijmeijer et al., 2008). Therefore it is vital to enhance the understanding of the exact nature of these impairments, aetiology and neurobiology, in order to enable effective evaluation of current treatments and to find novel therapeutic targets.

11.2.1 Neurocognitive deficits

The key cognitive deficits identified in this thesis in unmedicated adults with ADHD, include deficits in sustained attention, spatial working memory, executive function and response inhibition. This is the first study to assess the four cognitive domains outlined above in medicated and unmedicated patients with ADHD. Previous studies have reported deficits in working memory, response inhibition and attentional set-shifting in
adults withdrawn from medication 24 hours before assessment (Aron et al., 2003a; Dowson et al., 2004; McLean et al., 2004). This work extends previous efforts by using a larger sample of unmedicated patients (n=41), the majority of which had no history of ADHD medication use (n=34). This work is also the first to compare, using CANTAB, gender differences in cognitive impairments in unmedicated adults with ADHD. Males were more impulsive on the RVIP task making more false alarm responses, and took longer in the SWM task. However in the attentional set-shifting task, females made more errors compared with males. This work needs to be followed up in a larger sample of both males and females. The importance of this work is rooted in the need to establish the cognitive deficits in adult ADHD and the effectiveness of neuropsychological testing in assessing these. Identification of these core cognitive deficits using CANTAB, will potentially allow for the targeting of new treatments to improve the deficits, alongside the core symptoms of ADHD. This will ultimately improve the understanding of the biological basis of ADHD.

The usefulness of neuropsychological tests as part of a diagnostic and monitoring process is highlighted by these results. As predicted, unmedicated patients differed to controls across all symptom domains of a self-report ADHD symptom rating scale, and across all cognitive tasks. In addition, the results of medicated ADHD patients significantly differed from controls across all domains in the self-rating scale, apart from their ADHD index and self-concept scores. Interestingly, however, when tested on the sustained attention and response inhibition CANTAB tasks (RVIP and SST), there were no differences in this group (medicated) compared with controls. These findings indicate that either medicated patients are not accurate in self-reporting symptoms of inattention and impulsivity, or that CANTAB tasks are not sensitive in measuring sustained attention and response inhibition in medicated patients with ADHD. Further work in this area is required to explore these assertions.

An interesting future study would involve comparison of adult ADHD levels of impairments (scores on CANTAB tasks) with scores from children and adolescents with ADHD. This could be further extended by evaluating the effects of prior medication exposure/treatment during childhood in adults with ADHD. The inclusion of fMRI techniques would be extremely useful to elucidate the region-specific neurological differences throughout the lifetime of ADHD, i.e. compare adults who had been treated in childhood with those that were untreated. This would enable more efficient and quantitative diagnostic and monitoring processes and possible cognitive targets for intervention at different stages in development.
11.2.2 Social cognition

There is an overwhelming need to increase understanding of the social impairments in ADHD; as these are consistently reported to be seriously impairing for patients living with ADHD (McQuade and Hoza, 2008; Nijmeijer et al., 2008). To date there are a limited number of studies investigating social cognition in ADHD (Kochel et al., 2014). One aspect of social cognition includes effective emotion regulation, which has been shown to be dysregulated in ADHD (Barkley, 2010). It is widely argued that emotional dysregulation should be incorporated as an additional feature of ADHD in current diagnostic criteria (Barkley, 2010; Martel, 2009; Skirrow et al., 2009). Barkley (2010) suggests that individuals with ADHD are less likely to inhibit their emotions, particularly those involving frustration, impatience, and anger, as a result of deficient cognitive control. This will ultimately result in emotional impulsivity, which refers to the heightened speed and the increased likelihood that individuals with ADHD will react with negative emotions in response to events compared to others without ADHD (Barkley, 2010). The exact nature of these responses needs to be further investigated.

Impairments in emotion regulation have been shown to be vital in determining functional impairments and comorbidity outcomes in children with ADHD (Anastopoulos et al., 2011). Interestingly, a recent study in adults with ADHD has shown that poor emotional self-control was predictive of impairments in areas including; occupational, educational, criminal history, driving outcomes and marital satisfaction, independent of the core inattentive, impulsive and hyperactive symptoms (Barkley and Murphy, 2010). Thus, treatment of emotion dysregulation impairments should be a focus of future research. Further research in this area will help to identify key targets for pharmacological intervention and an insight into understanding the neural mechanisms that support emotional bias.

11.2.2.1 Emotion recognition - general cognitive dysfunction?

The work in this thesis focused on a vital area of social cognition - emotion recognition. Successful emotion recognition is imperative in effective social functioning, peer relationships and behaviour (Morris et al., 2009). Facial emotion processing appears to be the social cognitive process that is most affected in adult ADHD (Marsh and Williams, 2006). Facial expressions convey emotional cues that effect cognitive control, including response execution and inhibition in adults without ADHD (Hare et al., 2005; Schulz et al., 2007). It still remains poorly understood if emotion recognition impairments in ADHD are as a result of impairment in cognition functioning e.g.
inattention and impulsivity, or are specific impairments in social perception. Studies in children and adolescents report mixed findings; some support the notion that emotion recognition deficits in ADHD are due to a general cognitive dysfunction (Cadesky et al., 2000; Shin et al., 2009; Sinzig et al., 2008), and thus if the symptoms of ADHD are treated then the emotion recognition impairments should improve. Others argue that the emotion recognition deficits in ADHD show a primary cognitive deficit in social perception (Yuill and Lyon, 2007, Da Fonseca et al., 2009). Interestingly, deficits in emotion recognition have also been shown to directly effect cognition; a very recent study in children with ADHD has shown that deficits in emotion recognition negatively impacts on cognitive control (Kochel et al., 2014). These findings have been extended in adults with a history of childhood ADHD, showing again that emotional cues from facial expressions influence cognitive control (Schulz et al., 2014).

The study reported in this thesis is the largest study of adults with ADHD to examine the effects of inattention, impulse control, working memory and executive function on emotion recognition. The literature is limited in terms of facial emotion recognition deficits in adults with ADHD (Friedman et al., 2003; Miller et al., 2011; Rapport et al., 2002), and sample sizes are small to moderate (n<35). Although not directly assessing emotion recognition deficits, there are a number of studies examining emotional processing deficits using EEG techniques (Herrmann et al., 2009; Ibanez et al., 2011). The results in this thesis extend these findings and highlight the specific deficits present in unmedicated adults with ADHD. The sample of 41 unmedicated ADHD patients showed impairments in recognition of the negative emotions – sadness, anger, fear and disgust compared with controls. Interestingly, the medicated group (n=38) also showed deficits in anger and disgust recognition compared with controls. A number of previous studies in children support these findings (Pecl et al., 2006; Singh et al., 1998; Williams et al., 2008), for example, Williams et al., (2008) have shown event-related potential alterations to correlate with deficient anger and fear recognition in unmedicated adolescents with ADHD. This thesis also demonstrates the specific cognitive domains can directly influence emotion recognition; inattention and impairments in working memory showed to directly effect fear, sadness and disgust recognition in both ADHD groups (medicated and unmedicated). Impulsivity and attentional set-shifting did not appear to influence emotion recognition in either ADHD groups, and controls. Inattention and working memory deficits did not influence the recognition of anger in both ADHD groups, indicating a specific deficit in the social perception of anger.
Deficient emotion recognition could potentially have serious detrimental effects on interpersonal interactions containing negative emotion displays. Hence, adults with ADHD could miss important social cues that indicate that the interaction is not progressing well, which could ultimately result in a negative altercation. Aggression is known to be an important associated feature of ADHD (King and Waschbusch, 2010); earlier research (in children) suggests that aggression is associated with problems experienced in social cognition (Heiburn, 1990). Thus is it reasonable to assume that deficits in emotion recognition, hence personal interaction could lead to episodes of aggressive behaviour. Importantly, if improvements in social cognition could be achieved a subsequent reduction in aggressive behaviour could also be achieved.

Future research exploring which emotion is being incorrectly assigned to the negative emotion shown would be important and could potentially have extremely beneficial implications for patients. For example, when a fearful face is presented, an emotion is being incorrectly selected, possibly anger, thus fear is perceived as anger. Clinical interventions could then focus on social skills training, teaching patients to correctly appraise the social situation and correctly identify the emotion being displayed. The reaction times taken to select the positive and negative emotions should also be evaluated; adults with ADHD may take longer in processing certain emotions compared to controls. This work may even reveal differences in positive emotion recognition. Thus, despite the lack of difference in the accuracy of positive emotion recognition in ADHD, it may take longer for this group to process the emotion and select an appropriate response compared with controls.

For the first time, a group of ADHD patients were followed-up after starting treatment and re-assessed on the emotion recognition task. Before treatment, this group showed deficits in the recognition of all four negative emotions (anger, fear, sadness and disgust). Interestingly, the results from this follow-up demonstrate that methylphenidate is effective in reducing the negative emotion recognition deficits in adults with ADHD to a level comparable with controls. This is an extremely exciting and novel finding as it has potential implications for treating the emotion recognition deficits in ADHD. In line with these findings, a study by Williams et al., (2008) found that 4-week treatment with methylphenidate improved dysfunctional neural activity in adolescents with ADHD (n=51), which in turn predicted improvements in emotional lability. The improvements seen in sadness, fear and disgust recognition following methylphenidate could be due to the improvement in inattention. However the improvement in anger recognition following methylphenidate was independent to an
improvement in inattention. There remains a distinct lack of studies in female ADHD patients. The results in this thesis were generated from males and females; future analysis should involve a male/female comparison to investigate any sex differences in emotion recognition and treatment responses. One example includes the ventral tegmental area (VTA), which is suggested to play an important role in processing socially relevant cues (Groppe et al., 2013). Important sex differences (both structural and functional) have been reported in the VTA, highlighting the necessity to realise and appreciate the differences between males and females (Gillies et al., 2014).

Improvements in emotion recognition should potentially lead to improvements in social functioning. The implications of improving social functioning in ADHD, could enhance occupational functioning (interacting more effectively in the workplace), relationship functioning (fewer separations), less confrontations and greater peer-group acceptance.

The clinical implications of these findings may extend to other psychiatric conditions with emotion recognition deficits (depression, autism) as well as ADHD. These results suggest that the use of methylphenidate may reduce the emotion recognition deficits, potentially across these disorders. It also highlights the requirement to acknowledge emotion recognition deficits as an important characteristic of adult ADHD, one that needs to be assessed and reassessed after treatment. These findings also highlight the potential for the use of psychological therapy.

Future studies should address the limitations of this work, including the modest sample size (n=15) and the different formulations of methylphenidate taken by patients (Concerta and Ritalin). It is possible that there may have been practice/learning effects, however the CANTAB ERT did not provide feedback or reinforcement after correct or incorrect responses. Thus patients were not aware of whether or not they made a correct or incorrect response, either during or after the task. Improvements in overall cognitive function including inattention and impulsivity induced by treatment with methylphenidate could have impacted on performance. It is important to note that, despite improvements in negative emotion recognition, there were no differences in surprise and happiness recognition (% correct). Future work should also evaluate effects of the non-stimulant; atomoxetine, on emotion recognition deficits.

11.3 Conclusion

This work provides further evidence that ADHD is a debilitating, heterogeneous and multifaceted disorder that persists into adulthood. Firstly, the results illustrate that, just
like in humans, rats display a spectrum of attentive and impulsive behaviour, with differential responses to ADHD treatments observed. The clinical group of unmedicated patients exhibited deficits in response inhibition and sustained attention in the SST and RVIP, and subgroups of animals modeling the symptoms of ADHD (LA, HI and ADHD-C rats) exhibited the same deficits in the 5C-CPT. These findings show the potential for translational and back-translational approaches using the CANTAB tasks – SST and RVP and the 5C-CPT. Medicated patients were significantly less impulsive and more attentive compared with unmedicated patients; similarly, rats with response inhibition deficits and attention deficits that were treated with atomoxetine and methylphenidate improved to a level comparable to controls. The interesting finding in these animals was the differential effects of ADHD medication on attention and impulsivity. This has yet to be explored in a clinical population, but these results suggest that atomoxetine may be more effective in impulsive patients (responders), and methylphenidate in predominantly inattentive patients (responders). Data suggest that the DRD4 may be a potential target for improving vigilance and response inhibition deficits in ADHD, but was not effective in improving sustained attention in ADHD-C animals. Similarly, COMT inhibition enhanced vigilance, response inhibition and sustained attention in ADHD-C animals. The exploration of these targets in a clinical population is an exciting prospect. Secondly, the work outlined in this thesis demonstrates that unmedicated adults with ADHD have significant impairments across all of the cognitive domains assessed (sustained attention, response inhibition, attentional set-shifting and spatial working memory). This group also showed significant impairments in the recognition of negative emotions, which were linked to deficits in inattention and working memory, apart from anger recognition. Importantly, this work has also shown that these deficits can be substantially improved following short-term treatment with methylphenidate. This finding highlights the importance of pharmacotherapy in ADHD, not only to attenuate the behavioural symptoms of inattention, impulsivity and hyperactivity but also to improve the disabling social cognition deficits of this debilitating disorder.

11.4 Final comment

As is the case for any contemporary research, the work presented in this thesis is a work in progress. Hopefully, the incremental findings reported in these studies will lead to greater use of the patient stratification approach and neuropsychological assessments with a focus on impairments in social cognition. This will hopefully help us to move away from the ‘one-treatment fits all’ approach to medication, and to also recognise the
importance of improving social functioning in patients, realising that ADHD is not just a condition of inattention, impulsivity and hyperactivity.
APPENDIX
Appendix 1: *The use of the different vITI in the different chapters*

<table>
<thead>
<tr>
<th>vITI Mean (s)</th>
<th>Utilised in Study</th>
<th>Number of processed trials</th>
<th>ITI range (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Training and baseline determination (chapter 7)</td>
<td>111-120</td>
<td>4.0 4.5 5.5 6.0</td>
</tr>
<tr>
<td>10</td>
<td>LA and HA (chapter 7) ADHD-C and HA (chapter 8)</td>
<td>78-86</td>
<td>8.0 9.0 11.0 12.0</td>
</tr>
<tr>
<td>20</td>
<td>LI and HI (chapter 7)</td>
<td>41-53</td>
<td>16.0 18.0 22.0 24.0</td>
</tr>
</tbody>
</table>

vITI; variable inter-trial interval.

A descriptive table of the individual ITIs used to formulate the average vITI and a breakdown of the studies utilising each set vITI. The range of total trials processed using each vITI is also reported.
Appendix 2: Schematic diagram of study protocol, and participant numbers

Justification for sample size

Power for the clinical neuropsychological studies was based on existing literature showing a medium to large effect of drug treatment on ADHD symptoms and neuropsychological performance. A total recruitment of 40 patients per group would result in 80% power at a 0.05 level, and to adjust to expected drop-out; the study plan aimed to recruit 50 individuals per treatment group.
Appendix 3: Schematic diagram of study protocol, and participant numbers
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