Long-term Effects of Chronic Treatment for ADHD on Cognition and Neural Development in an Adolescent Rat Model

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Statement of Authentication and Ethical Accordance

This thesis is submitted to Macquarie University in fulfilment of the requirements for the degree of Combined Doctor of Philosophy/Masters in Clinical Neuropsychology.

The work presented in this thesis is, to the best of my knowledge and belief, original expect as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

All animal research carried out in this thesis was approved by the Macquarie University Animal Ethics Committee (ARA's 2006/019, 2009/001, 2010/001) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition (National Health and Medical Research Council, 2004).

Margery Claire Pardey

Signature:……. Date …./…./….

Co-Author Declaration

The following authors acknowledged that this work represents that of Margery Claire Pardey, and where appropriate accurate information regarding co-author contribution is supplied.

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List of Publications

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Abbreviations

ADHD	attention deficit hyperactivity disorder
ADHD-C	attention deficit hyperactivity disorder, combined subtype
ADHD-HI	attention deficit hyperactivity disorder, predominately hyperactive/impulsive subtype
ADHD-I	attention deficit hyperactivity disorder, predominately inattentive subtype
ANOVA	analysis of variance
CaMKII	calcium/calmodulin-dependent protein kinase II
cm	centimetre
°C	degree centigrade
DA	dopamine
D1	dopamine D1 receptor
D2	dopamine D2 receptor
dopa	3,4-dihydroxyphenylanine
DOPAC	3,4-dihydroxyphenylacetic acid
DBH	dopamine β-hydroxylase
g	gram
GABA	gamma aminobutyric acid
h	hour
HCl	Hydrochloride
IL	infralimbic cortex
i.p.	intraperitoneal
kg	kilogram
L	litre
LC	locus coeruleus
lux	luminous emittance
m	metre
µg	microgram
µL	microlitre
µm	micrometre
mg	milligram
min	minute
mPFC	medial prefrontal cortex
MPH	methylphenidate
NA	noradrenaline
α_1	noradrenaline α_1 receptor
α_2	noradrenaline α_2 receptor
β	noradrenaline β receptor
NHS	normal horse serum
NAc	nucleus accumbens
OFC	lateral orbitofrontal cortex
PBS	phosphate buffered saline
PBT	phosphate buffered saline with 0.1% Tween 20
PFA	paraformaldehyde
PFC	prefrontal cortex
PIR	passive infrared detector
PND	postnatal day

PrL	prelimbic cortex
sec	second
SEM	standard error of the mean
SHR	Spontaneously Hypertensive rat
SD	Sprague-Dawley rat
s.d.	standard deviation
TPBS	Tris- phosphate buffered saline
TH	tyrosine hydroxylase
vs	versus
VTA	ventral tegmental area
WKY	Wistar-Kyoto rat

Abstract

Attention Deficit/Hyperactivity Disorder (ADHD) has become the most commonly diagnosed childhood disorder. However, ADHD diagnosis involves subjective interpretation of diagnostic criterion which could lead to misdiagnosis. ADHD symptoms are thought to arise due to irregularities in the function of the neurotransmitters dopamine and noradrenaline. In order to normalise these irregularities, powerful psychostimulant medications such as Ritalin® (methylphenidate, MPH) are front line treatments for this disorder. MPH is generally administered over long periods of time where the onset and duration of chronic treatment corresponds with significant periods in brain development and maturation. This thesis was conducted to 1) investigate the long-term effects of chronic MPH treatment during adolescence on cognitive and neural development of misdiagnosed individuals; and 2) investigate the neural mechanisms underlying MPH's impact on impulsive behaviours.

Appropriate animal models are necessary to conduct these investigations. The Spontaneously Hypertensive rat (SHR) has been extensively studied and is a commonly used animal model for ADHD. However, the methodology of previous research was recently criticized by Alsop (2007). The aim of the first experimental chapter of this thesis (Chapter 2) was to re-assess the validity of the SHR as an animal model of ADHD, in light of Alsop's criticisms. The results showed that the SHR had increased locomotor activity and were more sensitive to increases in delay in an impulsivity task when compared to the control strain, Wistar-Kyoto rats (WKY). These findings provide further support for the SHR as a valid animal model of ADHD.

Previous pre-clinical research has employed drug administration methods which limit the generalisation of their findings to the human condition. When children are treated with MPH

they receive low oral doses, whilst the majority of pre-clinical research administers large doses of MPH via intraperitoneal injection. The aim of the second experimental study (Chapter 3) was to develop an oral method of drug administration that was less invasive than dosing by gavage and that required minimal training. For this, the rats learned to consume a MPH suspension through a drinking spout. Following this novel oral drug administration, there was a dose-dependent increase in locomotor activity that was similar in effect to MPH administration by gavage.

The following experimental study (Chapter 4) incorporated this novel oral administration method to assess the long-term effects on cognitive development of chronic MPH treatment during adolescence. While the animal model of ADHD is the SHR, the focus of this study was on the WKY as the non-ADHD or misdiagnosed strain. The rats were orally treated with either MPH or distilled water over 4 weeks throughout adolescence (PND 27 – 52). MPH was administered twice daily to model clinical dosing schedules in children. Locomotor activity was measured at the beginning of each week of treatment and cognitive-behavioural tests were completed in adulthood after cessation of treatment. The findings of this study suggest there are enduring behavioural changes in adulthood when rats inappropriately received chronic MPH treatment throughout adolescence. However, when chronic MPH treatment was appropriately given to the ADHD rats, there were no long-term effects observed in adulthood.

The effects of chronic MPH treatment on neural development were assessed in the following study (Chapter 5). Following 4 weeks of MPH treatment, immunostaining for Tyrosine Hydroxylase (TH) positive neurons in the prefrontal cortex (PFC) was performed at 3 stages. Group 1 and Group 2 were euthanized 1 week and 12 weeks, respectively, after cessation of treatment for neural tissue analysis. Group 3 (from Chapter 4) were euthanized upon completion of cognitive-behavioural testing for neural tissue analysis (12 weeks post

treatment). The results suggest that pre-exposure to MPH in non-ADHD rats may interfere with the maturation of the PFC and may subsequently alter future neural adaptations to behavioural experiences.

The final experimental chapter of this thesis was conducted to elaborate on the neurochemistry which may underlie the persistent behavioural changes in the adult WKY. Specifically, the final study investigated the role of dopamine and noradrenaline on impulsivity mediated by regions within the PFC (Chapter 6). Alterations in impulsivity were assessed following local infusions of dopamine antagonists or noradrenaline agonists into regions of the PFC. The results indicated that blockade of different dopamine receptors increased impulsivity depending on their location within the PFC, while noradrenaline receptor activation of the PFC was found to have no impact on impulsivity.

In conclusion, using a method of drug administration that closely models clinical treatment in children, the findings of this thesis suggests inappropriate chronic childhood treatment with MPH has long-term effects on cognition and may interfere with brain maturation. Furthermore, the potential cause of these deficits may be alterations in DA functioning. The findings of this thesis highlight the need for more stringent diagnostic criteria for ADHD.

Chapter 1

Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is one of the most commonly diagnosed childhood disorders (Biederman & Faraone, 2005). Children diagnosed with the disorder are often chronically treated with psychostimulants to alleviate the symptoms of hyperactivity, impulsivity and inattention. There is a common belief among the general public that ADHD is over-diagnosed, with 76% of people responding affirmatively to a CNN (2002) online poll asking "Do you think ADHD is over-diagnosed". This belief has been sustained by the publicity which ADHD receives in the press and mass media. For example, the influential talk show host Dr Phil McGraw stated that ADHD is "so over-diagnosed", following an episode on parenting hyperactive children (McGraw, 2004). Furthermore, a recent article in the Sydney Morning Herald discussed the increased risk of ADHD misdiagnosis of the youngest children in their school year (Sydney Morning Herald, 2010). It has also been suggested that ADHD may have become a desirable diagnosis for some parents to explain away poor school performance or unruly behaviour of their children (Smelter, Rasch, Fleming, Nazos, & Baranowski, 1996).

While there are no studies to date that adequately address the issue of over-diagnosis, there is evidence to suggest that primary care physicians vary greatly in the assessment methods and do not always follow 'best practice' guidelines when diagnosing ADHD (Handler & DuPaul, 2005). Such variations in diagnostic procedures would likely increase misdiagnosis of the disorder (Sciutto & Eisenberg, 2007). Misdiagnosis is of particular concern as during childhood and adolescence neural connections are being refined to attain their adult pattern (Andersen, 2003; Sowell, Thompson, Tessner, & Toga, 2001) and external influences, such as medications, have the ability to alter the morphology of neural circuitry (Crombag, Gorny, Li,

Kolb, & Robinson, 2005; Heijtz, Kolb, & Forssberg, 2003).

The current thesis used a rodent model to investigate the long-term effect of chronic administration of the common ADHD medication Ritalin® on brain development and behaviour. This introductory chapter will begin with a brief outline of the aetiology, prevalence, diagnosis and treatment of ADHD. A discussion of an appropriate animal model will follow, detailing the rodent literature that has previously assessed the acute and chronic effect of Ritalin® (methylphenidate) administration. The development of the human and rodent central nervous system will then be outlined, as will the role of key neurotransmitters in cognitive function mediated by the frontal lobes. The chapter will conclude with a discussion of behavioural tasks thought to reflect frontal lobe function in rodents, drawing to a close with the hypotheses and aims of the thesis.

1.1 What is ADHD?

The nomenclature and symptom profiles of ADHD have evolved over the years. ADHD has been referred to as brain injured child (Strauss & Lehtinen, 1947), minimal brain dysfunction (Wender, 1973) and hyperkinetic reaction of childhood (American Psychiatric Association, 1968). In an earlier version of the DSM-III (1980), the first subtypes were described with a distinction of Attention Deficit Disorder with and without hyperactivity.

The current version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 2000) states that ADHD is characterised by combinations of the symptoms of hyperactivity, impulsivity and/or inattention. The onset of ADHD symptoms usually occurs during childhood with the majority of sufferers attaining either partial or full remission of symptoms in adulthood (Biederman & Faraone, 2005). However, there are a proportion of sufferers that continue to meet full diagnostic criteria for ADHD in

adulthood, albeit with varying symptom profiles across age groups (Faraone, Biederman, & Mick, 2006).

1.1.1 Aetiology & Prevalence of ADHD

A clear cause of ADHD has yet to be identified. Increases in the risk of ADHD have been linked to both genetics and environmental components. ADHD has been shown to be highly heritable (Albayrak, Friedel, Schimmelmann, Hinney, & Hebebrand, 2008) and there are several environmental risk factors for developing ADHD. These include prenatal exposures to toxins such as tobacco, alcohol and lead (Braun, Kahn, Froehlich, Auinger, & Lanphear, 2006; Fryer, McGee, Matt, Riley, & Mattson, 2007; Rodriguez & Bohlin, 2005; Thapar et al., 2003); perinatal issues such as low birth weight (Nigg & Breslau, 2007); and postnatal factors such as adverse early childhood experiences, childhood illness and brain injury (Millichap, 2008).

The DSM-IV (American Psychiatric Association, 2000) reports that the prevalence of ADHD is estimated to be 3% to 7% in school-aged children, with a higher incidence in males than females. Reviews assessing the worldwide prevalence of ADHD (Polanczyk, de Lime, Horta, Biederman, & Rohde, 2007; Skounti, Phialithis, & Galanakis, 2007) estimate a rate of 4% to 10%, although the validity of such an estimate is limited by the nonstandardized design and methodology used across these studies.

1.1.2 Diagnosis of ADHD

At present, there are two commonly used diagnostic systems for mental disorders, the DSM-IV (American Psychiatric Association, 2000) and the International Statistical Classification of Diseases (ICD-10; World Health Organization, 1992). The DSM-IV criterion for ADHD requires that some symptoms which cause impairment must be present before the age of 7

years. There must be at least six symptoms of either inattention or hyperactivity-impulsivity which are inconsistent with developmental level and have been present for over six months. These symptoms must result in significant impairment in at least two settings (e.g. school and home), and they must not be better accounted for by another mental disorder (e.g. Mood Disorder, Anxiety Disorder).

The DSM-IV classifies ADHD into subtypes depending on symptom presentation. The three subtypes are: 1) ADHD, Combined type (ADHD-C) if the patient has at least six symptoms of both inattention and hyperactivity-impulsivity; 2) ADHD, Predominately Inattentive type (ADHD-I) if the patient exhibits six symptoms of inattention only; and 3) ADHD, Predominately Hyperactive-Impulsive type (ADHD-HI) if the patient exhibits at least six symptoms of hyperactivity-impulsivity only. Poor impulse control and inhibition have long been thought to be the underlying cause of the symptoms in ADHD-HI and ADHD-C, while deficits of focused attention and speed of information processing are thought to underlie ADHD-I (Barkley, 1999). It has been suggested that ADHD-HI may be the precursor to the more commonly presented ADHD-C (Hart, Lahey, Loeber, Applegate, & Frick, 1995).

The constellation of symptoms of hyperactivity, impulsivity and inattention are classified as Hyperkinetic Disorder in the ICD-10. Similar to the DSM-IV, the ICD-10 criterion requires onset at an early age of persistent symptoms, the presence of the symptoms in more than one setting, and exclusion of other psychiatric disorders. However, the ICD-10 requires a higher level of symptom expression than the DSM-IV. The ICD-10 requires at least six symptoms of inattention, at least three symptoms of hyperactivity and at least one impulsive symptom. The ICD-10 does not distinguish subtypes of the disorder as diagnosis requires exhibition of symptoms encompassing all symptom types and therefore can not be distinguished from one another. Diagnosis using the ICD-10 criteria is more stringent and would reflect a

significantly impaired individual with ADHD-C as diagnosed using the DSM-IV criteria.

The ICD-10 system is predominantly used throughout Europe, while North America and Australia follow the diagnostic criteria of the DSM-IV. The evolution of the ADHD criteria to include subtypes of this disorder in the current DSM-IV has been shown to increase the rate of diagnosis by 57% (Wolraich, Hannah, Pinnock, Baumgaertel, & Brown, 1996). This may explain the recent increase in diagnosis as the current DSM-IV allows for identification of children with ADHD-I and ADHD-HI subtypes, in addition to children exhibiting the classic triad of ADHD symptoms (Barkley, 2005; Wolraich et al., 1996).

The symptoms of ADHD are not specific to ADHD and a range of comorbid conditions need to be considered. Draft Australian guidelines on 'best practice' for ADHD have recently been released by The Royal Australasian College of Physicians (RACP, 2009). Despite the development of such guidelines, there is evidence to suggest that not all primary care physicians follow these diagnostic guidelines and that they can vary greatly in the assessment methods they employ to measure ADHD symptoms (Handler & DuPaul, 2005). Such departures from 'best practice' principles would affect the accuracy of diagnosis and potentially increase the possibility of misdiagnosis.

The draft guidelines recommend that ADHD should be diagnosed following the DSM-IV criteria. Diagnosis should be based on a comprehensive assessment including medical, developmental and psychosocial assessment, and gathering evidence of impairment in different settings from multiple informants. The draft guidelines recommend using rating scales or interviews for collecting such evidence. Rating scales such as the Child Behaviour Checklist (Achenbach & Edelbrock, 1991), Behavioural Assessment Schedule for Children (Reynolds & Kamphaus, 1992), and Strengths and Difficulties Questionnaire (Goodman,

1997) can assist with differential diagnosis, while scales such as the Conners Rating Scales (CRS; Conners, Sitarenios, Parker, & Epstein, 1998a; Conners, Sitarenios, Parker, & Epstein, 1998b) and the Swanson, Nolan and Pelham-IV (SNAP-IV; Swanson, 1992) assess the severity of ADHD symptomatology. All of these rating scales are available in appropriate forms for parent or teacher informants, and cover a range of developmental ages.

It is important to note that diagnosis of ADHD involves subjective ratings of behaviour, which need to take into account developmental appropriateness of behaviours. Due to the context-dependent nature of the disorder (American Psychiatric American Psychiatric Association, 2000; Thapar, Holmes, Poulton, & Harrington, 1999), diagnosis in the clinic relies on information from parents and teachers about the behaviour of the child in relevant contexts, as indicated by the draft RACP guidelines. Additionally, parent and teacher ratings are integral in determining symptom severity (Solanto & Alvir, 2009). Although incorporation of rating scales is stipulated by the draft RACP guidelines, the psychometric properties of such rating scales have recently been questioned. Solanto and Alvir (2009) compared intrarater reliability on two ADHD rating scales, CRS and SNAP-IV, which are both scales based on the DSM-IV criteria for ADHD. Solanto and Alvir (2009) found a difference in the ratings of the identical symptoms in the same child made by the same rater. They suggest this may occur for two main reasons. Firstly, variability in behaviour over different situations and times would make it difficult to rate behaviours such as hyperactivity consistently (Barkley, 1990). Secondly, they also suggested that the language used in the DSM-IV, and therefore the DSM-IV based rating scales, is overly complex and would likely affect the raters' comprehension of individual items. For example, inadequate definition of the term 'impairment' and use of the term 'often' in all of the DSM-IV symptoms entail subjective decisions and has lead to previous criticism of the language used in the DSM-IV criteria (Adler, 1995; Skounti et al., 2007). With a lack of any physiological diagnostic

criteria for ADHD (Wallis, 2010), clinicians are restricted by these subjective limitations of the DSM-IV which may lead to misdiagnosis of the disorder.

1.1.3 Treatment following ADHD diagnosis

The draft RACP guidelines recommend pharmacotherapies as a first line of treatment for severe ADHD (2009; pg xvii). ADHD medications come in the form of stimulants; such as methylphenidate (MPH; Ritalin®, Attenta®) and dexamphetamine, and non-stimulants, such as tricyclic antidepressants and clonidine (Catapres®). It is recommended that stimulant medications are initially trialled, and if the child does not respond to or is intolerant of stimulants, subsequent trials of non-stimulant medications is recommended (RACP, 2009; pg xviii).

In New South Wales (NSW), the prescription of stimulant medications is restricted to Paediatricians, Paediatric Psychiatrists and other clinicians with appropriate qualifications, and are regulated and monitored by the NSW Department of Health. Prescription rates of stimulants to treat ADHD have significantly increased in Australia over the past two decades (Berbatis, Sunderland, & Bulsara, 2002; Hollingworth et al., 2011; Preen, Calver, Sanfilippo, Bulsara, & Holman, 2007; Prosser & Reid, 1999, 2009). In their recent review which concluded that there was an 87% increase in the number of prescriptions of stimulant medications in Australia from 2002-2009, Hollingsworth et al. (2011) speculated that this trend arose from a number of factors including better affordability and evidence for efficacy of treatment, increased awareness of treatment options, changes in societal expectations leading to children staying at school longer, and as an outcome of marketing by pharmaceutical companies.

The increase in prescription rates may also be associated with an elevation in the misuse of

MPH. Prescription stimulants are commonly misused (Kollins, 2008) and MPH has acquired many street names including “Vitamin R” and “the smart drug” (Kollins, MacDonald, & Rush, 2001). The nonmedical to medical ratio for stimulant use is much higher than that for opiates, despite more opiate prescriptions per se (McCabe, Teter, & Boyd, 2006).

Given the limitations of the current diagnostic methods of ADHD and the recommendation of stimulant medication as a first line of treatment, together with the research evidence that suggests the developing nervous system has substantial plasticity (reviewed below in 1.3), the current thesis was conducted to investigate the long-term behavioural and neural effects of chronic MPH treatment in misdiagnosed adolescents using an animal model.

1.1.4 The Spontaneously Hypertensive rat as a model of ADHD

The Spontaneously Hypertensive rat (SHR) was developed by inbreeding its genetic control, the Wistar-Kyoto rat (WKY), selecting for hypertension. Over several decades, the SHR has been extensively studied and has become a widely used model for ADHD.

The SHR exhibits behaviours which are consistent with the core features of ADHD. The SHR has been found to have increased motor activity which corresponds to the characteristic hyperactivity in ADHD (Sagvolden, Petterson, & Larsen, 1993). Impulsive behaviours in the SHR have been demonstrated by their excessive responding for food and sucrose reinforcers in fixed-interval schedules (Berger & Sagvolden, 1998; Sagvolden, 2000; Sagvolden et al., 1992; Sagvolden et al., 1993), in addition to their preference for immediate behavioural reinforcement (Fox, Hand, & Reilly, 2008). Deficits of attention and learning have also been observed in the SHR with poor performance during extinction tasks (Berger & Sagvolden, 1998; Sagvolden et al., 1993) and difficulty acquiring operant tasks when behavioural reinforcement was delayed (Hand, Fox, & Reilly, 2006; Sagvolden, Johansen, Aase, &

Russell, 2005; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005).

In addition to showing behavioural symptoms of ADHD, the SHR also has altered neurobiology that corresponds with the postulated underlying cause of ADHD symptoms. Impairments in the release of the neurotransmitter dopamine (DA) have been reported in the SHR and DA metabolism is reduced compared to the WKY (Leo et al., 2003; Russell, 2002). Furthermore, altered function of the neurotransmitter noradrenaline (NA) has been reported in the SHR. It has been suggested that there may be a deficit in the inhibitory control of the NA auto-receptor, resulting in increased central NA transmission (Russell, Allie, & Wiggins, 2000; Russell & Wiggins, 2000). It is likely that the altered levels of the catecholamines DA and NA also contribute to hypertension in this strain (De Brito Gariepy, Carayon, Ferrari, & Couture, 2010; Kasparov & Teschemacher, 2008), which is confounding factor of the SHR as a model of ADHD. However, the behavioural characteristics of ADHD have been observed in young SHRs prior to the onset of hypertension (Sagvolden, Russell et al., 2005), and the neurocognitive deficits of the SHR have been shown to be unrelated to hypertension (Kantak et al., 2008).

The methodologies of research validating the SHR as a model of ADHD was recently criticised (Alsop, 2007). Alsop (2007) reanalysed selected data sets and determined that behavioural differences which had previously been interpreted as impulsivity were better explained by the increased locomotor activity levels of the SHR. One aim of this thesis was to re-assess the SHR as an animal model of ADHD, taking into consideration Alsop's observations.

1.1.5 The Wistar-Kyoto rat as a model of non-ADHD

The WKY is the genetic control for the SHR and has been widely used as the comparison or

'normal' strain in previous research of animal models of ADHD. The use of additional control strains has been advocated as the WKY has also been suggested as a model for anxiety and depression, and that previously reported differences attributed to the SHR, can alternatively be attributed to the underactivity of the WKY (Bull, Reavill, Hagan, Overend, & Jones, 2000; Drolet, Proulx, Pearson, Rochford, & Deschepper, 2002; van den Bergh et al., 2006). Bull and colleagues (2000) used a task requiring rats to withhold a lever press response for a pre-determined period of time to assess motor impulsivity in the SHR and WKY with an addition control strain, the Sprague-Dawley (SD) rat. Their results indicated that the WKY had a reduced ability to inhibit responding compared to both the SHR and SD, while there was no reported difference between the SHR and SD. Reduced locomotor activity of the WKY when compared to the Wistar strain, an alternate 'normal' strain, has also been reported (Drolet et al., 2002; van den Bergh et al., 2006). These findings have led some researchers to suggest that the SHR is not *hyper*active, but it is the WKY that is *hypo*active. However, there is also research that demonstrates similar reductions in impulsive responses over consecutive weeks of testing between SD and WKY rats, as measured by the number of incomplete arm entries on a water maze task (Clements & Wainwright, 2006). Furthermore, similar locomotor responses to acute and chronic MPH administration has been measured between SD and WKY rats (Yang, Amini, Swann, & Dafny, 2003; Yang, Swann, & Dafny, 2006).

A significant strength of the SHR as an animal model of ADHD is that the characteristic symptoms are set in the genome similar to the human condition (Albayrak et al., 2008; Faraone & Biederman, 1998; Thapar et al., 1999). The use of outbred strains, such as SD or Wistar rats, as a non-ADHD control for the SHR would introduce significant genetic variability. To keep genetic variability to a minimum, this thesis employed the WKY as the non-ADHD control strain, and ensured that any differences can not be explained by the inactivity of the WKY. Importantly, the focus of this thesis was on the inappropriate treatment

of the non-ADHD strain with MPH as a model of 'misdiagnosis'. While the SHR has also been studied, it is the results of the effect of MPH treatment in the WKY that were of primary interest.

1.2 Methylphenidate (Ritalin®)

Methylphenidate (MPH, Ritalin®, Figure 1) is a commonly prescribed treatment for ADHD (Engert & Pruessner, 2008). Although there is marked individual differences in the behavioural response to the clinical administration of MPH (Leonard, McCartan, White, & King, 2004), it is effective in treating the core symptoms of ADHD in approximately 70% of children with the disorder (Greenhill et al., 2002). The most common side-effects of MPH treatment include appetite suppression and insomnia, with other reported side-effects including stunted growth, seizures, blurred vision, stomach aches, headaches, and nervousness (Ahmann, Waltonen, Theye, Olson, & Van Erem, 1993; Barkley, McMurray, Edelbrock, & Robbins, 1990; Rapport & Moffitt, 2002). Serious cardiovascular problems and increased psychiatric symptoms have also been reported with use of MPH. However, to date there have been no reports of long-term side-effects of MPH treatment in children with ADHD.

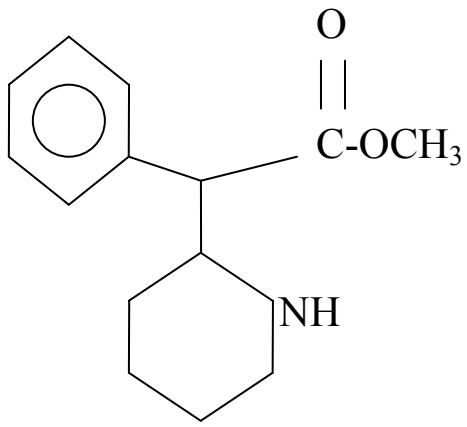

Figure 1. Chemical structural of methylphenidate.

1.2.1 Psychopharmacology of MPH

MPH is an orally administered medication that is rapidly absorbed and readily penetrates the blood brain barrier of the central nervous system (Wolraich & Doffing, 2004). Initially available in an immediate release formula, the relatively short duration of the drug action (4 hours) was inconvenient and reduced compliance as multiple doses were required throughout the day. More recently sustained-release and extended-release formulas have eliminated these problems. The withdrawal of MPH on weekends, or 'weekend holidays', is a common method of establishing a drug-free period and is based on the assumption that a medication free period may reduce side effects without affecting the efficacy of the drug (Coffey, 1997; Taylor, 1994). Indeed, weekend holidays have been shown to reduce side effects of insomnia and appetite suppression without significant increase in ADHD symptoms (Martins et al., 2004).

Early preclinical studies in rodents have not shown the same attenuation of ADHD behaviours with MPH treatment as observed in humans (Arnsten, 2006). Administration of MPH increased locomotor activity in rats (Kuczenski & Segal, 2002; Yang et al., 2006; Yang, Swann, & Dafny, 2010), therefore weakening the validity of using rodents to study the neuropharmacology of treatments for ADHD. However, these preclinical studies of MPH did not mirror the dosing regime used with ADHD children and did not take into consideration other factors which may affect the action of the drug (Dafny & Yang, 2006; Kuczenski & Segal, 2002). Typically, MPH is administered to children orally and in relatively low doses, while preclinical studies tended to administer MPH in very high doses via intraperitoneal (i.p.) injections. This results in significantly higher peak plasma levels of the drug than are obtained in therapeutic settings (Gerasimov et al., 2000). Other factors that have been shown to be critical for the behavioural and neurochemical response to the drug include the age at which the rats were treated, the duration and frequency of treatment, and the time of day that treatment is administered (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002;

Gaytan, Yang, Swann, & Dafny, 2000). Most preclinical studies administered MPH during the light phase or inactive 'sleep' time of rodents, in contrast to the clinical regime for treating children during their active phase (Kuczenski & Segal, 2002).

In light of the limitations of previous research, Kuczenski and Segal (2002) identified low, oral doses of MPH in rodents that produced similar blood plasma concentrations to those seen following therapeutic treatment of ADHD patients. The typical clinical blood plasma concentrations of MPH range from 8 to 40 ng/ml, allowing for variability in the behavioural response to MPH treatment (Swanson & Volkow, 2001). Kuczenski and Segal (2002) estimated that oral administration of 0.75 mg/kg to 3.0 mg/kg of MPH in rats would result in peak plasma levels covering this clinical range. The chronic treatment of rats with clinically relevant oral doses of MPH demonstrated that low doses (0.75 to 3 mg/kg) of MPH significantly reduced locomotor activity, while a higher oral dose of 5 mg/kg induced behavioural activation in line with the previous studies using high i.p. administration of MPH (Kuczenski & Segal, 2002).

MPH has been shown to dose-dependently increase levels of the catecholamine neurotransmitters DA and NA, with little effect on serotonin neurotransmission in the rat brain (Kuczenski & Segal, 1997, 2001a, 2002). Previous research using high doses of MPH (10, 20, and 30 mg/kg, intravenous) showed a significant increase in extracellular DA in the striatum and NA in the hippocampus (Kuczenski & Segal, 1997). However, in studies using clinically relevant doses of oral MPH an increase in hippocampal NA occurred, without affecting DA release in the nucleus accumbens (NAc; Kuczenski & Segal, 2002). Berridge and colleagues (2006) have also compared the effect of the administration of low doses of MPH on NA and DA efflux in cortical (prefrontal cortex, PFC) and subcortical (NAc and medial septal area, MSA) brain regions in the rat. It was shown that clinically relevant

doses of MPH significantly increased DA and NA efflux in the PFC, with little effect on subcortical regions. It should also be noted that the affect of MPH on the NA transporter may also contribute to the increase in DA efflux in the PFC (Bymaster et al., 2002; Madras, Miller, & Fischman, 2005). These findings suggest that regulation of catecholamines in the PFC may be an important therapeutic action of MPH treatment and implicate dysfunctional PFC catecholamine neurotransmission in ADHD.

MPH administration produces an increase in extracellular DA and NA through selective inhibition of DA and NA transporter function, thereby acting as indirect catecholamine agonists (Wilens, 2008). Therapeutic doses of MPH block more than 50% of DA transporters in human subjects (Volkow, Fowler, Wang, Ding, & Gatley, 2002; Volkow, Wang, Fowler, Logan, Franceschi et al., 2002). Volkow and colleagues (2002; 2001; 2002; 2004) have conducted studies using positron emission tomography (PET) to visualise changes in extracellular DA following MPH administration using the radio ligand carbon-11-labeled raclopride which competes with endogenous DA for D2 receptor occupancy. Their research has focused on the striatum, and has not assessed extracellular DA in the PFC. In contrast to the preclinical studies reporting no change in striatal DA following low clinically relevant doses of MPH (Berridge et al., 2006; Kuczenski & Segal, 2002), Volkow and colleagues (2002; 2001; 2002; 2004) reported significant increases in extracellular DA in the striatum following doses of MPH that were at the higher end of the therapeutic range. Furthermore, the enhanced DA levels following MPH treatment have been shown to occur only in the presence of a salient stimulus and are therefore dependent upon the environmental conditions in which MPH was administered (Volkow, Wang, Fowler, Logan, Jayne et al., 2002; Volkow et al., 2004). These results highlight the fact that the therapeutic action of MPH to increase DA levels by blockade of the DA transporter is modulated by individual differences in the amount of DA released from DA cells (Volkow, Wang, Fowler, Logan, Franceschi et al., 2002). Until

recently, neuroreceptor ligands to visualise NA in the human brain using PET and SPECT were not available. In the first study to assess the effect of MPH on NA in the human brain in vivo, Hannestad and colleagues (2010) used the NA transporter ligand (S,S)-[^{11}C] methylreboxetine to measure NA transporter occupancy of therapeutic doses of MPH. Their results indicate that MPH occupied over 80% of NA transporters in the locus coeruleus, raphe nuclei, and hypothalamus, with lower occupancy in the thalamus and thalamic subnuclei. The authors note that NA transporter density in the PFC is too low to be able to detect changes in occupancy via PET. Together, these studies support the role of DA and NA in the therapeutic efficacy of MPH in humans.

1.2.2 Proposed neurochemical mechanism underlying the therapeutic action of MPH administration

It is well described that catecholamines have different functions within the brain. The DA system is involved in regulating responses to reinforcement and motor control, while the NA system mediates perception and interest in the stimuli (Solanto, 1998). As such, dysfunction of the catecholamine system would produce ADHD symptoms of impulsivity, hyperactivity and inattention (Pliszka, 2005; Wilens, 2008). The following section will focus on the catecholamines within the striatum and PFC as they are the most extensively studied regions of the therapeutic action of MPH. A more detailed description of the catecholamine systems follows in section 1.4.1.

In general, the striatum regulates motor control behaviours such as response inhibition, motivation, reward and reinforcement learning (Hassani, Cromwell, & Schultz, 2001; Zandbelt & Vink, 2010), while the PFC is involved with higher cognitive functions such as decision making and working memory (Arnsten & Li, 2005). Both of these regions are highly innervated by the catecholamine systems (section 1.4.1; Berridge & Waterhouse, 2003;

Dahlstrom & Fuxe, 1964; Fuxe et al., 1974).

In the 1990's Grace proposed a tonic/phasic model of DA cell firing and subsequent DA release patterns in terminal regions such as the striatum and PFC (1991, 1995). Phasic DA release refers to the brief pulse of DA into the synaptic cleft following an action potential, which activates postsynaptic DA receptors and evokes a DA dependent behavioural response. Synaptic DA is then rapidly removed by DA transporters into the presynaptic terminal before it can diffuse into the extrasynaptic space. Low concentrations of DA in the extrasynaptic space activate inhibitory DA autoreceptors on the presynaptic terminal, known as tonic DA regulation. Activation of the autoreceptors will therefore inhibit DA synthesis and attenuate phasic DA release (Grace, 1991, 1995). The administration of MPH dose-dependently blocks DA transporter function to differentially impact the regulation of DA cell firing. The effect of MPH on the DA system most likely explains the dose-dependent effects of MPH on locomotor activity which is reduced by low doses of MPH, but increased following high doses (Seeman & Madras, 1998, 2002).

Seeman and Madras (1998, 2002) have demonstrated that extracellular subcortical DA briefly rises from baseline by 60-fold during a normal neuronal impulse. When the DA transporter is blocked by low doses of MPH, the resting level of extracellular DA is raised by approximately 6-fold. The elevated resting state of extracellular DA is hypothesized to engage the inhibitory presynaptic DA autoreceptors to reduce the amount of DA released following an action potential. This reduced DA release would cause less activation of the postsynaptic DA receptors, and would eventually result in reduced locomotor activity. In contrast, the administration of high doses of MPH are thought to significantly increase the DA output of the terminal to increase extracellular DA, triggering generalised activation of the nervous system by overcoming the presynaptic inhibition of the autoreceptors (Seeman & Madras,

1998, 2002). These findings support the therapeutic action of MPH to reduce hyperactivity at low and not high doses of MPH treatment.

Volkow and colleagues (2005) suggest two mechanisms to explain the therapeutic relevance of subcortical DA transporter blockade by MPH. Firstly, it has been postulated that the MPH-induced increase of the striatal dopamine signal could improve attention and concentration in individuals with ADHD. The release of DA decreases background neuronal firing, weakens inappropriate neural connections and reduces 'noise' in the neural circuit. Therefore, the signal-to-noise ratio of neuronal communication is increased by enhanced levels of extracellular DA (Kiyatkin & Rebec, 1996). Secondly, DA signals the saliency of stimuli and motivates goal-directed behaviour (Berridge & Robinson, 1998). Based on research demonstrating that oral MPH only increased extracellular dopamine in the striatum in the presence of salient stimuli (Volkow, Wang, Fowler, Logan, Jayne et al., 2002; Volkow et al., 2004), Volkow and colleagues (2005) argue that the enhanced dopamine signal could motivate the individual to engage in a specific task, improving attention and performance through an increased perception of the saliency of the stimulus.

Although they are generally considered mutually exclusive, Volkow and colleagues' (2005), and Seeman and Madras's (1998, 2002) accounts of the neurochemical mechanism underlying the MPH effect on striatal DA may be complimentary (Engert & Pruessner, 2008). Volkow and colleagues (2005) suggest that MPH increases extracellular DA in the presence of salient stimuli. While it appears that Seeman and Madras (1998, 2002) argue that MPH administration reduces phasic DA release, this decrease is *relatively lower* than would occur in the absence of MPH due to the elevated resting level of extracellular DA. Given the four possible states of extracellular DA levels 1) at rest; 2) during a neuronal impulse; 3) following MPH stimulation; and 4) during a neuronal impulse following MPH stimulation, the highest

total amount of extracellular DA should occur when a neuronal impulse triggers DA release following MPH administration. Therefore, Volkow and colleagues' (2002; 2004) findings showing that MPH increased extracellular DA only in the presence of a salient stimuli, would likely be predicted by Seeman and Madras' hypothesis.

While the above theories focus on the significance of the striatal DA system, findings reporting increased NA and DA efflux within the PFC following therapeutic doses of MPH (Berridge et al., 2006) highlight the importance of the catecholamines within this region. The available evidence suggests that low therapeutic doses of MPH facilitate the signal processing ability of PFC neurons, while preserving the phasic firing by selectively increasing catecholamines in this region only. The enhanced signalling may bias neural responses to more salient stimuli. In contrast, high doses of MPH suppress PFC neuron signalling resulting in impairment of PFC function. It is postulated that this differential effect of drug administration on PFC neurons is due to an inverted-U dose response effect of MPH on catecholamines in this regions (Berridge & Devilbiss, 2010). The significance of catecholamines in PFC function and their role in ADHD will be discussed in more detail in sections 1.4.2 and 1.4.3, respectively. As the long-term effect of MPH on PFC function has not been fully elucidated, a broad goal of this thesis was to expand and extend this understanding by investigating the role of catecholamines in PFC mediated behaviour.

1.2.3 Acute effects of MPH on animal behaviour and cognition

The locomotor activating properties of acute psychostimulant administration have been well documented. Similar to other psychostimulants, MPH increases locomotor activity in a dose dependent manner (Yang et al., 2006, 2010). In general, the administration of MPH doses below 0.6 mg/kg do not change baseline locomotor activity in rats, while doses above 2.5 mg/kg stimulate locomotor activity, and as doses increase above 10 mg/kg stereotypic

behaviours (e.g. repetitive movements such as head weaving) increase (Berridge et al., 2006; Gaytan et al., 2000; Gerasimov et al., 2000; Heyser, Pelletier, & Ferris, 2004; Kuczenski & Segal, 2002; Yang et al., 2006, 2010). However, age is an important consideration in the acute effect of MPH on locomotor activity. Heyser and colleagues (2004) demonstrated that an acute 5 mg/kg dose of MPH administered to weanling rats significantly increased locomotor activity, while the identical dose had no effect when administered to periadolescent rats. Together this research suggests that the MPH dose and the age at the time of administration are critical factors when considering the behavioural outcome of treatment.

Similar to the acute effect of MPH on locomotor activity, acute MPH administration has a dose-dependent effect on cognitive functions. The administration of low doses of MPH have been shown to improve performance on tasks measuring sustained attention and working memory (Arnsten & Dudley, 2005; Berridge et al., 2006) and high MPH dosing disrupted memory formation and increased perseverative errors on a delayed alternation 'T' maze task (Arnsten & Dudley, 2005; Chuhan & Taukulis, 2006). These studies demonstrate that at low, clinically relevant doses, acute administration of MPH improved PFC function, while high doses impaired performance on PFC related tasks (Arnsten & Dudley, 2005; Berridge et al., 2006; Chuhan & Taukulis, 2006).

1.2.4 Long-term effects of chronic MPH treatment/use in animals

1.2.4.1 Long-term effects of chronic MPH use on behaviour

Previous preclinical research assessing the enduring effects on behaviour of chronic MPH treatment has produced inconsistent findings. The majority of this research has investigated the effect of chronic MPH administration on the rewarding effects of the psychostimulant cocaine and suggests that pre-exposure to MPH during adolescence results in reduced rewarding effects, and in some instances aversion, to cocaine in adulthood (Andersen et al.,

2002; Augustyniak, Kourrich, Rezazadeh, Stewart, & Arvanitogiannis, 2006; Carlezon, Mague, & Andersen, 2003; Mague, Andersen, & Carlezon, 2005). However there are also limited reports of enhanced sensitivity to cocaine in adulthood following MPH administration during adolescence (Brandon, Marinelli, Baker, & White, 2001). Additionally, increases in long-term depressive and anxiety-like behaviours have been reported in rats chronically treated with MPH throughout adolescence (Bolanos, Barrot, Berton, Wallace-Black, & Nestler, 2003; Bolanos et al., 2008; Carlezon et al., 2003). In contrast, Gray et al. (2007) found that chronic MPH treatment during development in rats resulted in long-term decreases in anxiety-like behaviours.

The inconsistent findings reported above are likely due to methodological differences of the studies. These studies vary in the dose and route of administration of MPH. There is also a difference in the stage of the circadian cycle at which MPH was administered (these points will be addressed in more detail in Chapter 3). Additionally, an important consideration must be given to the age of first exposure to MPH and the duration of chronic MPH treatment, as they vary greatly from study to study. As demonstrated by Anderson and colleagues (2002), rats chronically treated with MPH from postnatal day (PND) 50 to 65 did not show the same aversion to cocaine 25 days after treatment, as was observed in rats receiving identical treatment from PND 20 to 35.

Recently, Griggs and colleagues (2010) attempted to address the methodological limitations of previous research mentioned above. However, their study did not accurately address concerns about the stage in the circadian cycle in which MPH was administered as treatment persisted for 24 hours per day. As this reduced the therapeutic relevance of their results, a goal of this thesis was administer MPH using a method of drug administration that more closely models the dosing regime employed in children with ADHD.

1.2.4.2 Long-term effects of chronic MPH on cognition

The majority of research that has been conducted assessing the long-term effects of chronic adolescent MPH treatment on cognition has focused on memory performance. LeBlanc-Duchin and Taukulis (2007) reported that rats chronically treated with 3 and 5 mg/kg oral MPH for 21 days during adolescence (treatment commenced on post natal day (PND) 35 – 39) exhibited an impairment in recognition memory which persisted for 42 days post treatment. They found similar long-term deficits of recognition and spatial memory when chronic MPH treatment was administered to adult rats (LeBlanc-Duchin & Taukulis, 2009). Transient memory deficits have also been reported following seven weeks of treatment with 5 mg/kg of oral MPH which commenced in adolescence (PND 27; Bethancourt, Camarena, & Britton, 2009). Additionally, impaired performance on a spatial memory task, as assessed using the Morris water maze, has been reported following chronic MPH treatment during adolescence (PND 15 - 45; Scherer et al., 2010). Object recognition memory deficits have also been reported following a much shorter exposures to MPH during early adolescence either between PND 15 – 21 or PND 28 – 34 (Heyser et al., 2004). However these researchers delivered a high (5 mg/kg), not therapeutically relevant, intraperitoneal injection of MPH twice a day.

A single study has been conducted assessing impulsivity following chronic MPH treatment. Adriani and colleagues (2007) reported reduced impulsive behaviour in adults rats that were treated with MPH during adolescence. However as discussed above (section 1.2.4.1 and detailed in Chapter 3) the dose and route of administration are vital to the pharmacokinetics of MPH (Gerasimov et al., 2000), as is the stage of the circadian cycle at which MPH is administered (Gaytan et al., 2000). The dosing regime employed by Adriani and colleagues (2007) reduces the relevance of their preclinical results to the human situation. As such, a goal of this thesis was to assess the long-term effects of therapeutically relevant

administration of chronic MPH on PFC functions, including impulsivity.

1.3 Central Nervous System development

The development of the central nervous system (CNS) in mammals continues after birth up until adulthood with periods of increased vulnerability to insult during this timeframe (Andersen, 2003; Bayer, Altman, Russo, & Zhang, 1993). From birth the brain undergoes periods of growth, from increase in cell number and size, and differentiation including increased connectivity of neurons (Barone, Das, Lassiter, & White, 2000). Interspecies comparisons between humans and rats revealed the progression of brain development are relativity parallel, however the time span is significantly shorter in rats, occurring over days compared to years in humans (Figure 2; Bayer et al., 1993).

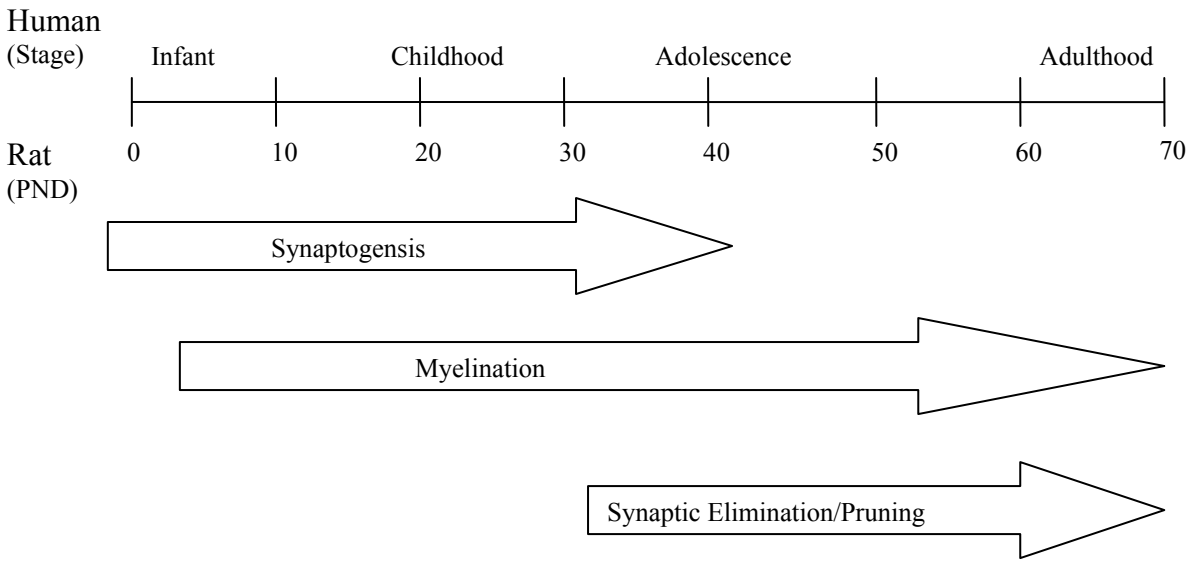

Figure 2. A comparison of stages of development for humans versus rats at days since birth (post natal day; PND). Redrawn and modified from (Andersen, 2003).

The maturation of the cerebral cortex occurs at different ages depending upon the region. The more posterior areas of the cortex reach maturity early in life while the development of the frontal cortices continues throughout childhood and adolescence (Kelley, Schochet, &

Landry, 2004; Laviola, Macri, Morley-Fletcher, & Adriani, 2003; Sowell, Thompson, Holmes, Jerigan, & Toga, 1999). Increases in white matter volume reflect increases in myelination during this period (Giedd et al., 1999; Paus et al., 1999). Grey matter volume initially increases during early to mid adolescence before decreasing during late adolescence, reflecting activity-dependent synaptic pruning (Bourgeois, Goldman-Rakic, & Rakic, 1994; Giedd et al., 1999; Huttenlocher, 1979; Huttenlocher & Dabholkar, 1997). PFC maturation has been reported to continue beyond adolescence and into the third decade of life (Sowell et al., 2001).

There are periods of sensitivity during development in which environmental influences may permanently impact future development (Andersen, 2003; Barone et al., 2000). An insult disrupting synaptogenesis in one region may allow neurons from neighbouring regions to encroach on that space, permanently altering development from that point forward. Artificial activation of a neuron by exogenous drug administration during adolescence may impact the elimination and pruning of superfluous synapses (Heijtz et al., 2003; Robinson & Kolb, 1999). The regions that are most vulnerable to such insults (PFC, amygdala, anterior cingulate and insular cortex), form the social brain which develop later in life (Andersen, 2003; Blakemore, 2008; Joseph, 1999). Therefore, early experiences can profoundly change the development of social and affective behaviour. Similar neuronal insults occurring outside of these periods of vulnerability do not have the same impact on brain function. Investigating the impact of chronic MPH treatment during these vulnerable periods in maturation was a major goal of this thesis. The focus of the long-term effect of MPH treatment was on the cognitive and neural development of the non-ADHD (misdiagnosed) rats.

1.4 Neurobiology of PFC function

1.4.1 Dopamine and noradrenaline

The brain employs DA and NA as neurotransmitters and collectively they are referred to as catecholamines. Catecholamines can be synthesised from dietary phenylalanine and tyrosine or the breakdown of brain proteins (Figure 3; Wurtman & Fernstrom, 1975). Catecholamines are involved in many cortical brain functions including attention (Tripp & Wickens, 2009), working memory and cognition (Arnsten, 1997, 2007; Arnsten, Mathew, Ubriani, Taylor, & Li, 1999), and emotion (Tully & Bolshakov, 2010).

The catecholamines are stored in synaptic vesicles in the neuron terminal (Potter, 1967) and following an action potential, an influx of calcium causes the vesicles to dock with the presynaptic membrane and release the neurotransmitter into the synaptic cleft (exocytosis; McClure & Robinson, 1996). The neurotransmitter binds to specific receptors (discussed in detail in section 1.4.1.1) and passes along the neural signal. The neurotransmitter then unbinds and can be metabolised within the synaptic cleft or can be taken up into the presynaptic terminal via specific DA and NA transporters (endocytosis). Within the terminal, the neurotransmitter is either metabolised or repackaged into synaptic vesicles for future use (Potter, 1967). As discussed previously (section 1.2.1), the action of MPH is to block the DA and NA transporters, therefore inhibiting the removal of these neurotransmitters from the synaptic cleft (Hannestad et al., 2010; Volkow, Fowler et al., 2002; Volkow, Wang, Fowler, Logan, Franceschi et al., 2002).

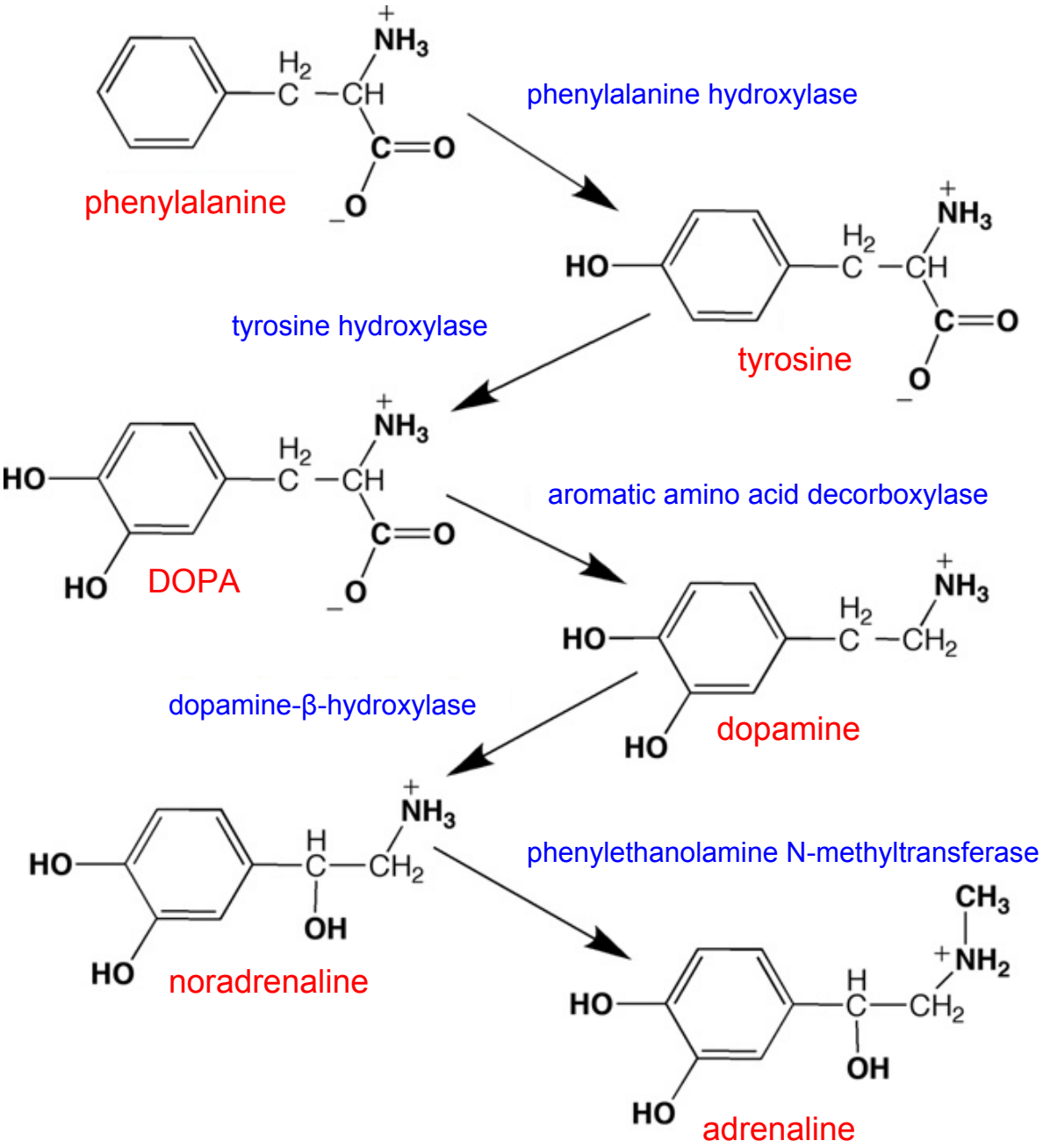

Figure 3. The biosynthesis of the catecholamine neurotransmitters.

Dopamine and NA neurons originate from specific nuclei within the brainstem. Three major dopaminergic pathways have been identified; 1) the nigrostriatal DA pathway; 2) the mesolimbic DA pathway; and 3) the mesocortical DA pathway. Briefly, the nigrostriatal DA pathway projects from the substantia nigra (A9 region) to the basal ganglia and is involved in motor control (Groenewegen, 2003). The mesolimbic DA pathway projects from the ventral tegmental area (VTA; A10 region) to subcortical limbic regions (NAc, olfactory tubercle and amygdala) and is involved in motivated behaviour and reinforcement learning (Hyman,

Malenka, & Nestler, 2006). The mesocortical DA pathway also originates in the VTA and projects to the PFC (Fuxe et al., 1974) and is of interest to this thesis as it is greatly involved in cognitive functioning (Arnsten & Li, 2005). The most extensively studied of the ascending noradrenergic nuclei project from the locus coeruleus (LC), or A6 region (Dahlstrom & Fuxe, 1964). The LC exclusively provides NA to subcortical and cortical structures and is involved in arousal and cognitive functioning (Berridge, 2008; Berridge & Waterhouse, 2003).

The arrival of the NA and DA afferents in the PFC occur at approximately the same time during development, however they reach their mature, adult patterns at different stages in development (Berger-Sweeney & Hohmann, 1997). In rats, the first DA and NA fibres can be seen entering the cortex on embryonic day 16 and 17, respectively (Coyle & Molliver, 1977; Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988; Levitt & Moore, 1979). The NA afferents achieve their adult pattern of connectivity by PND 7 (Levitt & Moore, 1979). By contrast, there is a continual increase in the density of the dopaminergic innervation to the PFC until early adulthood, with the adult patterns of DA connectivity not achieved until two months after birth (Kalsbeek et al., 1988). This protracted period of development of the mesocortical DA system suggests that it is vulnerable to external influences until adulthood and therefore has a greater capacity for synaptic plasticity (Gan, Kwon, Feng, Sanes, & Lichtman, 2003). There is the suggestion that the early maturation of the NA fibres is involved in cortical differentiation (Coyle & Molliver, 1977), while the later maturation of the DA fibres have a greater influence on cortical plasticity via cortico-cortico connections (Berger-Sweeney & Hohmann, 1997; Gaspar, Bloch, & Le Moine, 1995).

1.4.1.1 DA and NA receptors

Dopamine has two families of metabotropic receptors (Kebabian & Calne, 1979). The D1-like receptor subtype consists of D1 and D5 receptors and there is currently no pharmacological

agent available to distinguish these. D1-like receptors are located postsynaptically and stimulate cyclic adenosine monophosphate production (cAMP), causing excitation of ion channels (Stoof & Kebabian, 1981). The D1-like receptors are most abundant in the PFC (Goldman-Rakic, Lidow, & Gallager, 1990; Lidow, Goldman-Rakic, Gallager, & Rakic, 1991). The D2-like family consists of D2, D3 and D4 receptors, however the D4 receptor could be considered a catecholamine receptor as it also has a high affinity for NA (Van Tol et al., 1991). D2-like receptors are located both pre and postsynaptically and generally inhibit cAMP causing inhibition of ion channels (Stoof & Kebabian, 1981), although there are some exceptions (see Jackson & Westlind-Danielsson, 1994).

Three families of receptors have been identified for NA; α_1 receptor, α_2 receptor and β receptor. NA has the highest affinity for the α_2 receptor which has the subtypes α_{2A}, α_{2B}, and α_{2C}. All subtypes are found postsynaptically, while α_{2A} and to a lesser extent α_{2C} are found presynaptically (MacDonald, Kobilka, & Scheinin, 1997). The α_{2A} receptor is concentrated in the PFC (Aoki, Go, Venkatesan, & Kurose, 1994). α_2 receptors are generally coupled to G_i proteins which inhibit intracellular adenylyl cyclase/cAMP production pathways (Ramos, Stark, Verduzco, van Dyck, & Arnsten, 2006).

NA has a lower affinity for α_1 receptors which has 3 subtypes: the α_{1A}, the α_{1B}, and the α_{1D} (Hieble et al., 1995). The most prominent subtype in the rodent PFC are the α_{1A} and α_{1D} (Day, Campeau, Watson, & Akil, 1997). α_1 receptors are generally coupled to the phosphotidyl inositol/protein kinase C intracellular pathway via G_q proteins (Birnbaum et al., 2004).

Finally, NA has the lowest affinity for β receptors which has 3 subtypes, β_1, β_2 and β_3 all of which are found in the central nervous system (Insel, 1993). Compared to the other subtypes, β_1 receptors have the highest concentration in the adult rat cortex. β receptors are coupled via

G_s to adenylyl cyclase, increasing cAMP signalling (Ordway, O'Donnell, & Frazer, 1987).

1.4.2 Catecholamines in Prefrontal Cortex function

The PFC is responsible for higher order cognitive abilities, sometimes referred to as executive functions. Executive functions include inhibition and impulsivity, planning and organisation, working memory, and mental flexibility (Carlson, 2005; Diamond, 1991). There are age-related improvements in executive functioning during childhood and adolescence such that inhibition and working memory occur earlier in development, while more complex processes such as planning and organisation develop later (Diamond, 1991; Isquith, Gioia, & Espy, 2004; Zelazo & Muller, 2002). As children develop they are able to complete a greater variety of executive tasks (Becker, Isaac, & Hynd, 1987; Carlson, 2005), which correlate with the development of the different areas of the PFC (Mobini et al., 2002).

An inverted-U has been used to describe the change in PFC function as catecholamine levels fluctuate (Arnsten, 1997, 2007; Arnsten & Li, 2005). Low levels of catecholamines in the PFC, as seen during times of fatigue, impair PFC function, as does too much DA and NA as seen during times of stress (Deutch, Clark, & Roth, 1990; Foote, Aston-Jones, & Bloom, 1980). Optimal PFC performance occurs when there are moderate catecholamine levels.

The involvement of specific catecholamine receptor types for neuronal signalling is important in PFC function. Under optimal conditions in the PFC, moderate levels of NA activate the α_{2A} receptors and moderate levels of DA activate the D1 receptor (Arnsten, 1997). NA stimulation of the α_{2A} receptors enhances PFC function by strengthening appropriate networks, increasing the 'signal'. In contrast, D1 receptor stimulation enhances PFC function by weakening inappropriate connections, decreasing 'noise'. Excessive NA levels in the PFC have been shown to engage lower-affinity α_1 receptors, which suppresses PFC activation

(Birnbaum et al., 2004). High levels of DA in the PFC result in excessive D1 receptor stimulation which suppresses PFC activation by weakening not only inappropriate connections, but also connections that are required to carry out PFC function (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007).

It has been suggested by the work of Arnsten and her colleagues (1997, 2007; 2005) that the behavioural result of NA and DA activation in the PFC is the opposite of that for subcortical regions. For example, a DA agonist in the striatum stimulates locomotor activity, while a DA agonist in the PFC inhibits locomotor stimulation produced by administration of the striatal DA agonist (Heijtz, Kolb, & Forssberg, 2007). It has been proposed that the opposing role of the PFC to the striatum is based on arousal levels. Optimal levels of NA and DA in the PFC occur when we are alert, and therefore the PFC is 'turned on' and capable of controlling our behaviour. However, in times of fatigue and stress when we have either too little or too much NA and DA, PFC function is impaired and we rely on more primitive behavioural control, e.g. behaviours are driven by fear and/or anxiety which are regulated by subcortical regions. When we are aroused and alert, the PFC controls our behaviour and when we are resting the PFC is 'switched off' and we are under subcortical control (Ramos & Arnsten, 2007).

1.4.3 Catecholamines, the prefrontal cortex and ADHD

PFC dysfunction is significantly related to ADHD symptoms. Lesions in the PFC have been found to produce ADHD-like symptoms of distractibility and impulsivity (Eagle et al., 2008; Rossi, Bichot, Desimone, & Ungerleider, 2007). Neuroimaging studies have revealed volumetric reductions in the PFC, cerebellum and the striatum of ADHD patients (Castellanos et al., 1996; Filipek et al., 1997) and reduced prefrontal cortex activity in unmedicated ADHD children (Rubia et al., 1999). Furthermore, ADHD has been linked to suboptimal levels of catecholamines in the PFC (Pliszka, 2005; Wilens, 2008). In rats, subdivisions of the PFC

have been associated with different cognitive functions. Performance on impulsive decision making tasks has been linked to the orbitiofrontal cortex (OFC; Kheramin et al., 2004; Mobini et al., 2002), while the medial PFC regulates performance on maze tasks which measure working memory (Poucet, 1990; Taylor, Latimer, & Winn, 2003). Given the close association between the PFC and ADHD, keeping in mind that low therapeutic doses of MPH elevate DA and NA within the PFC (Berridge et al., 2006), this thesis used behavioural and neurochemical techniques to investigate the role of catecholamine receptors in a PFC mediated task.

1.5 Measures of change in behaviour of rat models

1.5.1 Locomotor activity

Locomotor activity can be defined and measured in a number of ways. It can refer to the distance a rat travels in the horizontal plane, the number of times a rat takes its forepaws off the floor (rearing), or even the amount of time a rat spends exploring an open field. Locomotor activity can also be used for an index of other behaviours such as novelty and anxiety. For the purposes of the current thesis locomotor activity will be used as a relatively simple measurement of the rats' general activity level and in determining physical responses to a drug (Kuczenski & Segal, 2001b) and strain differences (Commissaris et al., 2000). Alterations in locomotor activity following drug administration are generally accepted as an indication of the drugs effect on the dopaminergic system (Swanson, Heath, Stratford, & Kelley, 1997).

It is important that alterations in locomotor activity are accounted for in other behavioural tasks, as the variables being measured in these tasks require motor output. That is, if a hypoactive rat performs poorly on a maze task compared to a rat with normal activity, the poor maze performance is more likely due to the inactivity of the rat not completing the task

rather than a deficit of memory. This thesis employed locomotor activity measurements to investigate rat strain differences in addition to behavioural response to drug administration.

1.5.2 Impulsivity

Impulsivity can be broadly defined as action without considering consequences. Impulsivity is not thought to be a unitary construct (Evenden, 1999) as impulsive behaviours include decreased inhibitory control, intolerance of a delay to rewards and quick decision making with little consideration. All of these components of impulsivity can be observed in the behaviour of a child with ADHD-HI or ADHD-C (Winstanley, Eagle, & Robbins, 2006).

In the laboratory there are various behavioural paradigms for measuring different aspects of impulsive behaviour which can be divided into two categories: tasks measuring impulsive action or motoric impulsivity and tasks measuring impulsive choice or impulsive decision making (Winstanley et al., 2006). An impulsive action is the inability to withhold or inhibit a response. Impulsive choice can be conceptualised as the preference for a small reward delivered immediately over a large reward delivered after a delay. Two tasks that are commonly used to measure impulsive actions in both rats and humans are the go/no-go and the stop-signal reaction time tasks. Both of these tasks require the inhibition of a pre-potent response (Winstanley et al., 2006). Another task that can be employed to assess impulsive actions in rats is the five-choice serial reaction time task (5CSRT; Carli, Robbins, Evenden, & Everitt, 1983). While the 5CSRT task was based on the Continuous Performance Test (Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956) which is used to assess attentional function in humans, it can be utilised as an index of impulsive action as it also requires the inhibition of a pre-potent response. To assess impulsive choice, researchers employ delay-of-gratification, or delay-discounting paradigms which subjectively devalue the reward by increasing the delay experienced prior to reward delivery. Many delay-discounting paradigms

have been developed that require rats to choose between a small immediate or large delayed reward (Evenden & Ryan, 1996; Mobini et al., 2002), however similar operant tasks have been difficult to develop for human participants. Whilst questionnaires are usually employed to assess impulsive choice in humans, one operant delayed-discounting task has been published (Pietras, Cherek, Lane, Teheremissine, & Steinberg, 2003).

A benefit of measuring impulsive choice over impulsive action is that impulsive choice more closely reflects decision making processes, rather than motoric inhibition (Winstanley et al., 2006). Measuring impulsive actions assesses motor inhibition which would likely be adversely affected by increased activity levels, while tasks of impulsive choice require the rat to choose between levers and therefore are less likely to be affected by altered activity levels. Therefore, assessment of impulsive choice allows for a measure for impulsivity which is independent of hyperactivity, another core symptom of ADHD-HI and ADHD-C. As such, a delay-discounting paradigm was employed in this thesis to assess impulsive choice between strains and following drug treatment.

1.5.3 Working memory

Working memory is the ability to hold and manipulate information in mind in the absence of external cues. In humans, working memory is mediated by the PFC. In rodents, maze tasks are commonly used to analyse working memory as a measure of PFC function (Neave, Lloyd, Sahgal, & Aggleton, 1994; Poucet, 1990). Using an eight arm radial maze (RAM) to conduct a delayed non-match to sample task measures both spatial and working memory in rats. In such a task, the rats are forced to enter four baited arms during acquisition. Following a delay, the rats are required to select the remaining four baited arms during testing. This task requires the rats to continuously update and recall which arms have been entered during both acquisition and testing. Re-entering an arm from the acquisition phase is considered a spatial

memory deficit, whilst re-entering an arm in the testing phase is considered to be a working memory deficit. Performance on such maze tasks has been shown to be mediated by the medial PFC in the rat (Poucet, 1990; Taylor et al., 2003). A delayed non-matched to sample RAM task was employed in this thesis to assess spatial and working memory deficits following drug treatment. Assessing working memory in the current thesis was included given that PFC dysfunction is associated with ADHD and the RAM is a proven method of measuring PFC function in rats.

1.6 Thesis aims and hypotheses

The overall aim of this thesis was to determine what effect, if any, chronically treating misdiagnosed adolescent rats with Ritalin® has on their brain development. We know that Ritalin® exerts its effect through catecholamine systems and that these neurotransmitters are important regulators of neural development. As neural development continues into adulthood, chronic Ritalin® treatment could potentially cause functionally significant changes in neural circuitry if administered to those not requiring treatment. It was hypothesised that chronic MPH treatment to misdiagnosed adolescent rats would have enduring changes on cognitive and neural development. Furthermore, it was hypothesised that these changes resulted from alterations to the catecholamine system.

The experiments conducted for this thesis are presented as separate journal articles.

- Chapter 2 addressed the previous methodological issues surrounding the validation of the SHR as an animal model of ADHD.
- The aim of Chapter 3 was to determine the effectiveness of a novel oral drug administration method.
- The fourth and fifth chapters then used this novel method of oral drug administration to chronically administer Ritalin® to adolescent WKYs and SHRs. Rats were

administered oral Ritalin®, twice a day for four weeks (PND 27 to 52) to closely mirror clinical dosing schedules. These papers measured the long-term effects resulting from this chronic treatment on cognitive and neural development of the PFC.

- Chapter 6 of this thesis addressed the role the DA and NA receptors play in the impulsive behaviour mediated by the medial PFC and orbitofrontal cortex.

The final chapter discussed the major findings of this thesis and the clinical implications of this research, not only how it may impact ADHD diagnosis, but also the implications for other disorders that are characterised by impulsivity.

Co-Author Contribution

Cornish, J. L.	
Provided technical assistance with behavioural testing, contributed to research design and manuscript editing	4%
Homewood, J.	
Contributed to research design and manuscript editing	3%
Taylor, A.	
Contributed to data analysis	1%
Total	**8%**

Due to copyright laws, the following articles have been omitted from this thesis. Please refer to the following citations for details.

Pardey, M.C., Homewood, J., Taylor, A., & Cornish, J. L. (2009). Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods,* 76, 166-171. DOI: http://dx.doi.org/10.1016/j.jneumeth.2008.09.009

2.6 Addendum

Since the publication of this paper sub-strains within the WKY strain have been reported (Sagvolden et al., 2009). The origin of the WKY sub-strain employed in this study was the Charles River Laboratories, USA. This sub-strain, known as WKY/NCrl, has been suggested as an animal model for ADHD-Predominantly Inattentive subtype (Sagvolden, DasBanerjee, Zhang-James, Middleton, & Faraone, 2008). However, the results of Chapter 2 can not address this claim as measures of attention were not employed. In the locomotor activity, delayed reinforcement and extinction tasks in this laboratory, the SHR demonstrated hyperactivity, impulsivity and increased sensitivity to reinforcer delay, compared to the WKY/NCrl. Therefore, this WKY sub-strain was considered an appropriate non-ADHD control for Chapters 3, 4 and 5.

It is important to note that the single PIR sensor used to measure locomotor activity does not allow for the evaluation of horizontal and vertical exploration. Future research would benefit from assessing repetitive stereotypies and grooming to clarify strain differences. Furthermore, the results of this study are unable to determine whether the SHR has an altered sensitivity to the increasing delay or the value of the reward.

Research continues to support the SHR as an appropriate animal model of ADHD. In comparisons to the WKY, additional evidence for the SHRs preference for an immediate, over a delayed reinforcer has recently been reported (Sutherland et al., 2009). Furthermore, the SHR has demonstrated similar variation in their performance on reaction time tasks, known as intra-individual variation, as do children with ADHD (Perry, Sagvolden, & Faraone, 2010a, 2010b). In conclusion, the SHR continues to be the most widely used and

extensively validated animal model of ADHD and based on the tasks employed in the current study, the WKY is an appropriate control strain.

Text Amendment

The last sentence of 2.2.1 should read: Cameras in each chamber allowed observation of the rats to monitor their welfare during the session.

All figure caption: Error bars represent SEM.

2.7 Addendum References

Perry, G. M. L., Sagvolden, T., & Faraone, S. V. (2010a). Intra-individual variability in genetic and environmental models of attention-deficit/hyperactivity disorder. *American Journal of Medical Genetics Part B, 153B*, 1094-1101.

Perry, G. M. L., Sagvolden, T., & Faraone, S. V. (2010b). Intraindividual variability (IIV) in an animal model of ADHD - the Spontaneously Hypertensive Rat. *Behavioral and Brain Functions, 6*, 56-60.

Sagvolden, T., DasBanerjee, T., Zhang-James, Y., Middleton, F. A., & Faraone, S. V. (2008). Behavioral and genetic evidence for a novel animal model of attention-deficit/hyperactivity disorder predominantly inattentive subtype. *Behavioral and Brain Functions, 4*, 56.

Sagvolden, T., Johansen, E. B., Woien, G., Walaas, S. I., Storm-Mathisen, J., Bergersen, L. H., et al. (2009). The spontaneously hypertensive rat model of ADHD - The importance of selecting the appropriate reference strain. *Neuropharmacology, 57*, 619-626.

Sutherland, K. R., Alsop, B., McNaughton, N., Hyland, B. I., Tripp, G., & Wickens, J. R. (2009). Sensitivity to delay of reinforcement in two animal models of attention deficit hyperactivity disorder (ADHD). *Behavioural Brain Research, 205*, 372-376.

Co-Author Contribution

Cornish, J. L.	
Contributed to research design and manuscript editing	3%
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Contributed to research design and manuscript editing	3%
Clemens, K.	
Contributed to manuscript editing	1%
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Chapter 3

A novel non-invasive oral method of administration of Methylphenidate in rats

3.1 Introduction

Methylphenidate (MPH; Ritalin®) is a psychostimulant commonly used to treat children with Attention Deficit/Hyperactivity Disorder (ADHD; Engert & Pruessner, 2008). While it has been shown to be clinically effective in treating the symptoms of hyperactivity and impulsivity in this population (Greenhill et al., 2002), the findings of previous preclinical research assessing the attenuation of ADHD symptoms with MPH treatment have been inconsistent.

A vast quantity of animal research has aimed to elucidate the behavioural and therapeutic effects of MPH treatment. However the clinical relevance of these findings is limited by the methodological issues surrounding the administration of MPH. Clinically, MPH is administered orally to children at low doses in the morning to facilitate therapeutic drug effects during their most active period (Swanson & Volkow, 2002). The most common issues arising from rodent studies are that MPH is administered: i) at varying and often high doses, ii) during the light phase, which is the inactive period for rats and iii) by a non-oral route (commonly by parenteral injection; Dafny & Yang, 2006; Kuczenski & Segal, 2002).

The first limitation of past preclinical research that needs to be addressed is the use of identical MPH doses in rats that are employed in the clinical treatment of children. In comparison to therapeutic doses, the majority of previous animal research has used identical doses adjusted for body weight (mg/kg) or higher of MPH. While the mg/kg doses used in early preclinical studies roughly approximates mg/kg dosing to children, the peak plasma

concentrations achieved in rodents are significantly above the clinical therapeutic range of 8 to 40 ng/mL (Kuczenski & Segal, 2002; Swanson & Volkow, 2001). This disparity of peak plasma concentration is most likely due to altered pharmacokinetics of equivalent mg/kg doses caused by species differences in gastric absorption, volume of drug distribution, drug metabolism and excretion rates (Patrick, Ellington, & Breese, 1984; Wargin et al., 1983). These findings indicate that due to species differences in the pharmacokinetics of MPH, using equivalent human mg/kg doses in animal research will not produce therapeutically relevant findings.

The time of day of MPH administration is another limitation of previous preclinical research. Children are treated with MPH in the morning, which is considered their active phase. There is evidence to suggest that MPH's stimulant behavioural effects in rodents depend upon the time of the day it was administered (Gaytan, Yang, Swann, & Dafny, 2000), as measured by a progressive augmentation of locomotor activation with repeated MPH administration, known as behavioural sensitization (Robinson & Becker, 1986). Gaytan and colleagues (2000) reported differences in the expression of locomotor behavioural sensitization in rats when MPH was administered across the circadian cycle and reported that rats only developed behavioural sensitization to MPH when it was administered during their inactive (light) phase. When MPH was administered during the active (dark) phase, rats failed to sensitize, a finding that was replicated by Kuczenski and Segal (2002). These findings are consistent with reports that children with ADHD do not develop behavioural sensitization when chronically treated with MPH (Dafny & Yang, 2006), as their treatment is administered during their active phase.

The route of administration also greatly impacts on the pharmacokinetics of MPH (Gerasimov et al., 2000; Kuczenski & Segal, 2002). Intraperitoneal (i.p.) injections deliver the drug more rapidly and attain a higher peak plasma concentration faster than oral administration

(Gerasimov et al., 2000; Patrick et al., 1984). Together with the above findings, this suggests that previous studies have commonly administered large doses of MPH at inappropriate points in the diurnal cycle, using a method of drug administration that delivers the drug much faster than is clinically relevant.

There are preclinical studies in rodents that have assessed the pharmacokinetic profile of varying oral doses of MPH. Aoyama and colleagues (1990) systematically assessed the peak plasma concentrations of four oral MPH doses in rats. Their results indicated a nonlinear relationship with peak plasma concentrations at 15 minutes of 2.1, 36 and 62 ng/mL following doses of 0.5, 2.0 and 3.5 mg/kg, respectively. Kuczenski and Segal (2002) reported a peak plasma concentration of 2.3 ng/mL following oral administration of 1.0 mg/kg of MPH. Based on this, Kuczenski and Segal (2002) estimated that oral doses of 0.75 mg/kg to 3.0 mg/kg in rats would yield peak plasma levels within the therapeutic range. Consistent with this estimate, more recent research has reported plasma levels within the therapeutic range 15 minutes after the oral administration of 1 mg/kg (Wheeler, Eppolito, Smith, Huff, & Smith, 2007) and 2 mg/kg of MPH (Berridge et al., 2006; Wheeler et al., 2007).

Methods of oral MPH administration have included wet mash (Chuhan & Taukulis, 2006; LeBlanc-Duchin & Taukulis, 2004), spiked drinking water (Thanos, Michaelides, Benveniste, Wang, & Volkow, 2007) and crackers soaked in MPH solution (Arnsten & Dudley, 2005). However, these methods require voluntary consumption by the animal and the speed at which the dose is consumed can not be controlled. Gavage is a commonly used involuntary technique for rapidly delivered, oral administration. Whilst the gavage technique can administer the drug in a way that mimics oral drug administration to children, it is not without risks. To gavage an animal, a tube through which the drug is delivered is inserted down the animals' oesophagus into their stomach. Extreme care must be taken to ensure the tube is not

mistakenly placed in the lungs. In the hands of an inexperienced experimenter, gavaging can be stressful for the animal, increasing blood pressure, heart rate and glucocorticoid levels for several hours (Balcombe, Barnard, & Sandusky, 2004) and has the potential to cause damage to the oesophagus or respiratory system (Murphy, Smith, Shaivitz, Rossberg, & Hurn, 2001).

A non-invasive involuntary procedure for oral administration of MPH was recently proposed by Wheeler and colleagues (2007). They suspended MPH in apple juice. Whilst holding the rat, they slowly discharged the suspension from a micropipette for the rat to drink. The benefit of using this procedure was that it is possible to administer a dose to neonates (post-natal day; PND 5) due to their instinctive sucking reflex. However, they advocate that a rat over PND 15 should be trained with apple juice for 5 days before the introduction of the drug.

One of the major limitations of the oral administration procedure proposed by Wheeler et al., (2007) and of the gavage is the level of training that is required for the animal prior to drug administration. The time to habituate and train in these procedures can take up to a week which causes methodological restrictions when the research requires the animals to be treated throughout adolescence. Adolescence in the rat is considered to commence at weaning on PND 21 and continue to PND 60 (Andersen, 2002; Laviola, Macri, Morley-Fletcher, & Adriani, 2003), providing a narrow time-frame to conduct experiments in this clinically relevant age group.

The first aim of the present study was to determine whether an alternate treatment procedure would allow for oral drug administration without extended training in rats. Drug treatment could then commence sooner, allowing for the assessment of treatment effects during the early adolescent period. In the proposed novel method of MPH administration, treatment was delivered via a drinking spout, which was facilitated by a period of water restriction.

It is well established that psychostimulants increase locomotor activity. Although the administration methods of MPH varied in previous preclinical studies, in general increased locomotor activity was found following high, clinically irrelevant acute doses of MPH, while no activation was evident when lower, therapeutically relevant doses were administered (Askenasy, Taber, Yang, & Dafny, 2007; Berridge et al., 2006; Gerasimov et al., 2000; Kuczenski & Segal, 2002). In the present study the effect of oral administration of MPH on locomotor activity was investigated as a measure of the pharmacological effectiveness of the proposed novel technique of drug administration.

Therefore the second aim of this study was to conduct a dose-response experiment of MPH administration on locomotor activity to confirm that the oral method of MPH administration produces behavioural change. Based on previous findings, it was anticipated that oral administration of higher doses (5 and 10 mg/kg) of MPH would result in increased locomotor activity, with no change in locomotor activation following oral administration of the lower dose (2 mg/kg), compared to vehicle treatment.

3.2 Materials and Method

3.2.1 Subjects

Five male Wistar-Kyoto (WKY) rats were obtained from the Animal Resources Centre (Canning Vale, WA, Australia). Upon arrival in the laboratory, the rats were housed together in an opaque, plastic cage (60 x 21.5 x 36 cm, length x height x width) containing sawdust, a block of wood and shredded paper. The cage was covered with a raised wire mesh roof (27cm total height). They were allowed free access to water and standard laboratory rat chow, except during the drug administration procedure. During drug administration the rats were placed on water restriction (one hour access to water daily at 1000 hours) and individually housed to facilitate drug administration in the home cage without handling the animal. As handling

stress at the time of oral MPH administration has been shown to significantly increase neural dopamine (DA) efflux above that observed by both oral MPH administration and handling alone (Marsteller et al., 2002), administering MPH without handling the animal was a key aspect of this novel procedure. Importantly, no change in neural DA levels were reported in rats deprived of water for 24 hours (Alper, Demarest, & Moore, 1980). Furthermore, water restriction for a period of 48 hours has been shown to have no impact upon the baseline plasma levels of DA and noradrenaline (NA; Kiss, Jezova, & Aguilera, 1994). The animal holding room was held at a constant temperature of 21˚C. Rats were housed on a reverse light/dark cycle (lights on at 2000 hours until 0800 hours) and experiments were conducted during the rats' active (dark) cycle. At the beginning of the procedure, the rats were approximately 38 days old, weighed 130 - 143 grams, had been handled daily for one week by the experimenter and were experimentally naïve. Throughout the procedure the rats were weighed daily to assess the effect of restriction on general health as measured by body weight (Hughes, Amyx, Howard, Nanry, & Pollard, 1994). The study was conducted with the approval of the Macquarie University Animal Ethics Committee (reference number ARA 2006/019) and followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004).

3.2.2 Drug administration procedure

Ritalin tablets (10 mg/tablet, Novartis, East Hanover, New Jersey) were suspended in distilled water (1 mg/mL) and administered through a drinking spout. The drinking spout was a metal tube inserted into a rubber stopper, which had a ball-bearing at the end to hold the water in the tube. The drinking spout was assessed prior to drug administration to ensure that once it was sealed (thumb placed over the hole), it did not drip unless the ball-bearing was moved. The procedure consisted of two phases. The first phase determined whether the rats would voluntarily consume the drug suspension, and the second phase determined whether there was

a pharmacological effect of the drug.

3.2.2.1 Voluntary drinking

After the first 23 hours of water restriction, the drug suspension was individually administered via the drinking spout at 1000 hours (see Figure 1). The drinking spout was placed in the cage and 1 mL of water was inserted. Only when the rat began drinking was the drug dose (2 mg/kg) inserted into the spout via a syringe (based on body weight (mL/kg), typically 0.15 - 0.3 mL). Once the drug was added to the spout, an additional 1 mL of water was inserted into the spout to ensure the entire dose had been ingested. Immediately following consumption of the liquid in the drinking spout, the rats were given one hour *ad libitum* access to water. As the rats easily consumed the drug suspension, a dose-response study was then conducted to determine that this method of administration produced behavioural activation.

Figure 1. A Wistar-Kyoto Rat (WKY) voluntarily consuming Ritalin® tablets suspended in distilled water through a drinking spout, following water restriction.

3.2.3 Pharmacological effects of the drug on locomotor activity

A dose-response experiment on locomotor activity was conducted using a within subject design. The procedure was conducted every second day to ensure there was no interference from previous MPH doses. In rats the half-life of MPH is approximately 1 hour (Aoyama et al., 1990; Patrick et al., 1984).

3.2.3.1 Measure of Locomotor Activity

A more detailed description of the apparatus and procedure used to measure locomotor activity is available in Pardey et al., (2009, Chapter 2). Briefly, the rats were placed in operant conditioning chambers (purpose built by the University of Sydney, Australia) with two passive infrared detectors (PIR, Quantum passive infrared motion sensor, Ness Security Products, Australia) located opposite each other, 30mm above the floor. Locomotor activity was measured by detection of small movements of the subjects' head and body. These movements were tracked via "Workbench Mac" software running on Macintosh Computers every 60 seconds for three hours (McGregor, 1996). Cameras in each chamber allowed observation of the rats to monitor their welfare during the session.

3.2.3.2 Assessment of MPH oral administration on locomotor activity

Prior to administration of the drug, baseline locomotor activity of each rat was measured in the operant chamber for 30 minutes. A latin square design was used to assign each of the four doses (0 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg) to each rat, separated by at least 48 hours. Following oral drug administration as described above in section 3.2.2.1, the rats were given 5 minutes *ad libitum* access to water and returned to the locomotor chambers to measure their activity over the following three hours. Upon completion of the session, the rats were returned to their home cage and given one hour *ad libitum* access to water.

On the days between treatments, the rats were restricted to one hour *ad libitum* access to water to facilitate the administration of the drug on the following day. Once all locomotor sessions had been conducted for each of the test doses, the rats were taken off water restriction and given *ad libitum* access to water.

3.2.4 Statistical Analyses

Analyses were conducted using Analysis of Variance (ANOVA). The General Linear Model was used with Greenhouse-Geisser Epsilon (G-G) adjustments for the univariate statistics reported as the assumption of sphericity was violated. A within subjects design was used with four levels of the factor, 'dose' (0 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg) and seven levels of the factor, 'time' (baseline plus half hourly intervals for three hours). Contrasts were planned to compare the 0 mg/kg dose to each of the 2 mg/kg, 5 mg/kg, and 10 mg/kg doses with bonferroni adjustments made for multiple comparisons of dose ($p = 0.017$).

3.3 Results

The rats quickly consumed the entire solution from the drinking spout in approximately 30 seconds. There was no evidence to suggest a neophobic response to drug delivery in the rats. A small significant dip in weight was observed on experimental day 2, which was the result of the first 23 hour period of water restriction. From experimental day 2 onwards all rats continued to gain weight under the water restriction procedure and maintained good health (Figure 2).

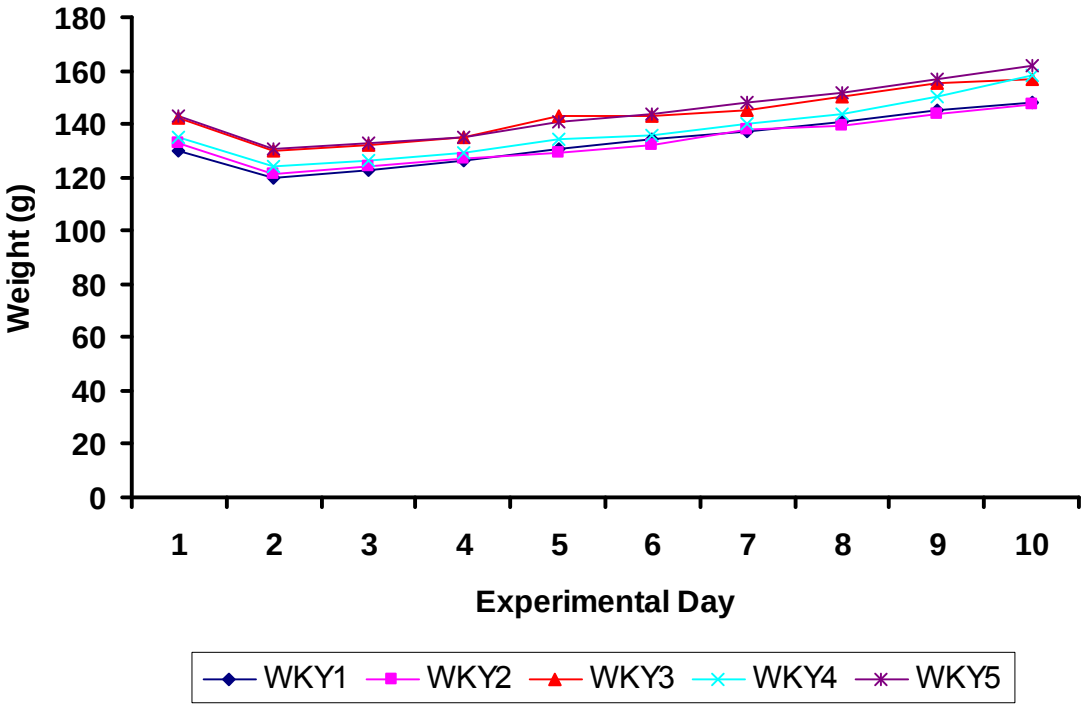

Figure 2. Weight of the Wistar-Kyoto rats (WKY) on each experimental day.

As expected there were significant main effects of dose, F (1.352, 4.055) = 19.654, p = 0.01, and time, F (2.071, 6.214) = 20.598, p = 0.002. The locomotor activity over the 3 hours was significantly higher for the 10 mg/kg dose compared to the 0 mg/kg dose, p = 0.001 (Figure 3). As time increased, the average locomotor activity decreased following drug administration. The dose by time interaction was also significant, F (2.689, 8.066) = 5.051, p = 0.032. As illustrated in Figure 3, compared to the 0 mg/kg dose, the 10 mg/kg dose significantly increased locomotor activity in the first, second, third and fifth 30 minute intervals, p's = 0.014, 0.001, 0.01, and 0.003, respectively. The increase in locomotor activity, compared to the 0 mg/kg dose, in the second 30 minute interval following the 5 mg/kg dose approached significance, p = 0.031. No significant differences were found between the 0 mg/kg and 2 mg/kg dose at any time interval, all p's > 0.05.

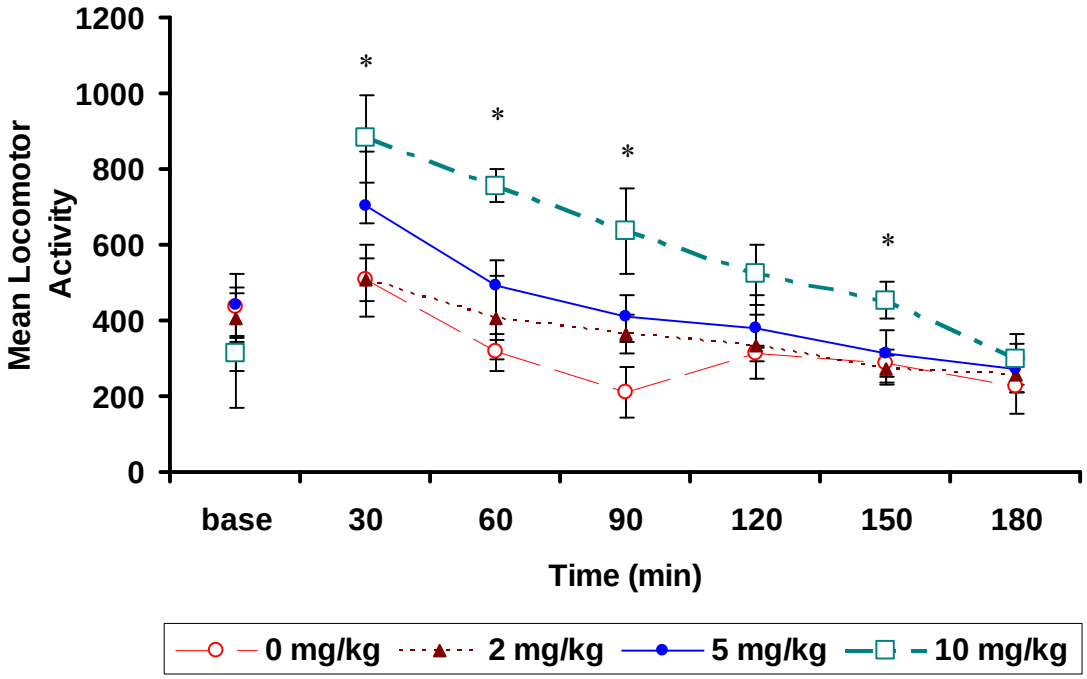

Figure 3. Mean (SEM) locomotor activity of Wistar-Kyoto Rats (n = 5) at half hourly intervals over a three hour period prior to (base) and following four doses of MPH. * $p <$ 0.017 for a significant difference between the 0 mg/kg and 10 mg/kg doses.

3.4 Discussion

The aim of the present study was to establish a procedure for oral drug administration, which did not require extended periods of training and was pharmacologically active as evident by changes in locomotor activity following administration. It was observed that the rats voluntarily drank the MPH suspension through a drinking spout following water restriction. The rats consumed all of the liquid administered through the spout in approximately 30 seconds which resulted in dose-dependent increases in locomotor activity.

Results from the dose-response experiment demonstrate that a high dose (10 mg/kg) of MPH, administered using the novel oral method, produced locomotor activation. In line with previous research reporting peak neurotransmitter levels and associated locomotor activity 40 minutes after intragastric administration of 5 mg/kg and 10 mg/kg of MPH (Gerasimov et al.,

2000), the current study found a significant increase in locomotor activity during similar time periods following administration of high dose MPH (10 mg/kg), with a trend toward increased locomotor activity following the 5 mg/kg MPH dose compared to vehicle. No increase in locomotor activity was observed when a lower dose of MPH (2 mg/kg) was orally administered, consistent with previous findings (Berridge et al., 2006; Gerasimov et al., 2000). The consistency of the current findings with previous research suggests that the proposed water restriction method of drug administration produces a similar pharmacological effect of MPH to other oral administration methods.

Importantly, the dose-dependent change in locomotor activation was observed during the dark or active phase for the rats. The time of day when the drug is administered is an important consideration as the pharmacokinetics of MPH have been shown to vary throughout a 24 hour period (Gaytan et al., 2000). As rats are nocturnal, administering MPH during the dark phase is necessary for the results to be considered clinically relevant.

A potential explanation for the dose-dependent change in locomotor activity involves the effect various MPH doses have on different brain regions. High doses of MPH increase the neurotransmitters DA and NA consistently throughout the brain. Specifically, increases in extracellular DA within the locomotor activating areas of the striatum and nucleus accumbens have been reported following administration of high MPH doses (Bymaster et al., 2002; Kuczenski & Segal, 2002; Volkow et al., 2001). However lower doses of MPH that are considered to be therapeutically relevant have been shown to specifically activate NA and DA transmission within the prefrontal cortex (Berridge et al., 2006). This difference in regional catecholamine efflux could explain the lack of locomotor activation associated with therapeutic administration of MPH (Volkow & Swanson, 2003), as there is no striatal activation with lower MPH doses (Berridge et al., 2006). These findings highlight the

importance of the administration of appropriate low dose MPH to rats as high doses induce behaviours which are not evident in clinically treated children.

The proposed water restriction method of administration does not require training which is a benefit of this procedure over a previous study using oral administration of MPH dissolved in apple juice (Wheeler et al., 2007). Wheeler and colleagues (2007) trained each rat with apple juice for 5 days prior to the introduction of the drug. Additionally, Wheeler states that following this period of training, they found it necessary to water restrict some rats before they would consume the apple juice suspension. The method of drug administration proposed in this study not only removes the need for training, but also eliminates the confounding variable of inconsistent water restriction across the treatment cohort. Furthermore, the current water restriction method allowed for MPH administration without handling of the rats. This is preferable to the procedure proposed by Wheeler and colleagues (2007) as handling stress has been shown to modulate MPH-induced DA efflux (Marsteller et al., 2002).

A limitation of the administration method used in the present study is the impact water restriction has on the effect of MPH treatment. While there have been no studies published addressing the pharmacokinetics of MPH at varying levels of hydration, the possibility remains that the water restriction procedure employed in this study may alter the MPH effect. However, the present study found behavioural activation very similar to studies employing the gavage technique in non-restricted animals (Berridge et al., 2006; Gerasimov et al., 2000), suggesting that the pharmacokinetics of MPH have not been affected by water restriction. In addition, the rats continued to gain weight and maintained good general health throughout the experiment suggesting that the water restriction paradigm did not impact on the general health or well-being of the animals. Furthermore, previous research has demonstrated that neural DA levels are unaffected by a similar period of water restriction to that employed in the current

study (Alper et al., 1980), suggesting that the current findings were not a result of altered baseline levels of DA.

The possibility of alterations in the dose volume using this oral method of administration can not be fully eliminated, due to potential leaks in the system. A great deal of care was taken to reduce this loss. Prior to drug administration, the drinking spout was assessed to ensure there was no leakage in the system. Furthermore, the drug was not inserted into the drinking spout until the rat had commenced drinking. Each rat consumed the entire liquid from the drinking spout very quickly and without interruptions so any drug loss would be negligible.

It is also important to note that the single PIR sensor used to measure locomotor activity does not allow for the evaluation of horizontal and vertical exploration. Future research would benefit from measuring repetitive stereotypies and grooming to elucidate the effect MPH has on these behaviours.

Previous research has demonstrated that the route of administration is central to the pharmacokinetics of MPH (Gerasimov et al., 2000; Kuczenski & Segal, 2002). This study has established an oral method of MPH administration which is non-invasive, does not require extensive training and easily delivers the drug over a short time period. From comparisons to other administration techniques, the procedure does not interfere with the pharmacology of MPH to activate locomotor behaviour and the ease of delivery allows for repeated administration of the drug. Using the present dosing method for future studies on the effects of MPH use would allow for the implementation of a dosing schedule in rodents which more closely mirrors clinical treatment regimes. The present dosing method will be employed in the following chapters to investigate the long-term effects of a chronic sub-threshold dose of MPH, with a focus on the WKY strain as the non-ADHD or misdiagnosed rat model.

3.5 References

Alper, R. H., Demarest, K. T., & Moore, K. E. (1980). Dehydration selectively increases dopamine synthesis in tuberohypophyseal dopaminergic neurons. *Neuroendocrinology, 31*, 112-115.

Andersen, S. L. (2002). Changes in the second messenger cyclic AMP during development may underlie motoric symptoms in attention deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research, 130*, 197-201.

Aoyama, T., Kotaki, H., & Iga, T. (1990). Dose-dependent kinetics of methylphenidate enantiomers after oral administration of racemic methylphenidate to rats. *Journal of Pharmacobio-dynamics, 13*, 647-652.

Arnsten, A. F. T., & Dudley, A. G. (2005). Methylphenidate improves prefrontal cortical cognitive function through α_2 adrenoreceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behavioral and Brain Functions, 1*, 1-9.

Askenasy, E. P., Taber, K. H., Yang, P., & Dafny, N. (2007). Methylphenidate (Ritalin): Behavioral studies in the rat. *International Journal of Neuroscience, 117* (6), 757-794.

Balcombe, J. P., Barnard, N. D., & Sandusky, C. (2004). Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science, 43*, 42-51.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F. T., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60*, 1111-1120.

Bymaster, F., Katner, J., Nelson, D., Hemrick-Luecke, S., Threlkeld, P., Heiligenstein, J., et al. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: A potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology, 27*, 699-711.

Chuhan, Y. S., & Taukulis, H. K. (2006). Impairment of single-trial memory formation by oral methylphenidate in the rat. *Neurobiology of Learning and Memory, 85*, 125-131.

Dafny, N., & Yang, P. (2006). The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: A review of its locomotor effects. *Brain Research Bulletin, 68*, 393-405.

Engert, V., & Pruessner, J. C. (2008). Dopaminergic and noradrenergic contributions to functionality in ADHD: The role of methylphenidate. *Current Neuropharmacology, 6*, 322-328.

Gaytan, O., Yang, P., Swann, A., & Dafny, N. (2000). Diurnal differences in sensitization to methylphenidate. *Brain Research, 864*, 24-39.

Gerasimov, M., Franceschi, M., Volkow, N. D., Gifford, A., Gatley, S. J., Marsteller, D., et al. (2000). Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *The Journal of Pharmacology and Experimental Therapeutics, 295* (1), 51-57.

Greenhill, L., Beyer, D. H., Finkleson, J., Shaffer, D., Biederman, J., Conners, C. K., et al. (2002). Guidelines and algorithms for the use of methylphenidate in children with attention-deficit/hyperactivity disorder. *Journal of Attention Disorders, 6 (Supplemental 1)*, S89-100.

Hughes, J. E., Amyx, H., Howard, J. L., Nanry, K. P., & Pollard, G. T. (1994). Health effects of water restriction to motivate lever-pressing in rats. *Laboratory Animal Science, 44*, 134-140.

Kiss, A., Jezova, D., & Aguilera, G. (1994). Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Research, 663*, 84-92.

Kuczenski, R., & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular noreprinephrine and absence of sensitization and

cross-sensitization to methamphetamine. *The Journal of Neuroscience, 22* (16), 7264-7271.

Laviola, G., Macri, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews, 27*, 19-31.

LeBlanc-Duchin, D., & Taukulis, H. K. (2004). Behavioral reactivity to a noradrenergic challenge after chronic oral methylphenidate (Ritalin) in rats. *Pharmacology, Biochemistry and Behavior, 79*, 641-649.

Marsteller, D. A., Gerasimov, M. R., Schiffer, W. K., Geiger, J. M., Barnett, C. R., Borg, J. S., et al. (2002). Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. *Neuropsychopharmacology, 27*, 163-170.

McGregor, I. S. (1996). Using Strawberry Tree Workbench Mac and Workbench PC for data acquisition and control in the animal learning laboratory. *Behavior Research Methods, Instruments and Computers, 28*, 38-48.

Murphy, S. H., Smith, P., Shaivitz, A. B., Rossberg, M. I., & Hurn, P. D. (2001). The effect of brief halothane anesthesia during daily gavage on complications and body weight in rats. *Contemporary Topics in Laboratory Animal Science, 40*, 9-12.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.).

Pardey, M. C., Homewood, J., Taylor, A., & Cornish, J. L. (2009). Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods, 176*, 166-171.

Patrick, K. S., Ellington, K. R., & Breese, G. R. (1984). Distribution of methylphenidate and *p*-hydroxymethylphenidate in rats. *The Journal of Pharmacology and Experimental Therapeutics, 231* (1), 61-65.

Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews, 11*, 157-198.

Swanson, J. M., & Volkow, N. D. (2001). Pharmacokinetic and pharmacodynamic properties of methylphenidate in humans. In M. V. Solanto, A. F. T. Arnsten & F. X. Castellanos (Eds.), *Stimulant drugs and ADHD: Basic and clinical neuroscience* (pp. 259-282). New York: Oxford University Press.

Swanson, J. M., & Volkow, N. D. (2002). Pharmacokinetic and pharmacodynamic properties of stimulants: implications for the design of new treatments for ADHD. *Behavioural Brain Research, 130*, 73-78.

Thanos, P. K., Michaelides, M., Benveniste, H., Wang, G. J., & Volkow, N. D. (2007). Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. *Pharmacology, Biochemistry and Behavior, 87*, 426-433.

Volkow, N. D., & Swanson, J. M. (2003). Variables that affect the clinical use and abuse of methylphenidate in the treatment of ADHD. *American Journal of Psychiatry, 160*, 1909-1918.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Gerasimov, M., Maynard, L., et al. (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *The Journal of Neuroscience, 21*, RC121.

Wargin, W., Patrick, K. S., Kilts, C., Gualtieri, C. T., Ellington, K. R., Mueller, R. A., et al. (1983). Pharmacokinetics of methylphenidate in man, rat and monkey. *The Journal of Pharmacology and Experimental Therapeutics, 226* (2), 382-386.

Wheeler, T. L., Eppolito, A. K., Smith, L. N., Huff, T. B., & Smith, R. F. (2007). A novel method for oral stimulant administration in the neonate rat and similar species. *Journal of Neuroscience Methods, 159*, 282-285.

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Chapter 4

Long-term effects of chronic Ritalin administration on cognitive development in non-ADHD adolescent rats

4.1 Introduction

Attention Deficit/Hyperactivity Disorder (ADHD) is the most common neurobehavioural childhood disorder (Biederman & Faraone, 2005). The psychostimulant methylphenidate (MPH; Ritalin®) is widely prescribed and has been shown to effectively reduce the ADHD symptoms of hyperactivity, impulsivity and inattention (Greenhill et al., 2002). However, diagnosis of ADHD relies heavily on subjective interpretations of the diagnostic criteria (Solanto & Alvir, 2009). There is evidence to suggest that primary care physicians vary greatly in the assessment methods and do not always follow 'best practice' guidelines when diagnosing ADHD (Handler & DuPaul, 2005). Such variations in diagnostic procedures would likely increase misdiagnosis of the disorder (Sciutto & Eisenberg, 2007). It has been reported that stimulants, such as MPH, are being administered to children who do not meet full diagnostic criteria of ADHD (Angold, Erkanli, Egger, & Costello, 2000; Sagvolden et al., 1992; Sawyer, Rey, Graetz, Clark, & Baghurst, 2002) and also to children as young as 2 years of age (Zito et al., 2000).

Inappropriate drug treatment throughout childhood and adolescence could have long-term effects on brain development. The human nervous system undergoes synaptogenesis and myelination through puberty, with continued remodelling of neural circuitry (synaptic plasticity) during adulthood (Rice & Barone Jr., 2000). Similar periods of neural development are evident in the rat brain, with the duration of this period reduced to days or weeks in the rat, compared to months or years in humans (Rice & Barone Jr., 2000).

Several lines of evidence suggest that dysfunction in the prefrontal cortex (PFC) is related to ADHD symptoms. Lesions in the PFC produce ADHD-like symptoms of distractibility and impulsivity (Eagle et al., 2008; Rossi, Bichot, Desimone, & Ungerleider, 2007). Neuroimaging studies have revealed volumetric reductions in the PFC, cerebellum and the striatum of ADHD patients (Castellanos et al., 1996; Filipek et al., 1997) and reduced PFC activity in unmedicated children diagnosed with ADHD (Rubia et al., 1999). Furthermore, ADHD has been linked to suboptimal levels of catecholamines in the PFC (Pliszka, 2005; Wilens, 2008). Optimal functioning of the PFC requires moderate levels of the catecholamines dopamine (DA) and noradrenaline (NA), where either too little or too much of these neurotransmitters results in impaired PFC function (Arnsten, 2009).

The therapeutic action of MPH is to increase synaptic levels of catecholamines by blocking their re-uptake at DA and NA transporters (Gatley, Pan, Chen, Chaturvedi, & Ding, 1996; Kuczenski & Segal, 1997; Volkow et al., 2002). Therapeutic doses of MPH administered to rats have been shown to increase both NA and DA in the PFC (Berridge & Devilbiss, 2008; Berridge et al., 2006). MPH is commonly administered during childhood and adolescence at an age where the PFC is undergoing extensive synaptogenesis and neural remodelling (Adriani & Laviola, 2004). Environmental stimuli and psychoactive drugs have been shown to influence synaptic plasticity in the PFC of the rat, and therefore have an ability to alter the function of this region (Kolb, Gibb, & Gorny, 2003; Robinson & Kolb, 2004). Furthermore, therapeutic doses of MPH improve performance on tasks that are dependent upon the integrity of the PFC, including those involving working memory and attention components (Arnsten & Dudley, 2005; Berridge et al., 2006). This suggests that the administration of MPH may have a pronounced effect on brain regions associated with higher cognition and may alter the functional development of these regions.

Appropriate animal models are necessary to assess the potential long-term effects of MPH treatment on development. Throughout this thesis the Wistar-Kyoto rat (WKY) was employed as the non-ADHD (i.e. misdiagnosed) rat, as it is the genetic control for the Spontaneously Hypertensive rat (SHR). The SHR has been widely used and extensively studied as an animal model for ADHD (see 1.1.4). The SHR model has been shown to have face validity (Fox, Hand, & Reilly, 2008; Pardey, Homewood, Taylor, & Cornish, 2009, Chapter 2; Sagvolden, Aase, Zeiner, & Berger, 1998), construct validity (Russell, Sagvolden, & Johansen, 2005), as well as predictive validity (Sagvolden et al., 1992). It must be noted that, the validity of the WKY as a 'normal' strain has been questioned as the hyperactivity measured in the SHR, could alternatively be interpreted as hypoactivity measured in the WKY (Alsop, 2007; Drolet, Proulx, Pearson, Rochford, & Deschepper, 2002; van den Bergh et al., 2006). Additionally, it has recently been suggested that a substrain of the WKY may be an appropriate animal model for the Predominantly Inattentive subtype of ADHD (Sagvolden et al., 2009). As discussed in Pardey et al. (2009, Chapter 2), in tasks employed in this laboratory WKYs were not found to be inactive compared to SHRs. The concern of a WKY substrain resembling the Predominantly Inattentive subtype of ADHD can not be addressed as attention tasks were not conducted in the previous study (Pardey et al., 2009, Chapter 2). Furthermore, the behavioural stimulation following a high oral dose of MPH administration to WKYs, reported in Chapter 3, was consistent with locomotor activation observed in Sprague-Dawley rats following equivalent dosing (Gerasimov et al., 2000). Together these findings provide support for the WKY as a 'normal' strain, and therefore it is appropriate in the following studies to employ the WKY as a non-ADHD model given the familial relationship to the genetic animal model of ADHD, the SHR (Sagvolden et al., 2009).

The current experiment was conducted to investigate the potential long-term effects on cognitive development of chronic MPH administration during adolescence in the rat. The

focus of this study is the impact such treatment has on a misdiagnosed (i.e. non-ADHD) population. To achieve this goal, WKYs and SHRs were treated with oral MPH throughout adolescence and their performance on cognitive tasks was assessed in adulthood.

In addition to the use of the WKY for exploration of chronic MPH effects in non-ADHD rats, the SHR was included in the current study for two reasons. Firstly, the SHR was included as a positive control. Acute therapeutic doses of psychostimulants have previously been shown to attenuate hyperactivity in the SHR (Myers, Musty, & Hendley, 1982). However, as therapeutic doses do not alter locomotor activity in 'normal' rats (Berridge et al., 2006; Gerasimov et al., 2000; Kuczenski & Segal, 2002; Chapter 3), the SHR was included to demonstrate the MPH dose employed was pharmacologically active. Secondly, inclusion of the SHR in this study enabled the investigation of recently reported strain differences in the effect of MPH (Thanos et al., 2010). Thanos et al. (2010) found that acute MPH administration increased distractibility in the WKY but did not in the SHR. Strain differences have also been reported in the short-term effects of chronic MPH administration (Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 2000).

The majority of previous pre-clinical research assessing the long-term effects on cognition of chronic treatment with MPH in adolescence has focused on memory performance. LeBlanc-Duchin and Taukulis (2007) reported that rats treated with 3 and 5 mg/kg of oral MPH for 21 days during adolescence exhibited an impairment in recognition memory which persisted for 42 days post treatment. They found similar long-term deficits of recognition and spatial memory when MPH treatment was administered chronically to adult rats (LeBlanc-Duchin & Taukulis, 2009). Transient memory deficits have also been reported following seven weeks of treatment with 5 mg/kg of oral MPH which commenced in adolescence (Bethancourt, Camarena, & Britton, 2009). Additionally, impaired performance on a spatial memory task, as

assessed using the Morris water maze, has been reported following chronic MPH treatment during adolescence (Scherer et al., 2010). Similar object recognition deficits have been reported in rats 30 minutes after their final dose of chronic MPH administration (Heyser, Pelletier, & Ferris, 2004). In the study by Heyser and colleagues (2004), there were no recognition deficits following an acute dose of MPH, suggesting the memory impairment is specifically related to chronic exposure of MPH and not acute intoxication.

A single study has been conducted assessing impulsivity following chronic MPH treatment. Adriani and colleagues (2007) reported reduced impulsive behaviour in adults rats that were treated with MPH during adolescence. However as discussed in Chapter 3, the dose and route of administration are vital to the pharmacokinetics of MPH (Gerasimov et al., 2000), as is the stage of the circadian cycle at which MPH is administered (Gaytan, Yang, Swann, & Dafny, 2000). The study conducted by Adriani and colleagues (2007) administered a single daily dose (2 mg/kg), via intraperitoneal (i.p.) injection during the light period or 'inactive' phase for the rats. This is not consistent with dosing regime employed clinically in which children are orally treated with low doses of MPH, during their 'active' phase of their circadian cycle (Kuczenski & Segal, 2002). The different method of drug administration used by Adriani and colleagues (2007) reduces the relevance of their results to the human situation.

Chronic administration of MPH has also been shown to have enduring effects on reward processing (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002; Augustyniak, Kourrich, Rezazadeh, Stewart, & Arvanitogiannis, 2006; Brandon, Marinelli, Baker, & White, 2001; Carlezon, Mague, & Andersen, 2003; Mague, Andersen, & Carlezon, 2005) and depressive and anxiety like behaviours (Bolanos, Barrot, Berton, Wallace-Black, & Nestler, 2003; Bolanos et al., 2008; Carlezon et al., 2003; Gray et al., 2007). However, the pattern of findings is inconsistent, possibly due to varying schedules, doses and routes of administration.

The aim of the present study was to assess the long-term effects of chronic MPH administration on cognitive functioning in non-ADHD (i.e. misdiagnosed) rats using a relevant animal model (Pardey et al., 2009, Chapter 2; Sagvolden et al., 2009). The cognitive functions examined in this research were impulsivity and working memory. Impulsive behaviour can be characterised as the selection of an immediate small reinforcer over a large reinforcer delivered after a delay (Evenden & Ryan, 1996). Working memory is the ability to hold information in mind while constantly updating and manipulating it (Baddeley & Hitch, 1974). Both of these cognitive tasks are altered by disturbances to PFC function (Mobini et al., 2002; Taylor, Latimer, & Winn, 2003).

The present study also aimed to mimic clinical dosing regimes in an animal model of MPH drug administration. As such, rats were treated orally with 2 mg/kg MPH, twice a day during their dark (active) phase. The drug administration method is important as the dose, time and route of administration all impact the pharmacokinetics of MPH, as discussed in Chapter 3. Treatment was administered for 4 weeks during adolescence (PND 27 – 52). Changes in behavioural response to chronic MPH treatment were assessed by measuring locomotor activation at the beginning of each week of treatment. It was anticipated that treatment would have no effect on the locomotor activity in the WKY, while attenuation of hyperactivity was expected for the SHR. Following MPH treatment, cognitive tasks assessing impulsivity and memory were conducted to test the hypothesis that inappropriate chronic drug treatment during adolescence would compromise cognitive development. It was anticipated that the enduring changes on cognitive performance of MPH treatment would only be expected in the WKYs, as the ability of MPH to correct the deficient dopamine system in the SHR is suggested to be transient (Russell et al., 2000).

4.2 Materials and Method

4.2.1 Subjects

Twenty-four male WKY and 24 male SHR rats were obtained from the Animal Resources Centre (Canning Vale, WA, Australia). One SHR was not well and was therefore excluded from the study. Upon arrival in the laboratory, the rats were housed individually in opaque, plastic cages (60 x 21.5 x 36 cm, length x height x width) containing sawdust, a block of wood and shredded paper. The cage was covered with a raised wire mesh roof (27cm total height). The animal holding room was held at a constant temperature of 21˚C ± 1. Rats were housed on a reverse light/dark cycle (lights on at 2000 hours until 0800 hours) and experiments were conducted during the rats' active (dark) cycle. At the beginning of the procedure, the rats were approximately 25 days old, weighed 51 – 67 (WKY) and 46 – 73 (SHR) grams, had been handled daily for one week by the experimenter and were experimentally naïve. They were allowed free access to water and standard laboratory rat chow, except during the drug administration and cognitive assessment procedures as detailed in the relevant sections below. The rats were individually housed to facilitate drug administration with minimal handling of the animals and to eliminate competition by littermates for food and water during these periods of restriction. Rats were weighed daily to determine treatment volume and to monitor growth.

The study was conducted with the approval of the Macquarie University Animal Ethics Committee (reference number ARA 2006/019) and followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004).

4.2.2 Drug administration procedure

Rats were placed on water restriction in order to facilitate drug administration via a drinking

spout (described in detail in Chapter 3.2.2). After an initial 23 hours of water restriction, rats were familiarized to the dosing procedure by exposure to a sham dose of water through the drinking spout. The following day treatment commenced using this procedure. Rats were chronically treated with either Ritalin (2 mg/kg oral; MPH) or water (dH2O) to model clinical dosing in children. Doses were based on the findings of the previous pilot study (Chapter 3); Kuczenski and Segal (2002); and Berridge et al., (2006).

Ritalin® tablets (10 mg/tablet, Novartis, East Hanover, New Jersey) were suspended in distilled water (1 mg/mL) and administered through a drinking spout twice a day, five days per week for four weeks. Drug free weekends were employed in the current study as 'weekend holidays' are used in the clinical setting and have been shown to reduce the incidence of side-effects (Martins et al., 2004). On the first week of treatment, the initial drug administration was given 23 hours after the sham dose during which time the rats were water restricted. The first drug administration for each of the following weeks was given after 23 hours of water restriction at the end of their rest days. The drinking spout was placed in the cage and 1 mL of water was inserted. As the rat consumed the first mL of water, the drug dose (2 mg/kg) was inserted into the spout via a syringe (typically around 0.1 to 0.3 of 1 mL, relative to body weight). An additional 1 mL of water was then inserted into the spout to ensure the entire dose had been ingested. Immediately following consumption of the liquid in the drinking spout, the rats were given five minutes *ad libitum* access to water. The second daily drug administration was identical and occurred five hours after the first dose, however the rats were allowed one hour *ad libitum* access to water following the second dose. After the second drug administration on the fifth day of the week, the rats were given *ad libitum* access to water for approximately 40 hours, at which time they were placed back on water restriction to facilitate drug administration in the following week. The schedule of water restriction was based on previous research allowing rats *ad libitum* access to water on rest days (Johansen,

Sagvolden, & Kvande, 2005; Sagvolden et al., 1992). Once the rats had completed four weeks of treatment, they were taken off water restriction and allowed *ad libitum* access to food and water for one week until the cognitive testing commenced.

4.2.3. Locomotor Activity

Locomotor activity was used as a simple method of measuring behavioural change throughout repeated MPH administration. A more detailed description of the apparatus and procedure used to measure locomotor activity is available in Pardey et al., (2009, Chapter 2). Briefly, the rats were placed in operant conditioning chambers (purpose built by the University of Sydney, Australia) with two passive infrared detectors (PIR, Quantum passive infrared motion sensor, Ness Security Products, Australia) located opposite each other, 30mm above the floor. Locomotor activity was measured by detection of small movements of the subjects' head and body. These movements were tracked via "Workbench Mac" software running on Macintosh Computers for one hour (McGregor, 1996). Cameras in each chamber allowed observation of the rats to monitor their welfare during the session. To habituate the rats to the chambers, they were placed in the chambers for 1 hour on the day prior to the initial data collection day. Locomotor activity of the rats was measured for 15 minutes prior to (weekly baseline measure) and one hour immediately following their morning dose on the first day of each week of treatment.

4.2.4 Cognitive tasks

The sequence in which the cognitive-behavioural tests were conducted is outlined in Figure 1.

Figure 1. The sequence of events for the rats performing the cognitive-behavioural tests. On the first day of each week of treatment, locomotor activity was measured. One week following cessation of treatment, rats were placed on food restriction and completed a Delayed Reinforcement (DR) and Extinction (EXT) task. Following one week of rest, rats completed the Radial Arm Maze (RAM) with water as their reinforcer. Rats were euthanized one week after the completion of the RAM.

4.2.4.1 Delayed reinforcement (DR) and Extinction (EXT) tasks

The delayed reinforcement (DR) and extinction (EXT) tasks have been described in more detail in Pardey et al., (2009, Chapter 2). The animals were placed on food restriction 24 hours prior to the commencement of the DR task to maintain their body weight at approximately 85%. There was *ad libitum* access to water in their home cage throughout the DR and EXT tasks. The food reinforcer used was 45 mg Noyes Precision Pellets, Formula A (Research Diets, Inc., New Brunswick, NJ, USA). For both tasks the rats were placed in operant conditioning chambers in which the test wall contained two cue lights, above two levers, on either side of a food magazine (Figure 1).

In the DR task, the rats were trained to press one lever to receive a small reinforcer (one pellet) immediately and the other lever to receive a large reinforcer (five food pellets) after a two second delay. To reduce the risk of partial reinforcement, during the delay the cue light above the activated lever flashed (0.6 seconds per on/off cycle). Once the animals had been trained, i.e. they pressed each lever 15 times on three consecutive days, they progressed to the

test phase. If a rat had not met this criterion within 7 days they were removed from the task.

For each test, the rats were allowed to choose which lever they pressed and therefore the size of reinforcer they would receive. The reinforcer associated with both levers remained constant throughout the test phase. If the rat chose the immediate lever, they received one pellet immediately. If the rats chose the large delayed lever, they received five pellets, however the delay between their response and reinforcer delivery increased during each test session, with increasing delay durations on each of the tests as shown in Table 1. A delay duration was experienced six times before proceeding to the longer delay on that particular test. Delay durations were based on Evenden and Ryan (1996) and Adriani and Laviola (2003). As with the training phase, during the delay the cue light flashed until the reinforcer was delivered. The data recorded was the longest delay accepted for each test, the total number of reinforcers attained from each lever and the total number of presses on each lever.

Table 1. Duration of delay (seconds) before reinforcer was delivered on each test.

Test	Delay duration in seconds (each experienced 6 times)
1	0, 1, 2, 3, 4
2	0, 2, 4, 6, 8
3	0, 5, 10, 15, 20
4	0, 10, 20, 30, 40
5	0, 10, 20, 40, 60
6	0, 20, 40, 60, 80
7	0, 30, 60, 90, 120

Following Test 7, all animals were placed on a constant 60 second delay schedule for one day prior to the EXT task. Extinction tasks have previously been employed as a measure of sustained attention (Berger & Sagvolden, 1998; Sagvolden et al., 1998; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). During the EXT task the animals were placed in the

operant conditioning chambers as per tests in the DR task, however no food reinforcers were delivered. The rats' lever pressing had no effect on the delivery of a reinforcer or the illumination of the cue lights. The rats' lever pressing was recorded at 20 second intervals over the five minute duration of the EXT task. At the end of the EXT task all rats were taken off food restriction and had *ad libitum* access to food and water for nine days until water restriction commenced for the RAM.

4.2.4.2 Radial Arm Maze (RAM)

4.2.4.2.1 Apparatus

The RAM was purpose built for Macquarie University by Allplastics Engineering Pty Ltd. (Sydney, Australia). It has 12 arms each measuring 610 mm x 80 mm x 145 mm (length x width x height) which radiate from a central hub (460 mm x 335 mm; diameter x height, Figure 1). The floor of the maze was opaque white polycarbonate and all the walls and doors were made from clear polycarbonate. Sessions were conducted in a dimly lit room (between 20 and 50 lux) with a radio playing softly to mask any background noise. Cardboard with vertically, horizontally or diagonally oriented black and white strips (50 mm thick each) were used as visual cues and were placed on the southern, eastern and northern walls, respectively. The experimenter was always positioned at the western wall. Doors were opened and closed via a pulley system resulting in minimal movement by the experimenter. Arm entries and the time to complete each session were recorded by the experimenter with a video camera capturing an aerial view to allow for later analysis of decision latency (the time it takes the rats to choose an arm) and arm latency (the time the rats spend in each arm).

4.2.4.2.2 Procedure

The rats were placed in the RAM daily for five out of seven days. Water was used as a reinforcer throughout the training and testing sessions of the RAM with 0.4 mL placed in a

bottle cap at the end of eight of the arms. The other four arms (those forming a 'T' in the maze) were never baited and had empty bottle caps placed at the end. Reinforcers were not replenished throughout a session. Between each rat and between the phases of the testing sessions the maze was thoroughly washed out with 10% ethanol to remove olfactory cues. At the end of each session, the rats were given one hour *ad libitum* access to water unless the following day was a rest day (Johansen et al., 2005; Sagvolden et al., 1992), in which case the rats had *ad libitum* access to water until 16 hours prior to their next session. The rats had *ad libitum* access to food throughout the RAM.

Habituation: The rats were given three days to acclimatize to the maze. On the first and second day, the rats were placed in the RAM for ten minutes. Sunflower seeds were scattered throughout the RAM and the doors remained open to encourage free exploration. On day one the rats were placed in the maze in groups of six and on day two in groups of three. On the third day, the rats were placed individually in the RAM for ten minutes. At the end of each arm was a bottle cap which contained sweetened condensed milk. As the rat moved throughout the maze, the doors were opened and closed. At the end of the third day the rats were placed on water restriction to facilitate training.

Training session: The rat was placed in the central hub of the RAM. After ten seconds, all the doors opened allowing the rat to enter an arm and attain the reinforcer. Once the rat entered an arm, the other eleven doors closed. Upon returning to the central hub the door to the entered arm closed, confining the rat to the central hub for ten seconds. Following the delay, all the doors were again opened and the cycle continued until the session was timed out at 14 minutes, the rat entered each of the 12 arms, or a maximum of 14 arm entries was reached. To meet the criteria to progress to the testing phase the rats had to enter each of the 12 arms, without making more than 14 arm entries, on three consecutive days. Rats that had not met

the first day of criteria to progress to the testing sessions in 15 days were removed from the task.

Testing session: On each test session the rats experienced a *forced-choice phase* and then a *free-choice phase*. During the *forced-choice phase* a rat was placed in the central hub and after ten seconds a single door was opened. Once the rat entered the arm, collected the reinforcer and returned to the central hub, the door was closed to confine the rat to the central hub for ten seconds. After the delay, another single door was opened. This cycle was repeated until the rat had been forced to 'choose' and therefore attain the reinforcer, from four predetermined arms. Once the rat had entered the four arms, it was removed from the maze and placed in a holding box for 15 minutes. Following this delay the *free-choice phase* began. During the *free-choice phase* the rat was returned to the central hub and after ten seconds all the doors were opened allowing the rat to choose which arm to enter. To complete this task efficiently, the rats must avoid entering arms that have never been baited, and the four arms it was forced to enter during the *forced-choice phase* on that particular day. The timing of the doors opening and closing continued as per the training sessions until the session timed out at 6 minutes, the rat entered each of the four arms which remain baited, or a maximum of six arms were entered during the *free-choice phase*. The RAM was concluded when each rat had completed ten testing sessions, at which time they were taken off water restriction and had *ad libitum* access to water and food.

4.2.5 Statistical Analyses

Analyses were conducted using Analysis of Variance (ANOVA). The General Linear Model was used unless otherwise specified with multivariate statistics reported when the assumption of sphericity was violated. Bonferroni adjustments were used when multiple comparisons were performed.

Data is presented for each strain separately. The data for the WKYs are presented first as they are the focus of this paper being the misdiagnosed or non-ADHD strain. The SHR data follows as they are the positive control.

A repeated measures analysis was conducted on the locomotor activity measured on the first day of each week of treatment. The analysis had 2 within subjects factors, with 4 levels of 'week' (week 1, week 2, week 3, and week 4) and 5 levels of 'time' (baseline, 15 min, 30 min, 45 min, and 60 min), and 2 levels of the between subjects factor, 'treatment' (MPH vs dH2O). There were 12 WKYs in each treatment group. Two rats were removed from the SHR analysis as their performance was ± 2 s.d. from the mean, leaving 9 SHRs treated with MPH and 12 SHRs treated with dH2O.

Data for the DR task was analysed by a separate repeated measures analysis for each test. The analysis included 5 levels of the within subjects factor, 'delay' (five different durations depending on the test, Table 1) and 2 levels of the within subjects factor, 'treatment' (MPH vs dH2O). Rats from each group and strain were removed from the analysis if their response for that test was ± 2 s.d. from the mean. A single SHR in the dH2O group failed to learn the task and was therefore removed from all analyses.

Repeated measures analysis assessed the number of immediate and delayed level presses at 20 second intervals over the 5 minute EXT task. There were 2 within subjects factors, with 2 levels of the factor, 'lever' (immediate and delayed) and 15 levels of the factor, 'time' (20 second intervals over 5 minutes), and 2 levels of the between subject factor, 'treatment' (MPH vs dH2O). All animals that completed the DR task, completed the EXT task. Due to a computer malfunction data was lost for a WKY treated with MPH resulting in final group numbers of: n = 11 WKY MPH, n= 12 WKY dH2O and n = 11 in each treatment for SHRs.

The *free-choice phase* of RAM yields several measureable behaviours such as percentage of correct arm entries, type and number of errors, decision latency and time spent within each arms. For brevity, only the data for error types are presented as there were no significant findings for the other variables. Within the RAM there were 3 different error types that could occur: Non-baited (NB) errors, when the rat enters an arm that has never been baited; Across-phase (AP) errors, when the rat enters an arm it was previously forced to enter during the *forced-choice phase*; and Perseverative (PER) errors, when a rat re-enters an arm previously chosen in the *free-choice phase*.

A repeated measures analysis was conducted to assess the number of errors averaged over the 10 test days. The within subjects factor 'error type', had 3 levels (NB, AP, and PER), and the between subjects factor had 2 levels, 'treatment' (MPH vs dH2O). A total of 10 rats failed to meet criteria to continue with testing and were therefore removed from the study (total participants in the MPH treated groups n = 9 for both the WKYs and SHRs and in the dH2O treated group n = 8 and 10 for the WKYs and SHRs, respectively).

4.3 Results

4.3.1 Locomotor activity (during chronic treatment)

4.3.1.1 Results for WKYs

There were significant main effects of week, Wilks' Lambda is 0.171, $F = 32.312$, $p < 0.001$, and time, Wilks' Lambda is 0.06, $F = 74.305$, $p < 0.001$. The average locomotor activity was significantly lower in week 1 compared to weeks 2, 3, and 4, p's < 0.05. Locomotor activity was significantly higher at baseline compared to all following time intervals, p's < 0.05. The week by time by treatment interaction was significant, Wilks' Lambda is 0.222, $F = 3.218$, $p = 0.031$. There was significantly higher locomotor activity in the MPH treated rats in first 45 minutes following treatment in week 1 only, p's < 0.05, illustrated in Figure 2. There was no

main effect of treatment and all other interactions involving treatment were not significant, *p*'s > 0.05. MPH treatment briefly increased locomotor activity in the WKYs during the first week of treatment only.

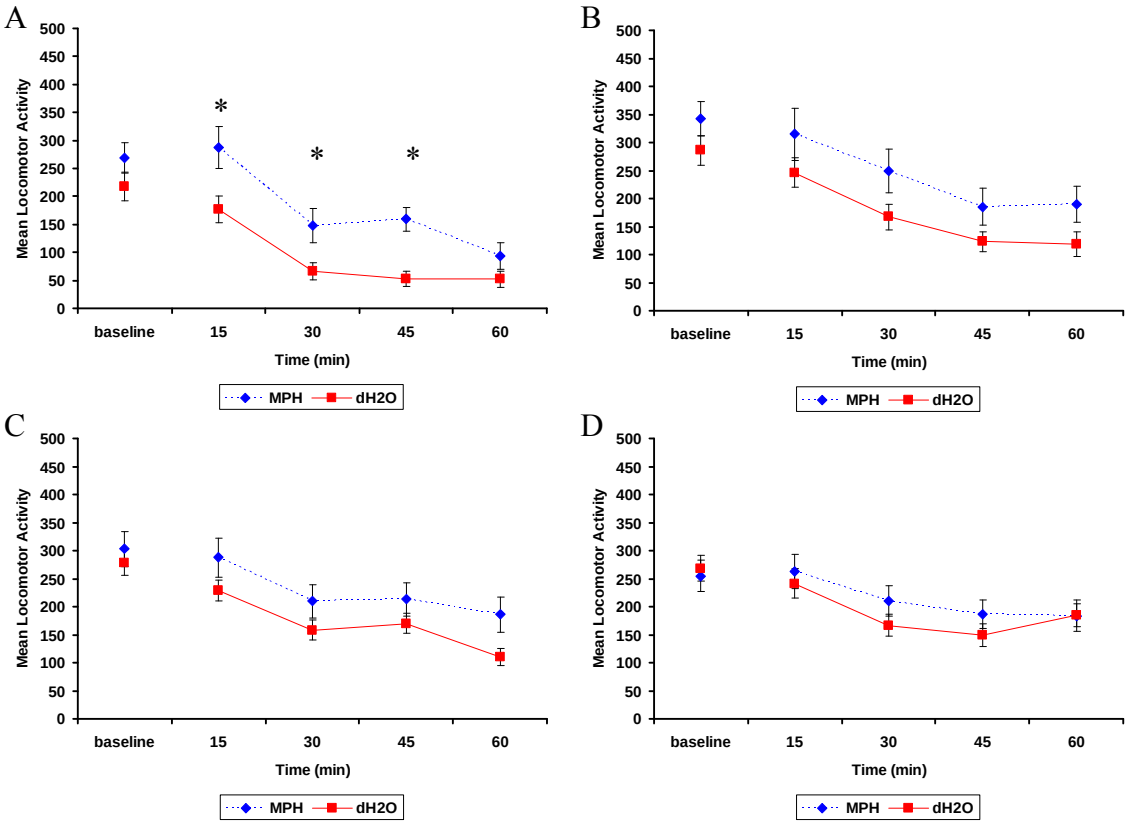

Figure 2. Mean (SEM) locomotor activity at the beginning of each week of treatment with methylphenidate (MPH; n = 12) or water (dH2O; n = 12), measured for 15 minute intervals prior to (baseline) and for 1 hour following treatment for Wistar-Kyoto rats. * Significant difference between treatment, $p < 0.05$, in week 1 (A) with no effect in week 2 (B), Week 3 (C), or week 4 (D).

4.3.1.2 Results for SHRs

Analysis revealed significant main effects of treatment, F (1, 19) = 14.698, p = 0.001, week, F (3, 57) = 44.33, $p < 0.001$, and time F (4, 76) = 103.083, $p < 0.001$. None of the interactions were significant, *p*'s > 0.05. On average, the MPH treated rats had significantly higher locomotor activity compared to dH2O treated rats. The average locomotor activity was

significantly lower in week 1 compared to weeks 2, 3, and 4, *p*'s < 0.05. Locomotor activity was significantly higher at baseline compared to all following time intervals, *p*'s < 0.05. As there was no treatment by time interaction, the main effect of treatment can be attributed to an elevated baseline activity and not a direct result of MPH administration, illustrated in Figure 3. MPH treatment did not affect locomotor activity in the SHRs.

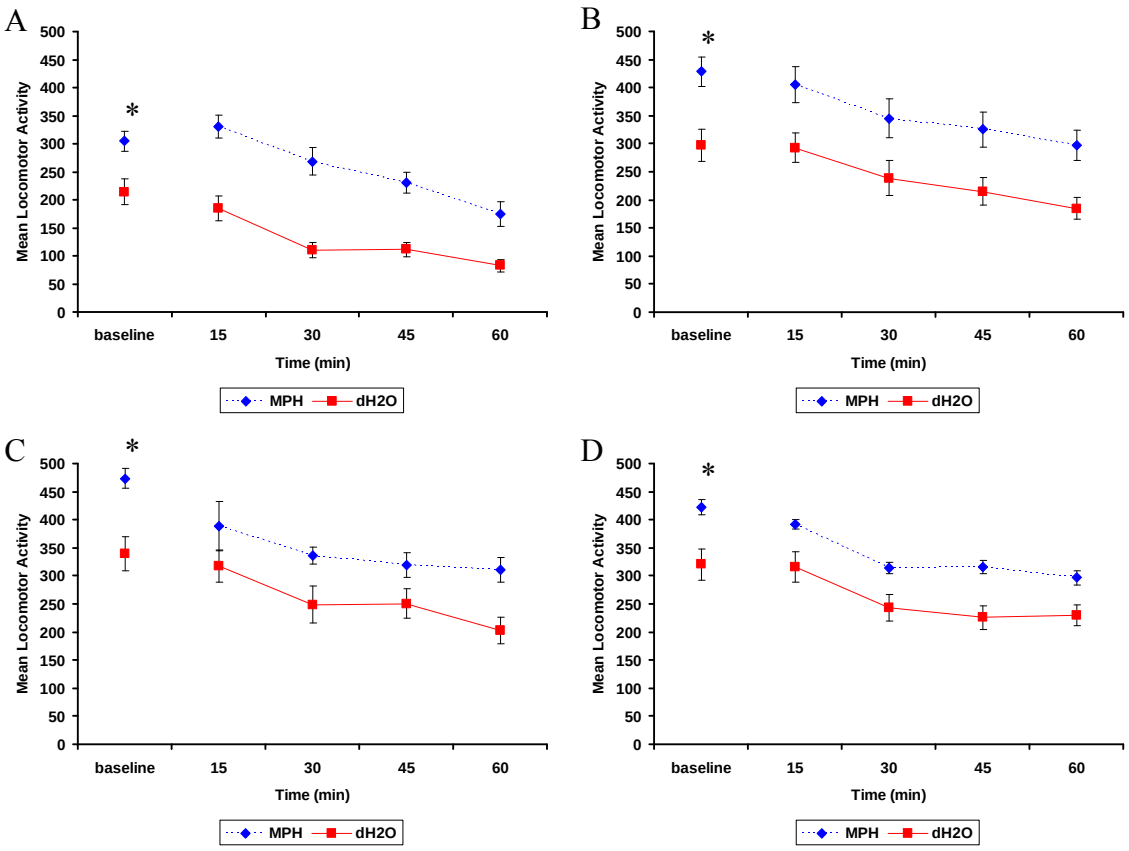

Figure 3. Mean (SEM) locomotor activity at the beginning of each week of treatment with methylphenidate (MPH; n = 9) or water (dH2O; n = 12), measured for 15 minute intervals prior to (baseline) and for 1 hour following treatment for Spontaneously Hypertensive rats in week 1 (A), week 2 (B), week 3 (C) and week 4 (D).

4.3.2 DR task (post chronic treatment)

4.3.2.1 Results for WKYs

Separate analyses for each test revealed significant results for test 1 and 5, with no effect of interest evident on the other tests as outlined below.

The analysis for test 1 included 12 rats previously treated with MPH and 11 rats previously treated with dH2O, as data from one rat in the dH2O group was excluded as an outlier. As illustrated in Figure 4A, there were significant effects of treatment, F (1, 21) = 4.402, p = 0.048, and delay, Wilks' Lambda is 0.351, F = 18.491, $p < 0.001$. The treatment by delay interaction was not significant, $p > 0.05$. Rats previously treated with MPH choose the delayed lever significantly less than rats that received dH2O pretreatment (Figure 4A).

The analysis for test 5 included 11 rats in each treatment group, as data from one rat in each group was excluded as an outlier. As illustrated in Figure 4E, there were significant main effects of treatment, F (1, 20) = 6.438, p = 0.02, and delay, Wilks' Lambda is 0.003, F = 3421.489, $p < 0.001$. The treatment by delay interaction was not significant, $p > 0.05$. Rats previously treated with MPH choose the delayed lever significantly less than rats that received dH2O pretreatment (Figure 4E).

The analyses for the other tests (2, 3, 4, 6 and 7) all returned similar results to each other. There was a significant effect of delay on each of the tests, p's < 0.05, such that the longer the delay duration the less the delayed lever was chosen (see Figure 4). The main effects of treatment and the treatment by delay interactions were not significant, p's > 0.05. These results indicate that on test 1 and 5, the WKYs previously treated with MPH were less willing to wait to receive a larger reinforcer and were therefore more impulsive.

4.3.2.2 Results for SHRs

The analyses for all tests (1 to 7) returned similar results. As illustrated in Figure 5, there was a significant effect of delay on each of the tests, p's < 0.05, such that the longer the delay duration the fewer times the delayed lever was chosen. The main effects of treatment and the treatment by delay interactions were not significant, p's > 0.05. There was no effect of prior

exposure to chronic MPH treatment on impulsivity in SHRs.

4.3.3 EXT task (post chronic treatment)

4.3.3.1 Results for WKYs

For the EXT task, analysis found a significant main effect of lever, Wilks' Lambda is 0.276, $F = 55.173$, $p < 0.001$. WKYs pressed the immediate more than the delayed lever in the EXT task. This is discrepant from the result in Chapter 2 which found that WKYs pressed the delayed lever more than the immediate lever in the EXT task. A probable explanation for this discrepancy is that this main effect was collapsed across treatment groups in the current study whilst the finding in Chapter 2 was attained in a homogenous group of WKYs. Figure 6 illustrates the significant lever by time by treatment interaction, Wilks' Lambda is 0.139, F = 3.540, $p = 0.039$. There was no main effect of treatment and all other interactions were not significant, p's > 0.05. When the EXT task commenced, WKYs previously treated with MPH pressed the immediate lever more than WKYs previously treated with dH2O. MPH treated WKYs continued to demonstrate sensitivity to delay.

4.3.3.2 Results for SHRs

Analysis found a significant main effect of lever, Wilks' Lambda is 0.491, $F = 20.701$, $p < 0.001$, and a significant main effect of time, Wilks' Lambda is 0.123, $F = 3.556$, $p = 0.049$, illustrated in Figure 7. SHRs pressed the immediate lever more than the delayed lever and as time progress lever pressing decreased. There was no main effect of treatment and all interactions involving treatment were not significant, p's > 0.05. MPH treatment of SHRs did not affect lever pressing during the EXT task.

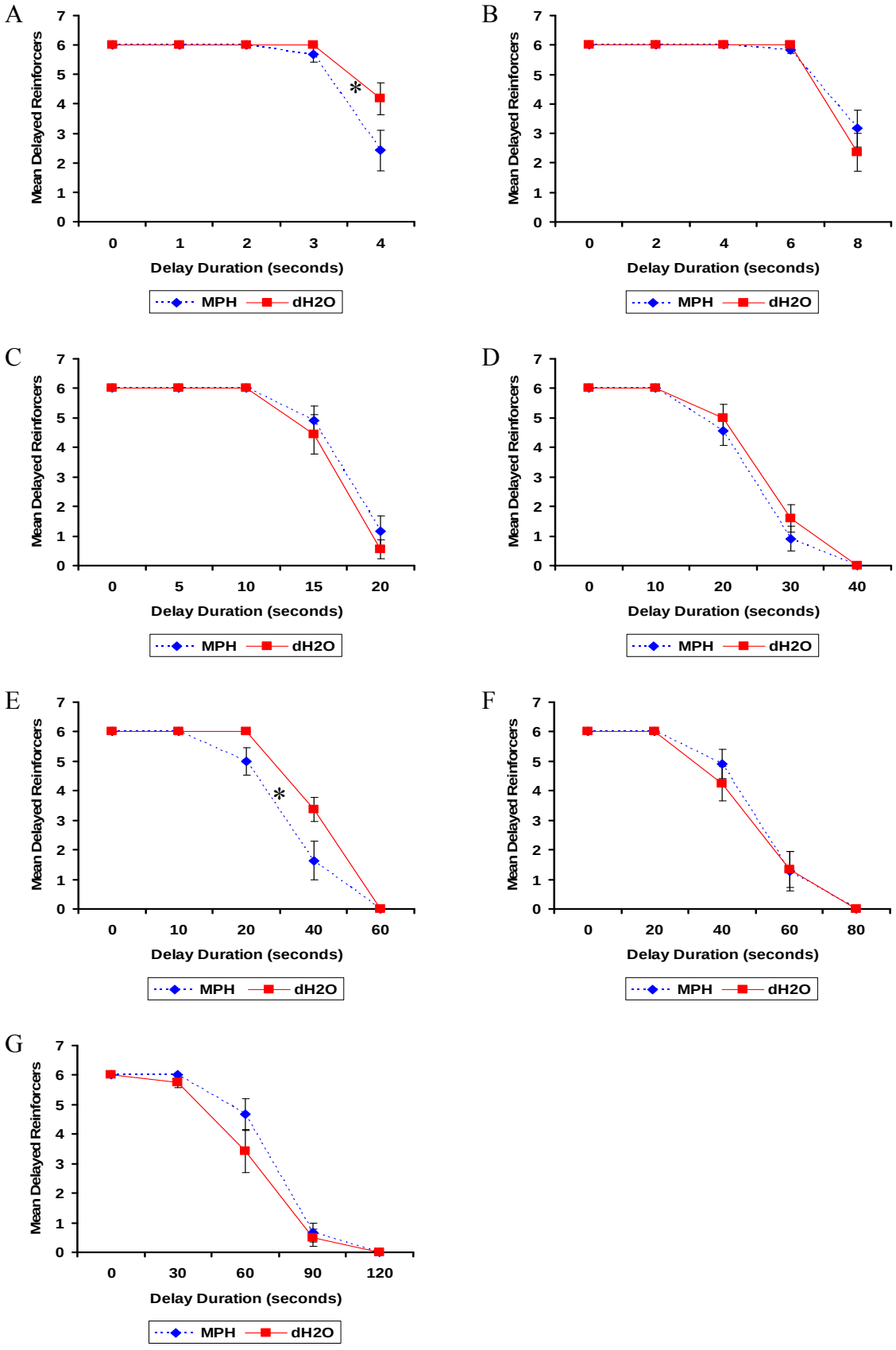

Figure 4. Mean (SEM) delayed reinforcers attained in the Delayed Reinforcement task by Wistar-Kyoto rats previously treated with methylphenidate (MPH) or distilled water (dH2O) on Test 1 to 7 (A to G, respectively). * Significant difference between treatment, $p < 0.05$.

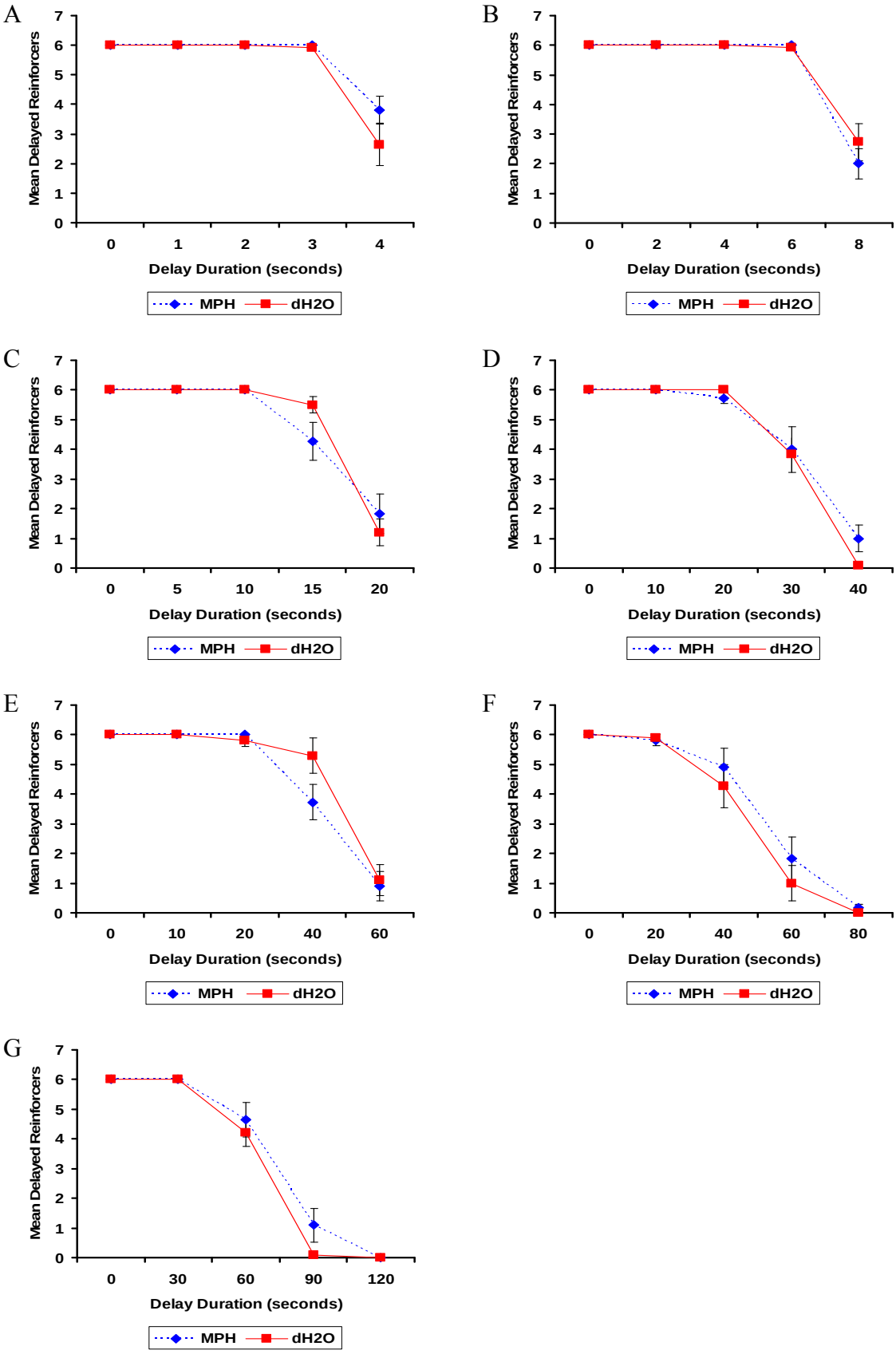

Figure 5. Mean (SEM) delayed reinforcers attained in the Delayed Reinforcement task by Spontaneously Hypertensive rats previously treated with methylphenidate (MPH) or distilled water (dH2O) on Test 1 to 7 (A to G, respectively).

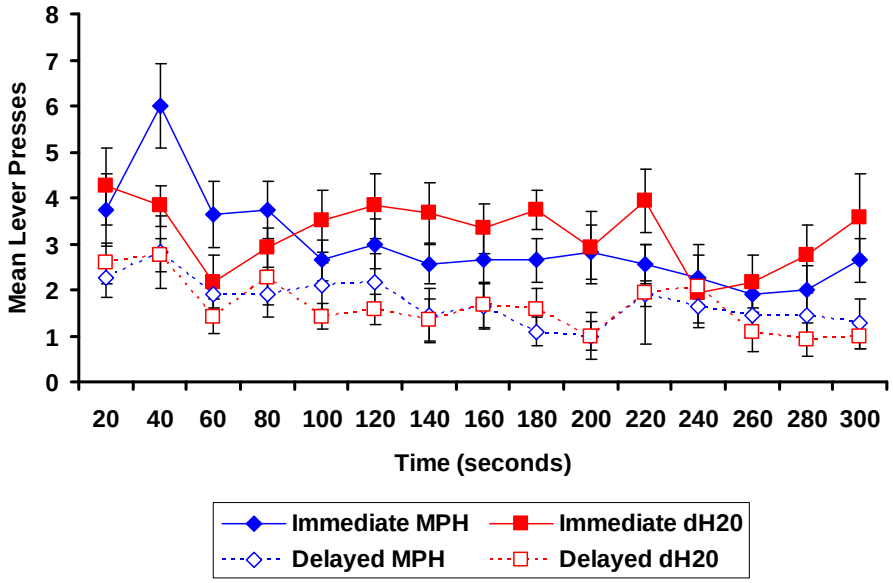

Figure 6. Mean (SEM) number of presses on the immediate (closed) and delayed (open) levers in consecutive 20 second intervals for Wistar-Kyoto rats previously treated with methylphenidate (MPH; blue diamond; n = 11) or distilled water (dH2O; red squares; n = 12), during the extinction task. There was a significant lever by time by treatment interaction, p = 0.039.

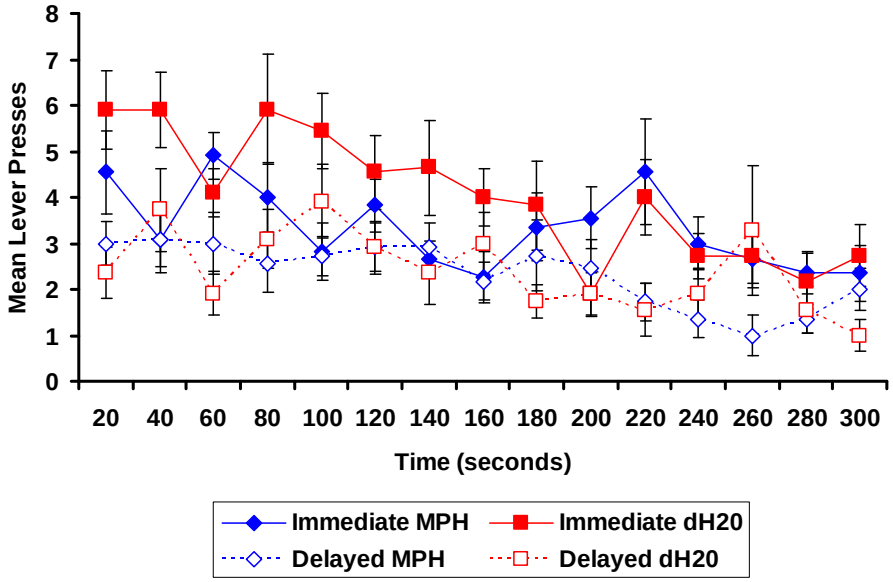

Figure 7. Mean (SEM) number of presses on the immediate (closed) and delayed (open) levers in consecutive 20 second intervals for Spontaneously Hypertensive rats previously treated with methylphenidate (MPH; blue diamond; n = 11) or distilled water (dH2O; red squares; n = 11), during the extinction task.

4.3.4 RAM (post chronic treatment)

4.3.4.1 Results of errors made by WKYs

There was no difference in the acquisition of the RAM task between WKYs in the MPH group and those in the dH2O control, $p = 0.532$, as illustrated in Figure 8. There was a significant main effect of error type, $F\ (2, 30) = 149.607$, $p < 0.001$. On average, WKYs made significantly fewer across phase errors compared to non-baited errors and significantly fewer perseverative errors than both non-baited and across phase errors, p's < 0.05, as illustrated in Figure 9. There was no main effect of treatment and no error type by treatment interaction, p's > 0.05. Hence, the data show that there was no effect of treatment with MPH on the behaviour of WKYs on the RAM task.

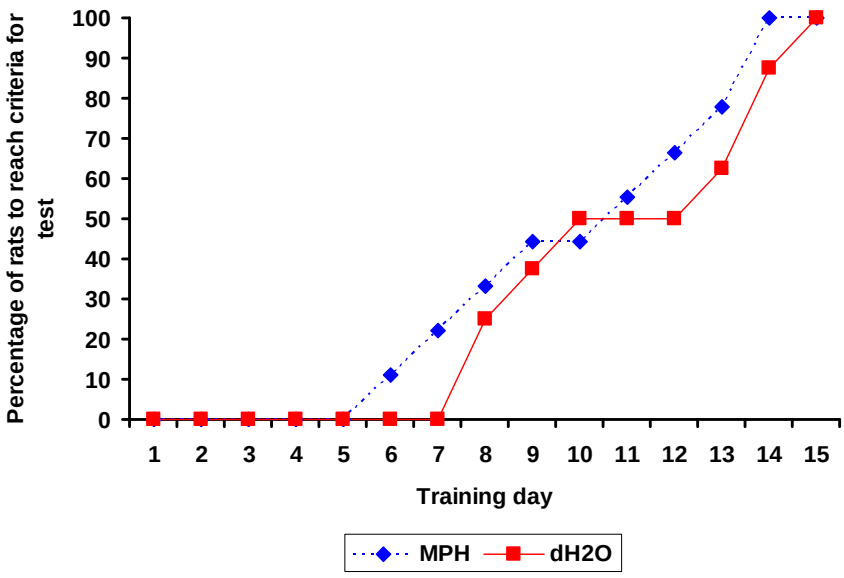

Figure 8: The number of training days required for Wistar-Kyoto rats previously treated with either methylphenidate (MPH) or distilled water (dH2O) to meet the first day of criteria to progress to testing. Following training the rats then completed 10 test days.

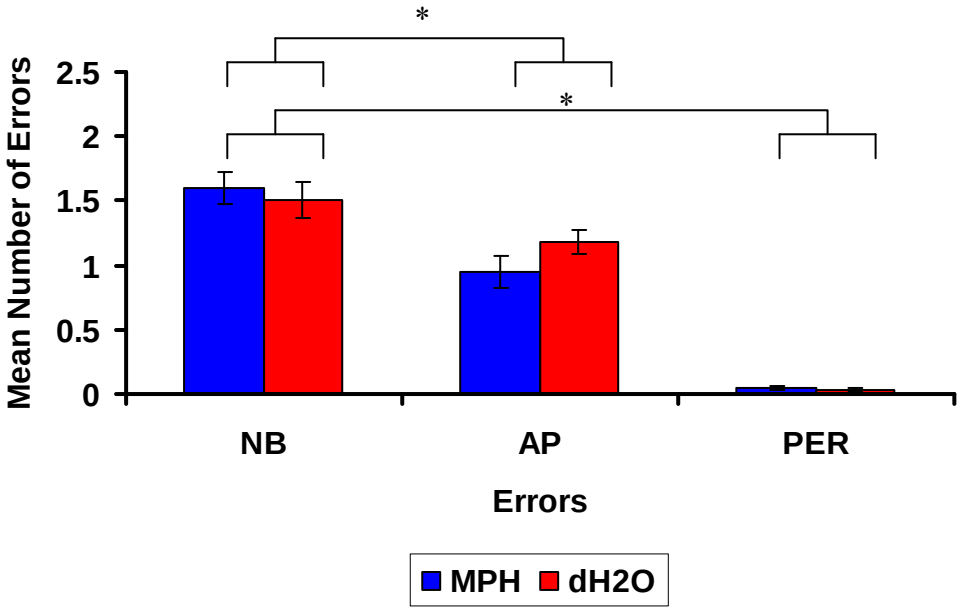

Figure 9. Mean (SEM) number of errors made in the Radial Arm Maze, averaged over 10 test days, for Wistar-Kyoto rats previously treated with either methylphenidate (MPH; n = 9) or distilled water (dH2O; n = 8). Error types recorded were non-baited errors (NB), across-phase errors (AP), and perseverative errors (PER). * Significant difference between error types. There was no effect of treatment on error type.

4.3.4.2 Results for SHRs

There was no difference between treatment in their acquisition of the RAM task, $p = 0.062$, illustrated in Figure 10. There was a significant main effect of error type, F (2, 34) = 160.303, $p < 0.001$. Parallel to the pattern described for the WKY, on average, SHRs made significantly fewer across phase errors compared to non-baited errors and significantly fewer perseverative errors than both non-baited and across phase errors, p's < 0.05, as illustrated in Figure 11. There was no main effect of treatment and no error type by treatment interaction, p's > 0.05. Previous MPH treatment of SHRs did not affect performance on the RAM.

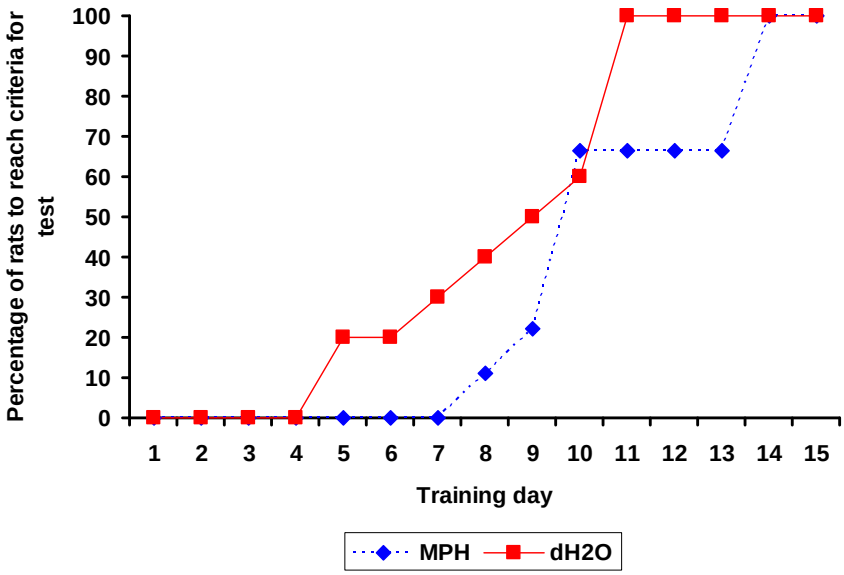

Figure 10: The number of training days required for Spontaneously Hypertensive rats previously treated with either methylphenidate (MPH) or distilled water (dH2O) to meet the first day of criteria to progress to testing. Following training the rats then completed 10 test days.

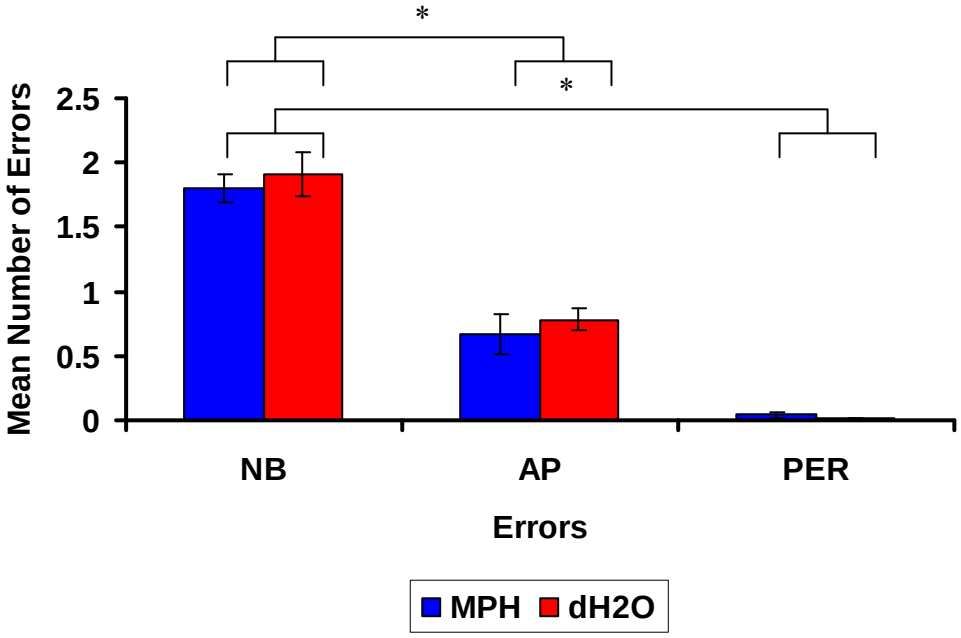

Figure 11. Mean (SEM) number of errors made in the Radial Arm Maze, averaged over 10 test days, for Spontaneously Hypertensive rats previously treated with either methylphenidate (MPH; n = 9) or distilled water (dH2O; n = 10). Error types recorded were non-baited errors (NB), across-phase errors (AP), and perseverative errors (PER). * Significant difference between error types. There was no effect of treatment on error type.

4.4 Discussion

The focus of this paper was to determine any enduring cognitive effects in non-ADHD subjects of chronic MPH treatment during development. This study chronically administered MPH to adolescent rats using a clinically relevant dosing regime. The findings suggest that chronic MPH treatment to a non-ADHD (misdiagnosed) rat, the WKY, results in altered cognitive performance in adulthood. WKYs treated with MPH throughout adolescence were found to have increased impulsivity and sensitivity to delay in adulthood, which was not evident in MPH treated SHRs (ADHD rats). These long-term effects of MPH treatment did not transfer across to performance on the RAM. Specifically, the significant effects of MPH on behaviour in the present study which were restricted to the WKY strain were: 1) on the first day of treatment MPH increased locomotor activity; 2) MPH treatment significantly increased impulsivity; and 3) increased sensitivity to delay in the absence of a reinforcer in MPH treated WKYs during the EXT task.

In contrast to the WKY, chronic MPH treatment during adolescence had no long-term cognitive effects on SHRs at adulthood. These results suggest that the enduring effects of chronic MPH treatment are different for SHRs and WKYs. These findings are consistent with previous research reporting strain differences in acute behavioural and locomotor effects of MPH (Thanos et al., 2010; Yang, Swann, & Dafny, 2006) and d-amphetamine (Hand, Fox, & Reilly, 2009). It was anticipated that MPH treatment would reduce SHR locomotor activity as previous research has demonstrated attenuated hyperactivity in the SHR following psychostimulant treatment (Myers et al., 1982). Differences in the acute locomotor effects of MPH between the strains provide a possible explanation for why the SHRs did not show the expected attenuation of hyperactivity during treatment in the current study, as the MPH dose may not have been therapeutically relevant for the ADHD strain.

The dose of MPH employed in this study was determined by a pilot study (Chapter 3), together with previous research assessing blood plasma levels following oral administration of MPH in non-ADHD rats (Berridge et al., 2006; Kuczenski & Segal, 2002). These previous studies both used Sprague-Dawley (SD) rats, while the pilot study showed in WKYs that the novel oral administration of MPH employed here produced similar behavioural responses to SDs treated with MPH via gavage (Gerasimov et al., 2000). Given the differences in MPH effect between WKYs and SHRs (Russell et al., 2000; Thanos et al., 2010; Yang et al., 2006), it is possible that the dose used in this study was not therapeutically relevant for the SHRs. Furthermore, the interpretation the effect of MPH in SHRs is complicated by a significant elevation in the baseline locomotor activity between the treatment groups. Therefore, the results of the effect of MPH treatment on SHR behaviours should be interpreted with some caution.

The locomotor results of MPH treatment for the WKYs were not entirely as expected. Based on previous research (Berridge et al., 2006; Gerasimov et al., 2000), the therapeutic dose of MPH administered in the current study was not expected to alter locomotor activity in the WKY. On the first week of MPH treatment, WKYs showed locomotor activation compared to vehicle controls. This acute increase in locomotor activity in the current study is not consistent with the results of Chapter 3 which found no locomotor activation following an identically administered 2 mg/kg dose of MPH. This inconsistency could have occurred as age has been shown to play a crucial role in the effect of MPH (Andersen et al., 2002). The rats in the current study were approximately 2 weeks younger than the rats in Chapter 3 when the first drug dose was administered. The age difference may therefore explain the small inconsistency between the studies. From the second week of treatment there was no increase in activity with MPH administration, which is consistent with expectations and previous research reporting no increase in locomotor activity following acute therapeutic doses of

MPH in 'normal' rats (Berridge et al., 2006; Gerasimov et al., 2000). The current study is the first to monitor locomotor activity throughout chronic therapeutically relevant administration of MPH.

Consistent with expectations, WKYs treated with MPH demonstrated long-term changes in cognitive performance. Following treatment, MPH pretreated WKYs were found to be more impulsive and sensitive to delays compared to WKYs pretreated with dH20. The current study is the first to report increased impulsivity following inappropriate, chronic MPH treatment in rodents. The findings of the impulsive choice task employed in the current study imply that WKYs chronically treated with MPH throughout adolescence are more susceptible to the influence of immediate gratification where their behaviour is not as strongly guided by the long-term consequences of their actions. However, the results of this study are unable to determine whether the previous chronic MPH treatment produced altered sensitivity to the increasing delay or the value of the reward. As the rats were drug free when impulsivity testing was conducted, it is unlikely that the appetite suppressive effects of MPH could explain the altered response pattern of WKYs previously treated with MPH.

The finding of increased impulsivity following chronic MPH administration during adolescence is not consistent with the findings of Adriani and colleagues (2007). Adriani and colleagues (2007) reported a reduction of impulsivity in adult rats that were pretreated with MPH. The inconsistent findings are likely due to different methods of MPH administration. The current study delivered a low (2mg/kg), oral dose of MPH twice a day during the rats' active period in their circadian cycle, while the Adriani study administered a single daily dose (2 mg/kg), via i.p. injection during the light period or 'inactive' phase for the rats. The method employed by Adriani and colleagues (2007) is not consistent with clinical dosing regimes in which children are orally treated with low doses of MPH, during their 'active' phase of their

circadian cycle (Kuczenski & Segal, 2002). Furthermore, as discussed in Chapter 3, an i.p. injection of 2 mg/kg MPH would result in rapid elevation and higher peak plasma concentrations of the drug than are considered therapeutically relevant (Gerasimov et al., 2000; Swanson & Volkow, 2001). The different method of drug administration used by Adriani and colleagues (2007) reduces the relevance of their preclinical results. Using a method of drug administration that more closely reflects the dosing regime employed clinically, the results of the current study indicate that inappropriate treatment with MPH may increase impulsivity in adulthood.

The MPH induced impulsivity in adulthood is consistent with previous reports of PFC dysfunction following chronic psychostimulant drug administration. Cocaine is a psychostimulant with a similar mechanism of action to MPH (Gatley et al., 1996; Kuczenski & Segal, 1997). In monkeys and rats, chronic treatment with cocaine has been shown to impair the PFC mediated task of reversal learning (Jenstch, Olausson, De La Garza, & Taylor, 2002; Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004). Reversal learning is the ability to adapt behaviour according to changing reward contingencies (Rolls, 2000). As reversal learning involves the inhibition of a previously rewarded response, impulsive behaviour has been associated with impairments of reversal learning (Jentsch & Taylor, 1999). The results from the current study expand and extend the previous findings by demonstrating similar long-term changes in impulsive-like behaviour following prior chronic treatment with the psychostimulant MPH.

The current findings have important implications for children chronically treated with MPH. If children have been misdiagnosed with ADHD and are inappropriately treated with MPH, these findings suggest that the chronic treatment may induce impulsive behaviour in adulthood. Increased impulsivity is a significant concern as this behaviour is an important

feature of substance use and abuse (Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009a, 2009b), conduct disorder and schizophrenia (American Psychiatric Association, 2000). Concerns have also been raised regarding adolescent treatment with psychostimulants, such as MPH, potentially leading to later substance abuse (Kollins, MacDonald, & Rush, 2001; Vitiello, 2001). Given the close relationship between substance abuse and impulsivity, the findings from the current study validate these concerns as the increased impulsivity following MPH treatment may increase the possibility of later substance abuse.

The MPH induced increase in impulsive behaviour in adulthood did not transfer to the WKYs cognitive performance in adulthood as assessed in the RAM task. There are a number of reasons why a global cognitive deficit was not identified in the current study. Firstly, the RAM model may not have had sufficient sensitivity to detect minor memory deficits. Longer intervals (≥ 2 hours) were required to show a psychostimulant effect on a novel object recognition task (Bisagno, Ferguson, & Luine, 2003), therefore the 15 minute delay employed in the RAM may not have provided sufficient difficulty to detect treatment effects. Second, performance on the DR and EXT tasks may be more responsive to fluctuations of catecholamine levels in the PFC compared to the RAM. Finally, the increased impulsivity identified in the DRT may be a transient behavioural effect and the underlying processes to affect memory function may have recovered by the time of RAM testing. The current study does not allow us to determine the persistence of this effect, as repeated assessment of impulsivity was not conducted. One way to elucidate the persistence of this deficit would be to repeat the current study assessing impulsivity at different time points in different subsets of animals.

Previous studies identified impairments of spatial and recognition memory after treatment with MPH (Bethancourt et al., 2009; LeBlanc-Duchin & Taukulis, 2007, 2009). Differences

in the methodologies of the previous and current research could explain this discrepancy. These previous studies assessed memory functioning with a novel object recognition task following the administration of higher doses of 3 and 5 mg/kg of MPH to result in memory impairment after cessation of treatment (Bethancourt et al., 2009; LeBlanc-Duchin & Taukulis, 2007, 2009). As noted earlier, higher doses of MPH can result in blood plasma concentrations which exceed therapeutic relevance (Swanson & Volkow, 2001). Both Bethancourt and colleagues (2009) and LeBlanc-Duchin & Taukulis (2009) reported no treatment effect on memory, with an equivalent dose of MPH to the current study (2 mg/kg). Therefore, when a more therapeutically relevant dose is employed in adolescence there is no evidence of long-term effects of chronic MPH treatment on memory function.

A possible explanation for the differences in the long-term effect of MPH on cognitive performance between WKY and SHR rats derives from current research on levels of catecholamines in the PFC. It is hypothesised that the therapeutic action of low doses of MPH in ADHD is to increase abnormally low levels of DA and NA in the PFC, increasing neural activity and improving cognitive performance (Arnsten & Dudley, 2005; Berridge & Devilbiss, 2008; Berridge et al., 2006). However, when MPH is administered to those with 'normal' catecholamine function in the PFC, the resultant increase in DA and NA produces catecholamine levels in excess of what is optimal, resulting in PFC impairment (Arnsten, 2006, 2009) . The long-term behavioural effects of MPH in the present study may be consistent with the hypothesis that psychostimulants produce a persistent reorganisation of patterns of synaptic connectivity in brain regions including the PFC, altering their function (Robinson & Kolb, 2004). The duration of these changes are yet to be identified for MPH.

The period of time between discontinuation of treatment and behavioural testing is critical. A period of withdrawal typically extends for one week following cessation of treatment and

these withdrawal symptoms may interfere with behavioural measures (Pierce & Kalivas, 1997). In a study with the goal of measuring enduring behavioural or neurochemical change, it is usual that experimental measures are collected at least a week following cessation of treatment to avoid measures of transient changes (Pierce & Kalivas, 1997). In the present study, it is likely that the rats had progressed past the withdrawal phase and were exhibiting enduring changes in impulsivity as these measures were attained over two weeks after the final dose of MPH. Similar periods between discontinuation of chronic psychostimulant treatment and assessment of enduring changes have been employed in previous studies (Floresco & Whelan, 2009; Li & Kauer, 2004).

There are some significant points that could be considered limitations of the current study and areas for future research. The rats in the present study were subjected to individual housing and food/water restriction. These factors have been reported as potentially stressful (Jones, Marsden, & Robbins, 1991). The decision was made to employ individual housing in the current study to facilitate drug administration without handling the rats, as handling has been shown to modulate MPH-induced catecholamine levels in the PFC (Marsteller et al., 2002). In regard to the water/food restriction, it is important to note that the rats showed continual appropriate weight gain throughout the experiment. With this in mind, the rats were not necessarily deprived of food and water, but were restricted to consuming their food and water during specific periods. Additionally, as discussed in Chapter 3, the water restriction method of drug administration employed in this study may have altered the pharmacological properties of MPH. However, as the findings of the pilot study (Chapter 3) were consistent with previous research administering MPH via gavage (Gerasimov et al., 2000), alterations in the pharmacological properties as a result of the administration method are unlikely. It is also important to note that the single PIR sensor used to measure locomotor activity does not allow for the evaluation of horizontal and vertical exploration. Future research would benefit from

measuring repetitive stereotypies and grooming to elucidate the effect MPH has on these behaviours. Finally, it should be kept in mind that the validity of the WKY as a 'normal' strain has previously been questioned (Alsop, 2007; Drolet et al., 2002; van den Bergh et al., 2006). However, as discussed in Pardey et al. (2009, Chapter 2), WKYs were not found to be hypoactive compared to SHRs in operant tasks as they are employed in this laboratory. It would be prudent to include additional 'normal' rat strains to establish that reported group differences are not the result of deficits in the WKY. However, consideration must be given to the large genetic variation that would be introduced by including such out-bred strains.

Further investigation of the neural mechanisms underlying persistent cognitive changes following chronic MPH treatment is necessary. MPH is commonly prescribed to children and adolescents to treat ADHD, with prescriptions rates rapidly rising in the last decade (Berbatis, Sunderland, & Bulsara, 2002; Preen, Calver, Sanfilippo, Bulsara, & Holman, 2007; Prosser & Reid, 1999, 2009). This emphasizes the need for more clinically appropriate research to elucidate the long-term effects of MPH treatment. It is imperative that future research employs dosing regimes that allow the results to be applicable to the human situation. Furthermore, future research would benefit from investigating the effects of chronic MPH treatment at different ages, early, mid, late adolescence, and even adulthood to determine if one period of development is more sensitive to pharmacological intervention than another.

In conclusion, the results of this study suggest there are elevated levels of impulsivity in adulthood when 'normal' rats inappropriately received chronic MPH treatment throughout adolescence. However, when chronic MPH treatment was appropriately given to an animal model of ADHD, there were no long-term effects observed in adulthood. Such a finding has particularly relevance for children that are misdiagnosed with ADHD and as a result, are chronically medicated with psychostimulants such as MPH. It could be inferred from the

results of this study that children misdiagnosed with ADHD may have enduring cognitive deficits in adulthood as a result of their treatment. This study highlights the importance of developing more sensitive, less subjective diagnostic criteria for ADHD.

4.5 References

Adriani, W., Canese, R., Podo, F., & Laviola, G. (2007). ^{1}H MRS-detectable metabolic brain changes and reduced impulsive behavior in adult rats exposed to methylphenidate during adolescence. *Neurotoxicology and Teratology, 29*, 116-125.

Adriani, W., & Laviola, G. (2003). Elevated levels of impulsivity and reduced place conditioning with *d*-Amphetamine: Two behavioral features of adolescence in mice. *Behavioral Neuroscience, 117* (4), 695-703.

Adriani, W., & Laviola, G. (2004). Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology, 15*, 341-352.

Alsop, B. (2007). Problems with spontaneously hypertensive rats (SHR) as a model of Attention-Deficit/Hyperactivity Disorder (AD/HD). *Journal of Neuroscience Methods, 162*, 42-48.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision ed.). Washington, DC: American Psychiatric Association.

Andersen, S. L., Arvanitogiannis, A., Pliakas, A. M., LeBlanc, C., & Carlezon, W. A. (2002). Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nature Neuroscience, 5* (1), 13-14.

Angold, A., Erkanli, A., Egger, H. L., & Costello, E. J. (2000). Stimulant treatment for children: A community perspective. *Journal of the American Academy of Child and Adolescent Psychiatry, 39* (8), 975-984.

Arnsten, A. F. T. (2006). Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology, 31*, 2376-2383.

Arnsten, A. F. T. (2009). Towards a new understanding of attention-deficit hyperactivity disorder pathophysiology. An important role for prefrontal cortex dysfunction. *CNS Drugs, 23* (supplementary 1), 33-41.

Arnsten, A. F. T., & Dudley, A. G. (2005). Methylphenidate improves prefrontal cortical cognitive function through α_2 adrenoreceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behavioral and Brain Functions, 1*, 1-9.

Augustyniak, P. N., Kourrich, S., Rezazadeh, S. M., Stewart, J., & Arvanitogiannis, A. (2006). Differential behavioral and neurochemical effects of cocaine after early exposure to methylphenidate in an animal model of attention deficit hyperactivity disorder. *Behavioural Brain Research, 167*, 379-382.

Baddeley, A. D., & Hitch, G. J. (1974). Working memory. In G. H. Bower (Ed.), *The psychology of learning and motivation: Advances in research and theory* (Vol. 8, pp. 647-667). New York: Academic Press.

Berbatis, C. G., Sunderland, V. B., & Bulsara, M. (2002). Licit psychostimulant consumption in Australia, 1984-2000: International and jurisdictional comparison. *The Medical Journal of Australia, 177*, 539-543.

Berger, D., & Sagvolden, T. (1998). Sex differences in operant discrimination behaviour in an animal model of Attention-Deficit Hyperactivity Disorder. *Behavioural Brain Research, 94*, 73-82.

Berridge, C. W., & Devilbiss, D. M. (2008). Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biological Psychiatry, 64*, 626-635.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F. T., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine

neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60*, 1111-1120.

Bethancourt, J. A., Camarena, Z. Z., & Britton, G. B. (2009). Exposure to oral methylphenidate from adolescence through young adulthood produces transient effects on hippocampal-sensitive memory in rats. *Behavioural Brain Research, 202*, 50-57.

Biederman, J., & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet, 366*, 237-248.

Bisagno, V., Ferguson, D., & Luine, V. N. (2003). Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. *Pharmacology, Biochemistry and Behavior, 74*, 859-867.

Bolanos, C. A., Barrot, M., Berton, O., Wallace-Black, D., & Nestler, E. J. (2003). Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biological Psychiatry, 54*, 1317-1329.

Bolanos, C. A., Willey, M. D., Maffeo, M. L., Powers, K. D., Kinka, D. W., Grausam, K. B., et al. (2008). Antidepressant treatment can normalize adult behavioral deficits induced by early-life exposure to methylphenidate. *Biological Psychiatry, 63*, 309-316.

Brandon, C. L., Marinelli, M., Baker, L. K., & White, F. J. (2001). Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology, 25* (5), 651-661.

Carlezon, W. A., Mague, S. D., & Andersen, S. L. (2003). Enduring behavioral effects of early exposure to methylphenidate in rats. *Biological Psychiatry, 54*, 1330-1337.

Castellanos, F. X., Giedd, J. N., Marsh, W. L., Hamburger, S. D., Vaituzis, A. C., Dickstein, D. P., et al. (1996). Quantitative brain magnetic resonance imaging in Attention-Deficit Hyperactivity Disorder. *Archives of General Psychiatry, 53* (7), 607-616.

Drolet, G., Proulx, K., Pearson, D., Rochford, J., & Deschepper, C. F. (2002). Comparisons of behavioral and neurochemical characteristics between WKY, WKHA, and Wistar Rat Strains. *Neuropsychopharmacology, 27*, 400-409.

Eagle, D. M., Baunez, C., Hutcheson, D. M., Lehmann, O., Shah, A. P., & Robbins, T. W. (2008). Stop-signal reaction-time task performance: Role of prefrontal cortex and subthalamic nucleus. *Cerebral Cortex, 18*, 178-188.

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*, 161-170.

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology, 48* (3), 589-601.

Floresco, S. B., & Whelan, J. M. (2009). Perturbations in different forms of cost/benefit decision making induced by repeated amphetamine exposure. *Psychopharmacology, 205*, 189-201.

Fox, A. T., Hand, D. J., & Reilly, M. P. (2008). Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behavioural Brain Research, 187*, 146-152.

Gatley, S. J., Pan, D., Chen, R., Chaturvedi, G., & Ding, Y.-S. (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sciences, 58* (12), PL 231-239.

Gaytan, O., Yang, P., Swann, A., & Dafny, N. (2000). Diurnal differences in sensitization to methylphenidate. *Brain Research, 864*, 24-39.

Gerasimov, M., Franceschi, M., Volkow, N. D., Gifford, A., Gatley, S. J., Marsteller, D., et al. (2000). Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *The Journal of Pharmacology and Experimental Therapeutics, 295* (1), 51-57.

Gray, J. D., Punsoni, M., Tabori, N. E., Melton, J. T., Fanslow, V., Ward, M. J., et al. (2007). Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *The Journal of Neuroscience, 27*, 7196-7207.

Greenhill, L., Beyer, D. H., Finkleson, J., Shaffer, D., Biederman, J., Conners, C. K., et al. (2002). Guidelines and algorithms for the use of methylphenidate in children with attention-deficit/hyperactivity disorder. *Journal of Attention Disorders, 6 (Supplemental 1)*, S89-100.

Hand, D. J., Fox, A. T., & Reilly, M. P. (2009). Differential effects of *d*-amphetamine on impulsive choice in spontaneously hypertensive and Wistar-Kyoto rats. *Behavioural Pharmacology, 20*, 549-553.

Handler, M. W., & DuPaul, G. J. (2005). Assessment of ADHD: Differences across psychology specialty areas. *Journal of Attention Disorders, 9* (2), 402-412.

Heyser, C. J., Pelletier, M., & Ferris, J. S. (2004). The effects of methylphenidate on novel object exploration in weanling and periadolescent rats. *Annals of the New York Academy of Sciences, 1021*, 465-469.

Jenstch, J. D., Olausson, P., De La Garza, R., & Taylor, J. R. (2002). Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. *Neuropsychopharmacology, 26*, 183-190.

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behaviour by reward-related stimuli. *Psychopharmacology (Berl), 146*, 373-390.

Johansen, E. B., Sagvolden, T., & Kvande, G. (2005). Effects of delayed reinforcers on the behavior of an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 162*, 47-61.

Jones, G. H., Marsden, C. A., & Robbins, T. W. (1991). Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: Neurochemical correlates. *Behavioural Brain Research, 43* (1), 35-50.

Kolb, B., Gibb, R., & Gorny, G. (2003). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory, 79*, 1-10.

Kollins, S. H., MacDonald, E. K., & Rush, C. R. (2001). Assessing the abuse potential of methylphenidate in nonhuman and human subjects: A review. *Pharmacology Biochemistry and Behavior, 68*, 611-627.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: Comparison with amphetamine. *Journal of Neurochemistry, 68*, 2032-2037.

Kuczenski, R., & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular noreprinephrine and absence of sensitization and cross-sensitization to methamphetamine. *The Journal of Neuroscience, 22* (16), 7264-7271.

Lawrence, A. J., Luty, J., Bogdan, N. A., Sahakian, B. J., & Clark, L. (2009a). Impulsivity and response inhibition in alcohol dependence and problem gambling. *Psychopharmacology, 207*, 163-172.

Lawrence, A. J., Luty, J., Bogdan, N. A., Sahakian, B. J., & Clark, L. (2009b). Problem gamblers share deficits in impulsive decision-making with alcohol-dependent individuals. *Addiction, 104*, 1006-1015.

LeBlanc-Duchin, D., & Taukulis, H. K. (2007). Chronic oral methylphenidate administration to periadolescent rats yields prolonged impairment of memory for objects. *Neurobiology of Learning and Memory, 88*, 312-320.

LeBlanc-Duchin, D., & Taukulis, H. K. (2009). Chronic oral methylphenidate induces post-treatment impairment in recognition and spatial memory in adult rats. *Neurobiology of Learning and Memory, 91*, 218-225.

Li, Y., & Kauer, J. A. (2004). Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. *Synapse, 51* (1), 1-10.

Mague, S. D., Andersen, S. L., & Carlezon, W. A. (2005). Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. *Biological Psychiatry, 57*, 120-125.

Marsteller, D. A., Gerasimov, M. R., Schiffer, W. K., Geiger, J. M., Barnett, C. R., Borg, J. S., et al. (2002). Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. *Neuropsychopharmacology, 27*, 163-170.

Martins, S., Tramontina, S., Polanczyk, G., Eizirik, M., Swanson, J. M., & Rohde, L. A. (2004). Weekend holidays during methylphenidate use in ADHD children: A randomized clinical trial. *Journal of Child and Adolescent Psychopharmacology, 14* (2), 195-205.

McGregor, I. S. (1996). Using Strawberry Tree Workbench Mac and Workbench PC for data acquisition and control in the animal learning laboratory. *Behavior Research Methods, Instruments and Computers, 28*, 38-48.

Mobini, S., Body, S., Ho, M.-Y., Bradshaw, C. M., Szabadi, E., Deakin, J. F. W., et al. (2002). Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology, 160*, 290-298.

Myers, M. M., Musty, R. E., & Hendley, E. D. (1982). Attenuation of hyperactivity in the Spontaneously Hypertensive rat by amphetamine. *Behavioral and Neural Biology, 34*, 42-54.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.).

Pardey, M. C., Homewood, J., Taylor, A., & Cornish, J. L. (2009). Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods, 176*, 166-171.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Reviews, 25*, 192-216.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*, 1385-1390.

Preen, D. B., Calver, J., Sanfilippo, F. M., Bulsara, M., & Holman, C. D. J. (2007). Patterns of psychostimulant prescribing to children with ADHD in Western Australia: Variations in age, gender, medication type and dose prescribed. *Australian and New Zealand Journal of Public Health, 31*, 120-126.

Prosser, B., & Reid, R. (1999). Psychostimulant use for children with ADHD in Australia. *Journal of Emotional and Behavioral Disorders, 7*, 110-117.

Prosser, B., & Reid, R. (2009). Changes in use of psychostimulant medication for ADHD in South Australia (1990-2006). *Australian and New Zealand Journal of Psychiatry, 43*, 340-347.

Rice, D., & Barone Jr., S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environmental Health Perspectives, 108* (supplement 3), 511-533.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology, 47*, 33-46.

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cerebral Cortex, 10*, 284-294.

Rossi, A. F., Bichot, N. P., Desimone, R., & Ungerleider, L. G. (2007). Top-down attentional deficits in Macaques with lesions of lateral prefrontal cortex. *The Journal of Neuroscience, 27* (42), 11306-11314.

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S. C. R., Simmons, A., et al. (1999). Hypofrontality in Attention Deficit Hyperactivity Disorder during higher-order motor control: A study with functional MRI. *The American Journal of Psychiatry, 156* (6), 891-896.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C. L., & Taljaard, J. J. F. (2000). Methylphenidate affects striatal dopamine differently in an animal model for attention-deficit/hyperactivity disorder - The spontaneously hypertensive rat. *Brain Research Bulletin, 53* (2), 187-192.

Russell, V. A., Sagvolden, T., & Johansen, E. B. (2005). Animal models of attention-deficit/hyperactivity disorder. *Behavioral and Brain Functions, 1*, 9.

Sagvolden, T., Aase, H., Zeiner, P., & Berger, D. (1998). Altered reinforcement mechanisms in Attention-Deficit/Hyperactivity Disorder. *Behavioural Brain Research, 94*, 61-71.

Sagvolden, T., Johansen, E. B., Woien, G., Walaas, S. I., Storm-Mathisen, J., Bergersen, L. H., et al. (2009). The spontaneously hypertensive rat model of ADHD - The importance of selecting the appropriate reference strain. *Neuropharmacology, 57*, 619-626.

Sagvolden, T., Metzger, M. A., Schiorbeck, H. K., Rugland, A.-L., Spinnangr, I., & Sagvolden, G. (1992). The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): Changed reactivity to reinforcers and to psychomotor stimulants. *Behavioral and Neural Biology, 58*, 103-112.

Sagvolden, T., Russell, V. A., Aase, H., Johansen, E. B., & Farshbaf, M. (2005). Rodent models of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57*, 1239-1247.

Sawyer, M. G., Rey, J. M., Graetz, B. W., Clark, J. J., & Baghurst, P. A. (2002). Use of medication by young people with attention-deficit/hyperactivity disorder. *Medical Journal of Australia, 177*, 21-25.

Scherer, E. B. S., da Cunha, M. J., Matte, C., Schmitz, F., Netto, C. A., & Wyse, A. T. S. (2010). Methylphenidate affects memory, brain-derived neurotrophic factor immunocontent and brain acetylcholinesterase activity in the rat. *Neurobiology of Learning and Memory, 94*, 247-253.

Schoenbaum, G., Saddoris, M. P., Ramus, S. J., Shaham, Y., & Setlow, B. (2004). Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. *European Journal of Neuroscience, 19*, 1997-2002.

Sciutto, M. J., & Eisenberg, M. (2007). Evaluating the evidence for and against the overdiagnosis of ADHD. *Journal of Attention Disorders, 11* (2), 106-113.

Solanto, M. V., & Alvir, J. (2009). Reliability of DSM-IV symptom ratings of ADHD: Implications for DSM-V. *Journal of Attention Disorders, 13* (2), 107-116.

Swanson, J. M., & Volkow, N. D. (2001). Pharmacokinetic and pharmacodynamic properties of methylphenidate in humans. In M. V. Solanto, A. F. T. Arnsten & F. X. Castellanos (Eds.), *Stimulant drugs and ADHD: Basic and clinical neuroscience* (pp. 259-282). New York: Oxford University Press.

Taylor, C. L., Latimer, M. P., & Winn, P. (2003). Impaired delayed spatial win-shift behaviour on the eight arm maze following excitotoxic lesions of the medial prefrontal cortex in the rat. *Behavioural Brain Research, 147*, 107-114.

Thanos, P. K., Ivanov, I., Robinson, J. K., Michaelides, M., Wang, G.-J., Swanson, J. M., et al. (2010). Dissociation between spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats in baseline performance and methylphenidate response on measures of attention, impulsivity and hyperactivity in a Visual Stimulus Position Discrimination Task. *Pharmacology, Biochemistry and Behavior, 94*, 374-379.

van den Bergh, F. S., Bloemarts, E., Chan, J. S. W., Groenink, L., Olivier, B., & Oosting, R. S. (2006). Spontaneously hypertensive rats do not predict symptoms of Attention-Deficit Hyperactivity Disorder. *Pharmacology, Biochemistry and Behavior, 83*, 380-390.

Vitiello, B. (2001). Long-term effects of stimulant medications on the brain: Possible relevance to the treatment of attention deficit hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology, 11*, 25-34.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Franceschi, D., Maynard, L., et al. (2002). Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeutic implications. *Synapse, 43*, 181-187.

Wilens, T. E. (2008). Effects of methylphenidate on the catecholaminergic system in Attention-Deficit/Hyperactivity Disorder. *Journal of Clinical Psychopharmacology, 28* (3), S46-S53.

Yang, P. B., Swann, A. C., & Dafny, N. (2006). Acute and chronic methylphenidate dose response assessment on three adolescent male rat strains. *Brain Research Bulletin, 71*, 301-310.

Zito, J. M., Safer, D. J., dosReis, S., Gardner, J. F., Boles, M., & Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. *JAMA, 283* (8), 1025-1030.

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Chapter 5

Long-term effects of chronic Ritalin administration on tyrosine hydroxylase immunostained neurons as a measure of neural development in adolescent rats

5.1 Introduction

Methylphenidate (MPH; Ritalin®) is a psychostimulant commonly used to treat Attention Deficit/Hyperactivity Disorder (ADHD) in children. Over the past 20 years, prescription rates for MPH and other psychostimulants used to treat ADHD have increased (Barbaresi et al., 2006; Prosser & Reid, 1999). A trend towards the starting age of psychostimulant treatment becoming younger has been reported (Prosser & Reid, 2009), with children as young as 2 years old receiving psychostimulant treatment (Zito et al., 2000). There have also been reports that children who do not meet full diagnostic criteria of ADHD are receiving MPH or another psychostimulant (Angold, Erkanli, Egger, & Costello, 2000; Sagvolden et al., 1992; Sawyer, Rey, Graetz, Clark, & Baghurst, 2002). This raises the possibility that children are being misdiagnosed with ADHD and are therefore inappropriately treated with powerful psychostimulants such as MPH.

The therapeutic action of MPH is to increase levels of catecholamines by blocking the dopamine (DA) and noradrenaline (NA) transporters (Gatley, Pan, Chen, Chaturvedi, & Ding, 1996; Kuczenski & Segal, 1997; Volkow et al., 2002). When therapeutically relevant doses of MPH are administered to rats, NA and DA increase within the prefrontal cortex (PFC; Berridge & Devilbiss, 2008; Berridge et al., 2006). These elevated levels of DA and NA have been associated with improved performance on PFC dependent tasks such as those involving working memory and attention (Arnsten & Dudley, 2005; Berridge et al., 2006).

A previous finding of this thesis was that PFC dependent behavioural deficits were shown in adulthood following chronic treatment with MPH throughout adolescence in a non-ADHD rat strain (Chapter 4). The findings of this study were that the Wistar-Kyoto rats (WKY; non-ADHD or misdiagnosed strain) treated with MPH during adolescence were more impulsive and sensitive to delays in a delayed reinforcement task when tested drug-free in adulthood, compared to WKYs treated with vehicle (water). In contrast, no effect of chronic MPH treatment was observed during this task in the animal model of ADHD, the Spontaneously Hypertensive rat (SHR). The selective effect of chronic MPH administration to only produce deficits in the behavioural function of the WKY suggest alterations to underlying brain function in this strain. To investigate this possibility, the current study was conducted to measure changes to catecholamine neurons of the PFC following chronic MPH treatment.

The PFC is innervated by DA and NA projections originating within the brainstem. The mesocortical DA pathway originates in the ventral tegmental area (VTA; A10 region) and projects to the PFC (Fluxe et al., 1974). This pathway is involved in cognitive functioning (Arnsten & Li, 2005). The ascending noradrenergic projections originate in the locus coeruleus (LC; A6 region; Dahlstrom & Fuxe, 1964). The LC exclusively provides NA to subcortical and cortical structures and is involved in arousal and cognitive functioning (Berridge, 2008; Berridge & Waterhouse, 2003).

The PFC undergoes extensive synaptogenesis and neural remodelling during childhood and adolescence (Adriani & Laviola, 2004). Alterations in neural remodelling, known as synaptic plasticity, are influenced by environmental stimuli and the administration of psychoactive drugs (Kolb, Gibb, & Gorny, 2003a; Robinson & Kolb, 2004). For example, repeated administration of amphetamine or cocaine changes the morphology of the pyramidal neurons within the PFC, increasing the dendritic branching and spine density of these neurons

(Robinson, Gorny, Mitton, & Kolb, 2001; Robinson & Kolb, 1997, 1999). Region specific morphological changes following self-administration of amphetamine have also been reported, with an increase in spine density observed in the medial PFC while spine density decreased in the orbitofrontal cortex (OFC; Crombag, Gorny, Li, Kolb, & Robinson, 2005). Together, the previous research poses the possibility that chronic MPH administration may affect the developing prefrontal regions to subsequently alter the higher cognitive function of these regions.

Higher cognitive functions such as impulsivity and sensitivity to delay are well-described to be regulated by the PFC. Clear distinctions have been made between the function of the medial and orbital regions of the PFC which are reflected by their differing connectivity throughout the brain (Ongur & Price, 2000). The OFC has been associated with encoding the value of reward (Cardinal, 2006; Homayoun & Moghaddam, 2006), while the medial PFC including the prelimbic (PrL) and infralimbic (IL) regions are responsible for functions such as decision making, judgements and motor inhibition (Dalley, Mar, Economidou, & Robbins, 2008; Mulder, Nordquist, Orgut, & Pennartz, 2003; Peters, O'Donnell, & Carelli, 2005; Walton, Bannerman, Alterescu, & Rushworth, 2003). Although the PrL and IL together are considered part of the medial PFC, research has shown that the projections from these regions disperse differently throughout the brain which suggests that they have different functional roles in the CNS (Vertes, 2004).

The administration of MPH at high doses has previously shown changes in the level of tyrosine hydroxylase (TH) in the prefrontal cortex of rats (Gray et al., 2007). This enzyme is the rate limiting enzyme for the production of DA and NA (Levitt, Spector, Sjoerdsma, & Udenfriend, 1965) and staining of the TH enzyme provides the neuroanatomical location of the catecholamines (Pickel, Joh, Field, Becker, & Reis, 1975). Therefore

immunohistochemical staining of TH positive neurons will identify the presence of DA and/or NA within the neuron (Pickel et al., 1975) and importantly for this study, changes to the level of TH availability following chronic treatment using a clinically relevant dosing regime.

Alterations in the TH positive immunoreactive fibres may represent a change in the amount of catecholamines within a fixed number of fibres, or a change in the number of fibres containing DA and NA, or both (Unger, Terenghi, Zhang, & Polak, 1988). Changes to the level of TH positive neurons may also indicate alterations in neural connectivity following treatment. Evidence suggests that there is an association between the total area of TH positive fibres and the optical density (measured via fluorescence) of that region (Urbanavicius et al., 2007). Changes in TH immunoreactive neurons have previously been used to indicate synaptic plasticity and changes in neural connectivity (Csakvari et al., 2008; Dey, Mactutus, Booze, & Snow, 2006). Previous research using this technique reported a significant increase in TH density in the PFC of MPH treated rats compared to controls, with a trend towards persistent long-term plasticity, following high doses (5 mg/kg) of MPH administered to young rats via intraperitoneal (i.p.) injection (Gray et al., 2007). Altered plasticity following chronic MPH administration have also been reported using different methods, for example DA transporter density and survival of new cells (Lagace, Yee, Bolanos, & Eisch, 2006; Moll, Hause, Ruther, Rothenberger, & Huether, 2001). However, treatment methods employed in these previous studies may be questioned in terms of therapeutic relevance.

The present study assessed the long-term effect of chronic MPH treatment on catecholamine fibres in the PFC when it was administered using a treatment regime reflecting clinical dosing in children. Consistent with previous chapters the SHR was employed as an animal model for ADHD as a control, yet the focus of this study was on the effect of MPH treatment on TH immunostaining in the WKY as the non-ADHD or misdiagnosed strain. In addition to the

measure of TH immunostaining in the PFC of two new groups of MPH treated rats (short term & long term withdrawal groups), this study also examined changes in TH immunostaining in the behavioural cohort of Chapter 4. The level of TH in terminal areas of DA and NA neurons of the PFC was visualised using immunofluorescence and measures of optical density (Urbanavicius et al., 2007). Based on previous research (Gray et al., 2007), it was hypothesised that adolescent MPH treatment would increase the density of TH positive neurons in the adult PFC. As chronic MPH treatment did not alter performance on cognitive-behavioural testing in adult SHRs (Chapter 4), increases in TH staining within the PFC are expected in the WKYs only.

5.2 Materials and Method

5.2.1 Subjects

Twenty-four male WKY and 24 male SHR rats (8 rats from each strain had completed the cognitive-behavioural study in Chapter 4) were obtained from the Animal Resources Centre (Canning Vale, WA, Australia). Upon arrival in the laboratory, the rats were housed individually in an opaque, plastic cage (60 x 21.5 x 36 cm, length x height x width) containing sawdust, a block of wood and shredded paper covered with a raised wire mesh roof (27cm total height). The animal holding room was held at a constant temperature of 21˚C. Rats were housed on a reverse light/dark cycle (lights on at 2000 hours until 0800 hours) and experiments were conducted during the rats' active (dark) cycle. At the beginning of the procedure, the rats were approximately 25 days old, weighed 51 – 85 (WKY) and 46 – 90 (SHR) grams, had been handled daily for one week by the experimenter and were experimentally naïve. They were allowed free access to water and standard laboratory rat chow, except during drug administration and behavioural testing. During drug administration the rats were placed on water restriction (one hour access to water daily), while during the cognitive assessment of rats involved in Chapter 4, the rats were either placed on food

restriction (food was restricted to maintain their body weight at approximately 85% for approximately 16 days during the delayed reinforcement and extinction tasks) or on water restriction (one hour access to water daily for approximately 25 days during the radial arm maze).

The study was conducted with the approval of the Macquarie University Animal Ethics Committee (reference number ARA 2006/019) and followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004).

5.2.2 General procedure

Following 4 weeks of chronic oral MPH administration during adolescence (postnatal day 27 - 52; Laviola, Macri, Morley-Fletcher, & Adriani, 2003), immunohistochemical staining for TH positive neurons was performed at 3 points. The short-term group (8 WKY, 8 SHR) and long-term group (8 WKY, 8 SHR) were euthanized 1 week and 12 weeks, respectively, after cessation of treatment for neural tissue analysis. The behavioural group (8 WKY, 8 SHR, from Chapter 4) were euthanized upon completion of cognitive-behavioural testing for neural tissue analysis (12 weeks post treatment). The short-term group was included to assess the potential for MPH to induce neural changes at a point in time that reflects when the rats in the behavioural group would have commenced the delayed reinforcement task. The long-term group was included to determine whether the potential for MPH to induce neural plasticity persisted over time, in the absence of environmentally enriching cognitive-behavioural tests.

With the exception of performing the cognitive-behavioural tests, rats in the long-term and behavioural groups had the same experiences. The long-term group rats experienced identical levels of food and water restriction during the 12 weeks following treatment as did rats in the

behavioural group.

5.2.3 Drug administration procedure

The drug administration procedure is outlined in more detail in Chapter 4. Rats were placed on water restriction in order to facilitate drug administration via a drinking spout. To familiarise them to the procedure, after the initial 23 hours of water restriction a sham dose (water only) was administered. The following day treatment commenced. Rats were chronically treated with either Ritalin® (2 mg/kg oral; MPH) or water (dH2O) to model clinical dosing in children. Doses were based on the findings of our previous pilot study (Chapter 3); Kuczenski and Segal (2002); and Berridge et al., (2006).

Ritalin® tablets were suspended in distilled water (1 mg/mL) and administered through a drinking spout twice a day, five days per week for four weeks. Drug free weekends were employed in the current study as 'weekend holidays' are used and have been shown to reduce the experienced side-effects in the clinical setting (Martins et al., 2004). Following the morning dose the rats were given five minutes *ad libitum* access to water. The second daily drug administration occurred five hours after the first dose. Following the second dose the rats were allowed one hour *ad libitum* access to water. After the second drug administration on the fifth day of the week, the rats were given *ad libitum* access to water for approximately 40 hours, at which time they were placed back on water restriction to facilitate drug administration in the following week. The schedule of water restriction was based on previous research allowing rats *ad libitum* access to water on rest days (Johansen, Sagvolden, & Kvande, 2005; Sagvolden et al., 1992). Once the rats had completed four weeks of treatment, they were taken off water restriction and allowed *ad libitum* access to food and water.

5.2.4 Immunohistochemistry

Rats were deeply anesthetized with lethobarb (pentobarbitone sodium, 325 mg/mL, 1 mL) and received an intra-cardiac injection of 1 mL solution of 1:10 dilution of 5% Sodium Nitrate in Heparin. Rats were then intra-cardially perfused with 300 mLs of phosphate buffered saline (PBS) followed by 4% paraformaldhyde (PFA). Brains were removed and stored in PFA at 4°C overnight. The following day, the brains were blocked at the hypothalamus and returned to cold PFA for a minimum of 3 hours. At this time the PFA was replaced with 15% sucrose solution and the brains held at 4°C overnight. The following day, the 15% sucrose solution was replaced with 30% sucrose solution and the brains were held at 4°C for a minimum of 2 days. The brains were then transferred to freezing solution and stored in a -20°C freezer until processed for TH immunohistochemistry.

The rostral portion of the brain was removed from cryoprotectant and washed twice for 10 minutes in PBS with 0.1% Tween 20 (PBT). Each brain was sectioned into 50 μm coronal slices using a vibrotome and placed sequentially across 4 pots containing PBT. One pot from each brain was reacted, with the other 3 pots transferred to freezing solution and stored in a -20°C freezer.

To minimize variance due to solution concentrations, pots were combined such that they contained the brain sections from 2 rats: one treated with MPH and one with dH2O of the same strain, from each group. A nick in both hemispheres of the brains of the MPH treated rats distinguished the sections.

The pots to be reacted for immunohistochemistry were washed in 5X Sodium citrate buffer with 0.1% Tween 20 for 10 min. The solution was refreshed and the pots were placed on a shaker overnight and kept at 58°C. Three 30 min washes at room temperature with cold Tris-

phosphate buffer saline (TPBS) followed. The primary antibody solution, consisting of 0.3% mouse anti-TH antibody (Sigma), 10% normal horse serum (NHS) and TPBS with 0.05% merthiolate was then applied and pots were placed on a shaker for one hour at room temperature and then transferred to 4°C for 48 hours. Following three 30 min washes in cold TPBS at room temperature, the secondary antibody, consisting of 0.2% donkey anti-mouse Cy3 (Jackson Immunoresearch), 5% NHS and TPBS with 0.05% merthiolate was applied and pots were placed on a shaker for one hour at room temperature and then transferred to 4°C for a minimum of 12 hours. Pots were washed thrice for 20 min in cold TPBS and stored in TPBS with 0.05% merthiolate while sections were mounted. Sections were mounted on non-gelatinized slides using Vectorshield and coverslipped.

5.2.5 Image analysis

Sections were visualised using an Axiocam MRM camera mounted on a Zeiss Z1 microscope attached to a PC computer. Using Axiovision software, mosaic images containing 70 - 90% of the section were captured at 10X magnification. All microscope and computer settings remained constant to obtain comparable fluorescence of each section. The exposure time was set to 400 ms for each section.

Quantitative optical densitometry of TH staining was performed. The average pixel density (of 65,000 grey levels) of each region of interest (ROI) was determined using a square probe (50,000 μm^2). Background staining and variations in the illumination levels between images was accounted for by subtracting the mean grey value from a section of tissue (400 μm^2) with minimal labelling, from the ROI. The adjusted grey value for a ROI was averaged across all the images on which it appeared (minimum of 3 images for each ROI) prior to statistical analysis.

5.2.6 Statistical Analyses

The General Linear Model was used to compare the adjusted grey value of each ROI (orbiotofrontal cortex (OFC), prelimbic cortex (PrL), and infralimbic cortex (IL)) between treatment and group factors. Separate analyses were conducted for each ROI with 2 levels of the between subjects factor 'treatment' (MPH vs dH2O), and 3 levels of the between subjects factor 'group' (short-term vs long-term vs behavioural). Fischers' least squared difference adjustments were used when multiple comparisons were performed.

The data were presented for each strain separately. The data for the WKYs were presented first as they are the focus of this paper as the misdiagnosed or non-ADHD strain. The SHR data follows to investigate effects in the ADHD strain.

5.3 Results

5.3.1 Results for WKYs

Immunohistochemistry identified TH positive fibres throughout all regions of the PFC. As can be seen in Figure 1, there is high density of TH staining in layers 5 and 6 of the PrL and IL. Analysis of the treatment and group differences in the mean grey value of the OFC revealed a main effect of group, F (2, 18) = 5.28, p = 0.016 (Figure 2). This effect was such that the short-term group had significantly less TH staining compared to the long-term (p = 0.035) and behavioural (p = 0.006) groups, averaged across treatment. There was no main effect of treatment, F (1, 18) = 0.006, p = 0.937, and the treatment by group interaction was not significant, F (2, 18) = 1.028, p = 0.378.

Analysis of the mean grey value of the PrL revealed a main effect of group, F (2, 18) = 23.508, $p < 0.001$ (Figure 2). This effect was such that the behavioural group had significantly more TH staining compared to both the short-term ($p < 0.001$) and long-term (p = 0.002)

groups, averaged across treatment. There was no main effect of treatment, F (1, 18) = 0.249, p = 0.624, and the treatment by group interaction was not significant, F (2, 18) = 0.938, p = 0.41.

Analysis of the treatment and group differences in the mean grey value of the IL revealed a main effect of group, F (2, 18) = 6.259, p = 0.009 (Figure 2). This effect was such that the behavioural group had significantly more TH staining compared to the long-term (p = 0.003) and short-term (p = 0.017) groups, averaged across treatment. There was no main effect of treatment, F (1, 18) = 0.068, p = 0.797. As seen in figure 2, the treatment by group interaction was significant, F (2, 18) = 3.853, p = 0.04, indicating that the group differences were different for each treatment. In WKYs treated with dH2O, there was significantly less TH staining in the long-term compared to the short-term group (p = 0.039), while the behavioural group had significantly more TH staining compared to the long-term group (p = 0.007). In WKYs treated with MPH, no group differences were evident (p's > 0.05). Thus, MPH treatment interfered with the alterations of TH positive fibres that were measured across the groups following dH2O treatment.

5.3.2 Results for SHRs

Immunohistochemistry identified TH positive fibres throughout all regions of the PFC. As can be seen in Figure 3, there is high density of TH staining in layers 5 and 6 of the PrL and IL. Analysis of the treatment and group differences in the mean grey value of the OFC revealed no main effect of group, F (2, 18) = 1.21, p = 0.321, or treatment, F (1, 18) = 3.628, p = 0.073 (Figure 4). The treatment by group interaction was not significant, F (2, 18) = 0.796, p = 0.466.

Analysis of the treatment and groups differences in the mean grey value of the PrL revealed no main effect of group, F (2, 18) = 3.326, p = 0.059, or treatment, F (1, 18) = 0.379, p = 0.546 (Figure 4). The treatment by group interaction was not significant, F (2, 18) = 0.061, p = 0.941.

Analysis of the treatment and groups differences in the mean grey value of the IL revealed a main effect of group, F (2, 18) = 5.995, p = 0.01 (Figure 4). This effect was such that the behavioural group had significantly more TH staining compared to the short-term (p = 0.004) and long-term (p = 0.021) groups, averaged across treatment. There was no main effect of treatment, F (1, 18) = 2.644, p = 0.121, and the treatment by group interaction was not significant, F (2, 18) = 0.947, p = 0.407.

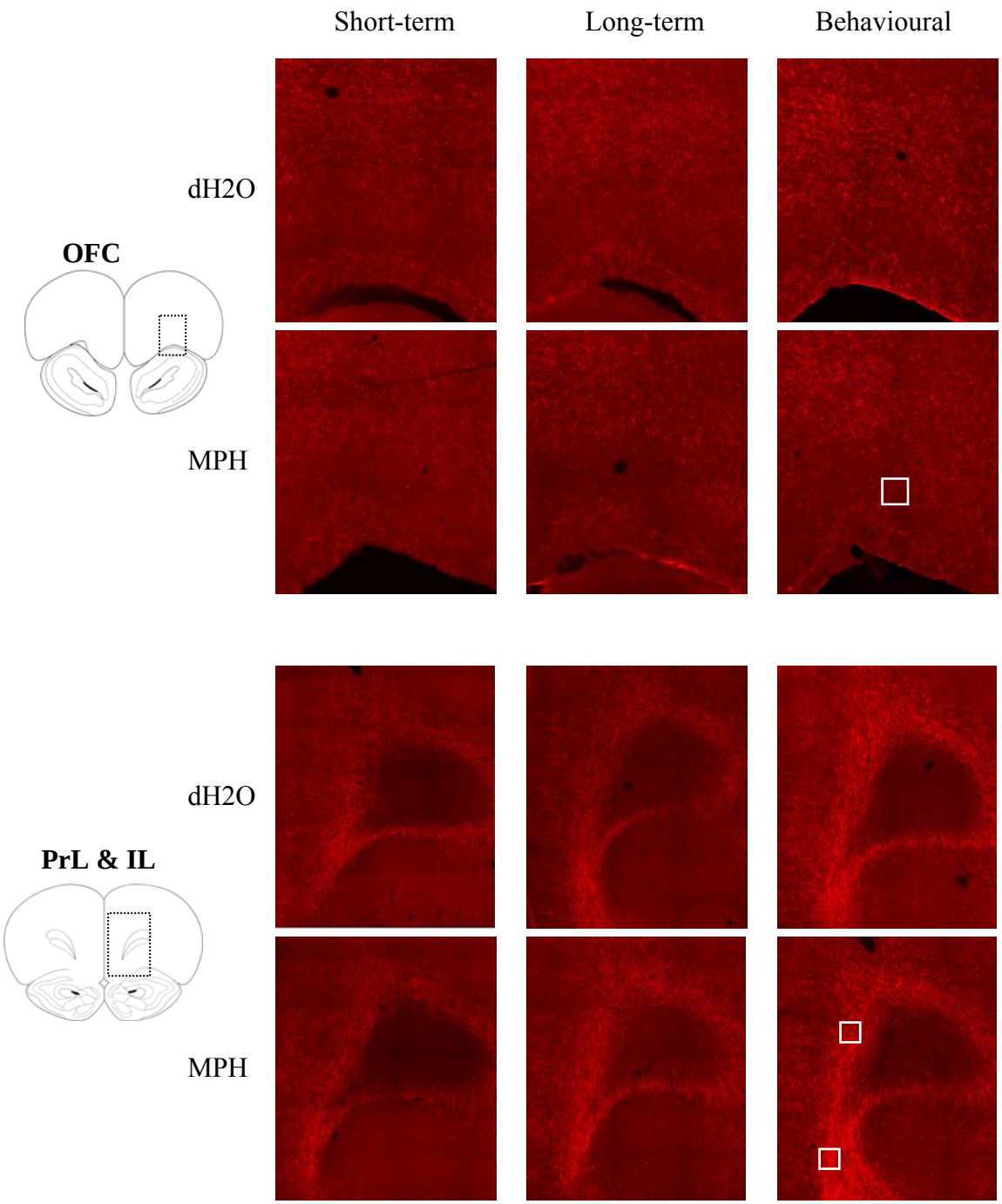

Figure 1. Mosaic pictures (10X) of tyrosine hydroxylase positive fibres in the orbitofrontal cortex (OFC), prelimbic cortex (PrL; dorsal) and infralimbic cortex (IL) of Wistar-Kyoto rats following chronic treatment with either methylphenidate (MPH) or water (dH2O). Immunohistochemical staining was conducted in the short- and long-term groups, 1 and 12 weeks after cessation of treatment, respectively, and following behavioural testing (12 weeks post treatment) in the behavioural group. The white square represents the analysis probe in each region of interest (50,000 μm^2). Images have been adjusted for presentation.

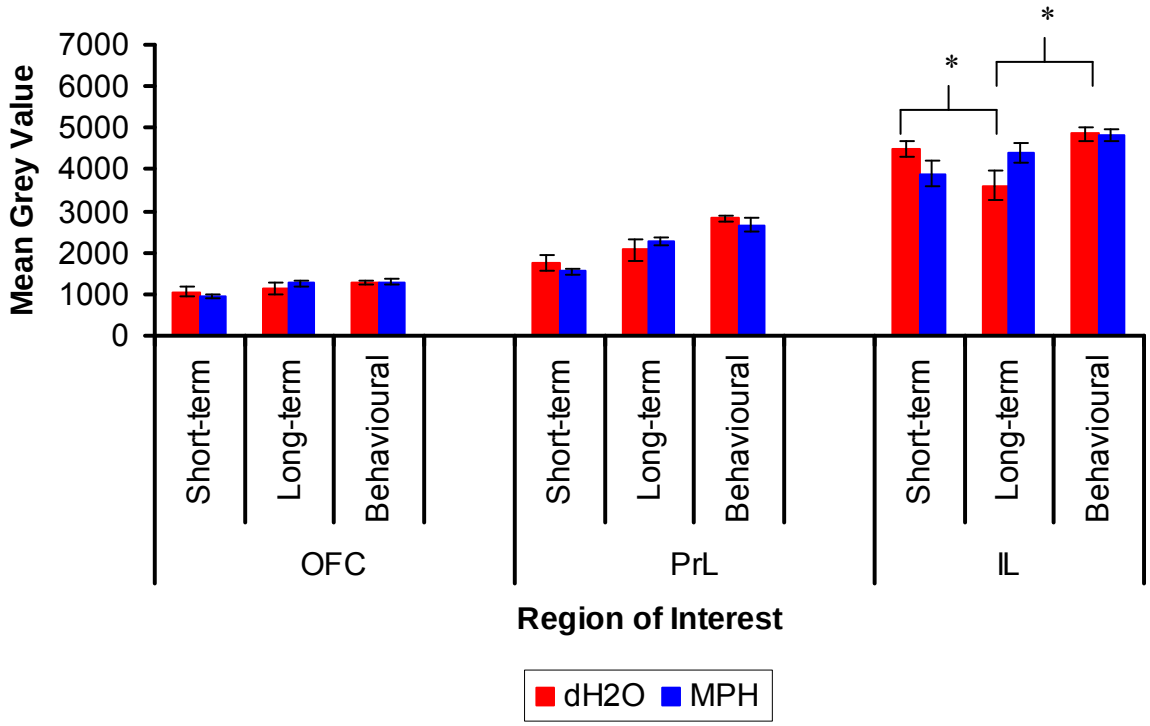

Figure 2. Mean (SEM) Tyrosine Hydroxylase staining in the lateral orbiotofrontal cortex (OFC), the prelimbic cortex (PrL), and infralimbic cortex (IL) of Wistar-Kyoto rats in the short-term group, long-term group, and behavioural group, previously treated with methylphenidate (MPH) or distilled water (dH2O). Within each region of interest, there was a significant main effect of group such that the behavioural group had significantly more TH staining compared to the short-term and long-term groups, averaged across treatment. * Significant difference for the comparisons of interest for water treated WKYs between the short-term and long-term groups, and the long-term and behavioural groups, *p*'s < 0.05.

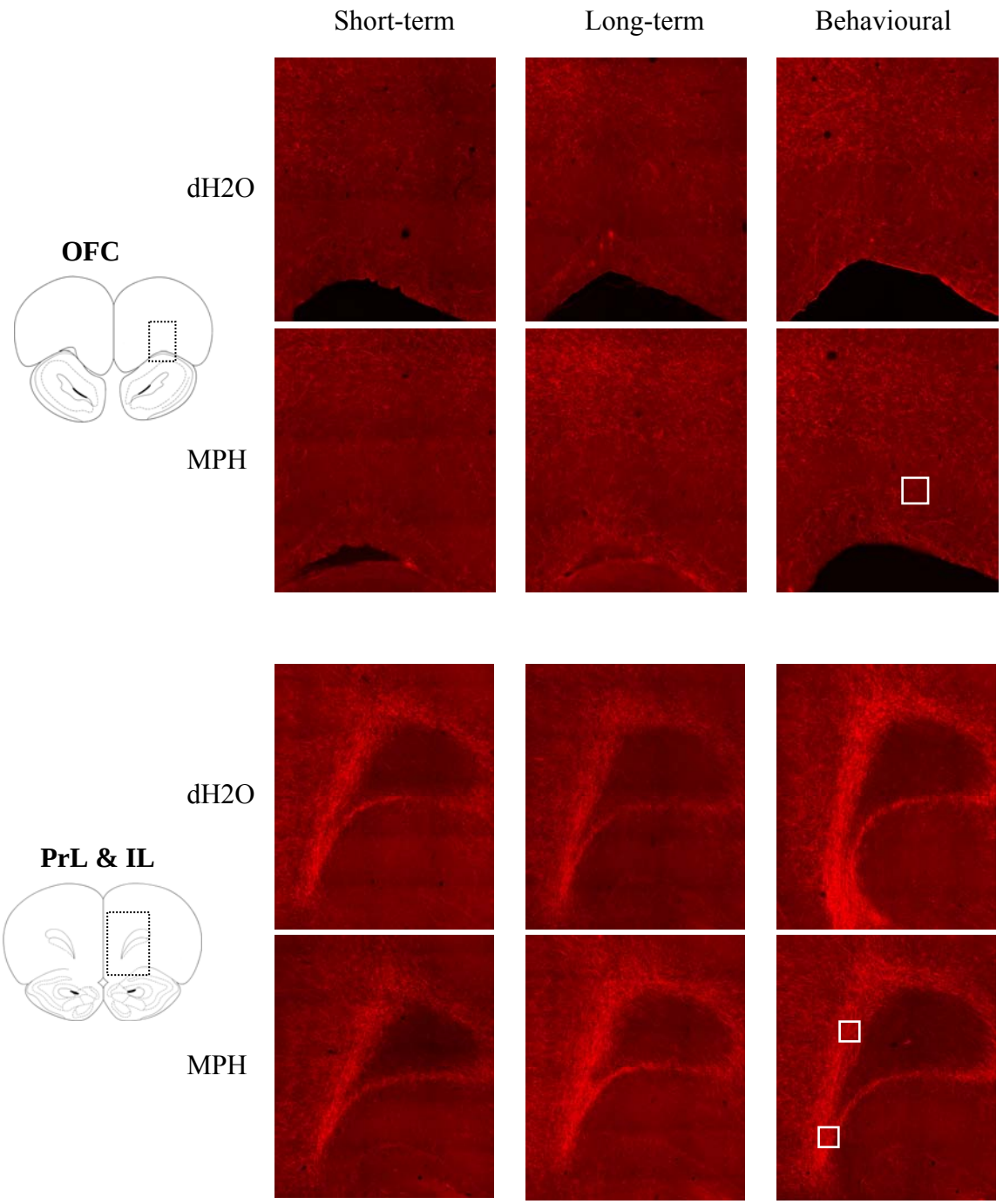

Figure 3. Mosaic pictures (10X) of tyrosine hydroxylase positive fibres in the orbitofrontal cortex (OFC), prelimbic cortex (PrL; dorsal) and infralimbic cortex (IL) of Spontaneously Hypertensive rats following chronic treatment with either methylphenidate (MPH) or water (dH2O). Immunohistochemical staining was conducted in the short- and long-term groups, 1 and 12 weeks after cessation of treatment, respectively, and following behavioural testing (12 weeks post treatment) in the behavioural group. The white square represents the analysis probe in each region of interest (50,000 μm^2). Images have been adjusted for presentation.

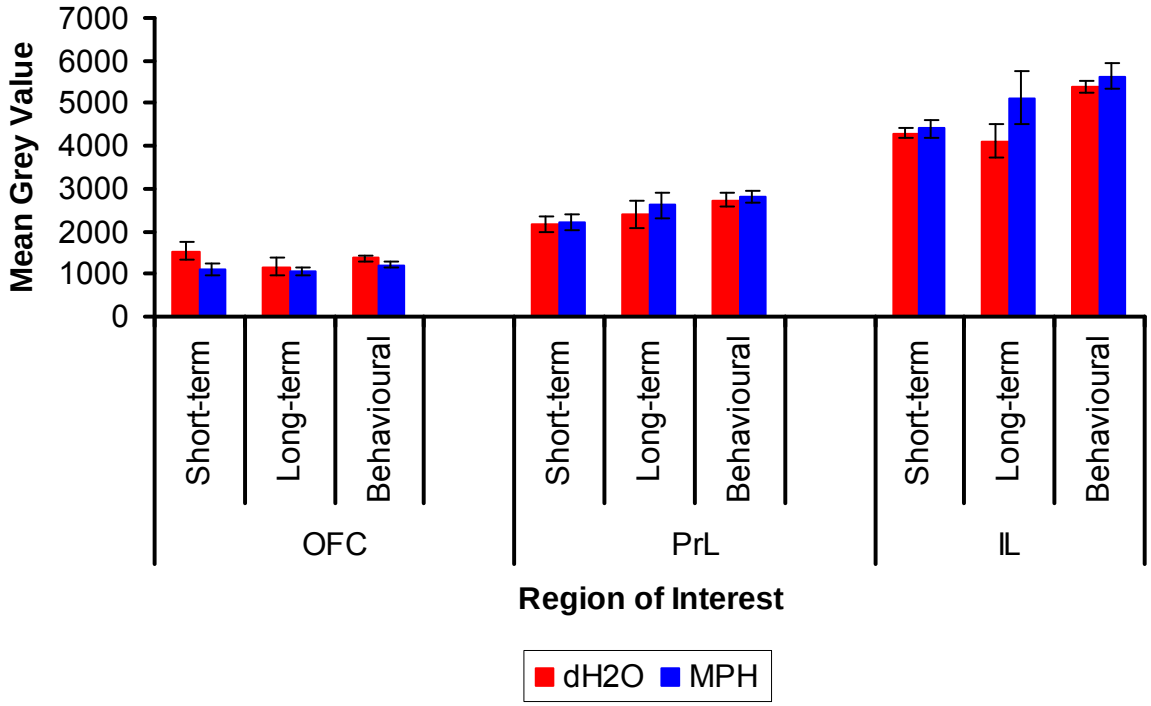

Figure 4. Mean (SEM) Tyrosine Hydroxylase staining in the lateral orbiotofrontal cortex (OFC), the prelimbic cortex (PrL), and infralimbic cortex (IL) of Spontaneously Hypertensive rats in the short-term group, long-term group, and behavioural group, previously treated with methylphenidate (MPH) or distilled water (dH2O). Within the IL region, there was a significant main effect of group such that the behavioural group had significantly more TH staining compared to the short-term and long-term groups, averaged across treatment.

5.4 Discussion

The current study was conducted to assess the effect of chronic adolescent MPH treatment on catecholamine neural development in the PFC. Following chronic oral MPH administration during adolescence, immunostaining for TH positive neurons was conducted at 1 (short-term) and 12 (long-term) weeks after cessation of treatment, and after cognitive-behavioural testing (from Chapter 4; behavioural) which was also 12 weeks after cessation of treatment. The findings suggest no direct effect of MPH treatment on TH density for each group. However, treatment differentially affected the TH density across each group when considering the TH immunostaining from the IL region of the PFC for the WKYs only. The effect was such that

group differences were observed in dH20 treated rats, but not MPH treated rats. The results for the SHRs revealed no significant effects of treatment on TH density within the PFC. These results suggest that MPH treatment in the WKYs, but not the SHRs, interferes with the catecholamine development that would normally occur following aging and exposure to enriching environments. Similar strain differences in TH activity following reserpine treatment have been reported (Renaud, Joh, & Reis, 1979).

The influence of MPH on the pattern of TH staining across the groups in the IL region of the WKYs suggests that MPH administration may interfere with brain maturation and response to experience. This is in line with previous findings of increased impulsivity in WKYs pretreated with MPH (Chapter 4) as the IL region has previously been shown to be involved with impulsive actions, an aspect of impulsivity (Chudasama et al., 2003). A comparison between the rats in the short-term and long-term groups determined the influence of time on catecholamine distribution, which could be considered a snap-shot of brain maturation. For the dH2O treated WKYs, this comparison revealed that the TH density of the older rats (long-term) was reduced compared to the younger rats (short-term). This finding is in line with the analysis of changes in TH immunostaining over the development of the PFC of the rhesus monkey (Rosenberg & Lewis, 1995). Rosenberg and Lewis (1995) reported a rapid rise in TH positive fibres during infancy and early childhood, followed by a decline from 2 – 3 year of age until reaching a stable adulthood level. These findings could also be considered consistent with human and animal research that demonstrates pruning or reduction of superfluous synapses and neurons that occurs in the frontal cortices until early adulthood (Aghajanian & Bloom, 1967; Huttenlocher, 1979). As there was no such reduction in the TH staining for the WKYs treated with MPH in the long-term group, this suggests that MPH treatment during adolescence interferes with the catecholaminergic maturation of the PFC.

Enriching experiences have been shown to alter brain morphology (Kolb et al., 2003a). The comparison between the WKYs in the long-term and behavioural groups investigated the alterations in TH density due to cognitive-behavioural testing. Such cognitive-behavioural tests are considered a component of behavioural environmental enrichment (van Praag, Kempermann, & Gage, 2000). The increased level of TH density in the IL of the dH2O treated WKYs in the behavioural group suggests increased neural complexity following behavioural enrichment. Notably, this difference between the long-term and behavioural groups was not evident for WKYs treated with MPH. The lack of neural adaptations as a result of behavioural enrichment of MPH treated WKYs may consistent with the previous findings of Kolb and colleagues (2003b) that psychostimulant pretreatment interfered with the ability of future environmental enrichment to shape the dendritic structure. Housing an adult rat in a complex, enriched environment increases dendritic branching and spine density in various brain regions, compared to rats housed in standard laboratory cages (Kolb et al., 2003a). However, when rats were repeatedly treated with cocaine or amphetamine prior to housing in a complex environment, the psychostimulant pretreatment interfered with the ability of the later experience in the complex environment to shape the dendritic structure (Kolb et al., 2003b). The findings of the current study may support previous research by demonstrating a similar effect with MPH, although a significant effect of treatment within the behavioural group would strengthen this conclusion.

Cocaine and amphetamine are psychostimulants with similar properties to MPH. D-amphetamine is also employed as a treatment for ADHD (MTA Cooperative Group, 2004). Previous research has shown that chronic treatment with these psychostimulants result in increased dendritic branching and arborisation of the neurons within the nucleus accumbens and the PFC (Crombag et al., 2005; Heijtz, Kolb, & Forssberg, 2003; Lee et al., 2006; Norrholm et al., 2003; Robinson et al., 2001; Robinson & Kolb, 1997, 1999). Using a similar

design to the current study, Heijtz and colleagues (2003) treated adolescent rats for 14 days with a low amphetamine dose (0.5 mg/kg; s.c.). Two weeks after the cessation of treatment they found increased dendritic length and branching, and spine density in the PFC, but not the nucleus accumbens in treated rats. The results of the current study are not consistent with those of Heijtz and colleagues (2003). There are obvious differences in the drug and method of administration employed between the current and previous study, however importantly the studies used different techniques to visualise the neurons and their fibres. In the current study, a TH-immunoreactive stain identified fibres containing DA and/or NA, while the previous research labelled pyramidal cells via the golgi-cox method. The golgi-cox method labels any cell irrespective of the neurotransmitter. Therefore, a potential explanation for the lack of direct effect of MPH treatment on TH staining is that the increases in neuron complexity following psychostimulant treatment of the previous study potentially reflect changes in neurons innervated by neurotransmitter such as DA and NA, rather than alterations to the catecholamine containing neurons themselves.

The limited effect of chronic MPH treatment on TH staining is inconsistent with previous research by Gray and colleagues (2007) that reported an increase of TH density in the PFC following the last dose of 28 days of chronic MPH treatment. Differences in methodologies used in the current study and Gray and colleagues (2007) research may explain the discrepant findings. Most significantly, in Gray et al (2007) the brain analysis that reported elevated TH density was conducted on the last day of their treatment regime, while the present study employed a minimum delay of 1 week between cessation of treatment and tissue analysis. It is usual that experimental measures are collected at least a week following cessation of treatment to avoid measures of transient changes brought about by withdrawal symptoms (Pierce & Kalivas, 1997). Therefore, the changes observed in Gray et al.'s (2007) research may have been transient as their analysis was conducted so close to the final dose

administration. Furthermore, Gray and colleagues (2007) reported a trend toward long-term increases in TH density in the PFC with chronic MPH treatment, however their study employed a high dose of MPH administered via i.p. injection. Such a high dose administered via i.p. injection would result in blood plasma concentrations well in excess of the therapeutic range (Swanson & Volkow, 2001), and therefore reducing the validity of the findings to the human situation. However, the therapeutically relevant dose administered in the current study did not directly affect the TH density at a specific point in time, rather it was the influence of MPH over time that affected TH density.

An alternate explanation for the lack of change in the TH density as a result of MPH treatment in each group is the different magnitude to which DA and NA increase following MPH treatment. While catecholamine levels both increase with MPH, therapeutic doses of MPH increase NA in the PFC significantly more than DA (Berridge et al., 2006). Therefore, changes in one neurotransmitter and not the other are not as likely to be evident when labelling TH positive neurons as they could contain either DA or NA fibres. In noradrenergic neurons, DA reacts further with dopamine β-hydroxylase (DBH) to produce NA (Pickel et al., 1975). Double labelling with a DBH-immunoreactive stain to differentiate the DA and NA fibres was not conducted as the available antibodies were not sufficiently sensitive to produce a clear image.

The null effect of MPH treatment on TH staining in the SHRs is consistent with the findings of Chapter 4. In Chapter 4, chronic adolescent MPH treatment did not alter performance on the cognitive behavioural tests in adult SHRs. Similarly, the current study found no effect of adolescent MPH treatment on the density of TH fibres within the PFC of SHRs. A possible explanation for this null effect is that the SHR has an abnormally functioning catecholamine system (Russell, 2002). As such, MPH may not be able to alter regulation of the SHRs

catecholamine systems as these systems are naturally up-regulated in the SHR (Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995). However, as discussed in Chapter 4, the results of the SHRs should be interpreted with some caution as the dose of MPH employed in this study may not have been therapeutically relevant for the SHR strain.

Further investigation into the MPH effect on each neurotransmitter is warranted. Highly selective DA and NA receptor antagonists are now available. Chronic simultaneous administration of such antagonists with MPH could elucidate the role of the catecholamines. Future studies employing co-administration of selective antagonists with MPH could identify the involvement of specific receptors in inducing neural adaptations of MPH treatment.

In conclusion, this study assessed the neurodevelopmental changes to TH containing neurons following chronic MPH treatment throughout adolescence. The results suggest that MPH treatment may interfere with catecholamine maturation of the PFC and may subsequently alter future neural adaptations to behavioural experiences, in the non-ADHD (i.e. misdiagnosed) strain only. Such findings hold significant implications for children misdiagnosed with ADHD. The data suggests that should a misdiagnosed child receive MPH treatment, they may experience long-term deficits in neural development.

5.5 References

Adriani, W., & Laviola, G. (2004). Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology, 15*, 341-352.

Aghajanian, G. K., & Bloom, F. E. (1967). The formation of synaptic junctions in developing rat brain: A quantitative electron microscopic study. *Brain Research, 6*, 716-727.

Angold, A., Erkanli, A., Egger, H. L., & Costello, E. J. (2000). Stimulant treatment for children: A community perspective. *Journal of the American Academy of Child and Adolescent Psychiatry, 39* (8), 975-984.

Arnsten, A. F. T., & Dudley, A. G. (2005). Methylphenidate improves prefrontal cortical cognitive function through α_2 adrenoreceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behavioral and Brain Functions, 1*, 1-9.

Arnsten, A. F. T., & Li, B. M. (2005). Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions. *Biological Psychiatry, 57*, 1377-1384.

Barbaresi, W. J., Katusic, S. K., Colligan, R. C., Weaver, A. L., Leibson, C. L., & Jacobsen, S. J. (2006). Long-term stimulant medication treatment of Attention-Deficit/Hyperactivity Disorder: Results from a population-based study. *Developmental and Behavioral Pediatrics, 27* (1), 1-10.

Berridge, C. W. (2008). Noradrenergic modulation of arousal. *Brain Research Reviews, 58*, 1-17.

Berridge, C. W., & Devilbiss, D. M. (2008). Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biological Psychiatry, 64*, 626-635.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F. T., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine

neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60*, 1111-1120.

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews, 42*, 33-84.

Cardinal, R. N. (2006). Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks, 19*, 1277-1301.

Chudasama, Y., Passetti, F., Rhodes, S. E. V., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: Differential effects on selectivity, impulsivity and compulsivity. *Behavioural Brain Research, 146*, 105-119.

Crombag, H. S., Gorny, G., Li, Y., Kolb, B., & Robinson, T. E. (2005). Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. *Cerebral Cortex, 15*, 341-348.

Csakvari, E., Kurunczi, A., Hoyk, Z., Gyenes, A., Naftolin, F., & Parducz, A. (2008). Estradiol-induced synaptic remodeling of tyrosine hydroxylase immunopositive neurons in the rat arcuate nucleus. *Endocrinology, 149*, 4137-4141.

Dahlstrom, A., & Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia, 20*, 398-399.

Dalley, J. W., Mar, A. C., Economidou, D., & Robbins, T. W. (2008). Neurobehavioral mechanisms of impulsivity: Fronto-striatal systems and functional neurochemistry. *Pharmacology, Biochemistry and Behavior, 90*, 250-260.

Dey, S., Mactutus, C. F., Booze, R. M., & Snow, D. M. (2006). Specificity of prenatal cocaine on inhibition of locus coeruleus neurite outgrowth. *Neuroscience, 139*, 899-907.

Fluxe, K., Hokfelt, T., Johansson, O., Jonsson, G., Lidbrink, P., & Ljungdahl, A. (1974). The origin of the dopamine nerve terminals in limbic and frontal cortex. Evidence for meso-cortico dopamine neurons. *Brain Research, 82*, 349-355.

Gatley, S. J., Pan, D., Chen, R., Chaturvedi, G., & Ding, Y.-S. (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sciences, 58* (12), PL 231-239.

Gray, J. D., Punsoni, M., Tabori, N. E., Melton, J. T., Fanslow, V., Ward, M. J., et al. (2007). Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *The Journal of Neuroscience, 27*, 7196-7207.

Heijtz, R. D., Kolb, B., & Forssberg, H. (2003). Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? *European Journal of Neuroscience, 18*, 3394-3399.

Homayoun, H., & Moghaddam, B. (2006). Progression of cellular adaptations in medial prefrontal and orbitofrontal cortex in response to repeated amphetamine. *The Journal of Neuroscience, 26* (31), 8025-8039.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Research, 163*, 195-205.

Johansen, E. B., Sagvolden, T., & Kvande, G. (2005). Effects of delayed reinforcers on the behavior of an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 162*, 47-61.

Kolb, B., Gibb, R., & Gorny, G. (2003a). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory, 79*, 1-10.

Kolb, B., Gorny, G., Li, Y., Samaha, A.-N., & Robinson, T. E. (2003b). Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the

neocortex and nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 10523-10528.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: Comparison with amphetamine. *Journal of Neurochemistry, 68*, 2032-2037.

Kuczenski, R., & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular noreprinephrine and absence of sensitization and cross-sensitization to methamphetamine. *The Journal of Neuroscience, 22* (16), 7264-7271.

Lagace, D. C., Yee, J. K., Bolanos, C. A., & Eisch, A. J. (2006). Juvenile administration of methylphenidate attenuates adult hippocampal neurogenesis. *Biological Psychiatry, 60*, 1121-1130.

Laviola, G., Macri, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews, 27*, 19-31.

Lee, K.-W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., & Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 3399-3404.

Levitt, M., Spector, S., Sjoerdsma, S., & Udenfriend, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *The Journal of Pharmacology and Experimental Therapeutics, 148*, 1-7.

Martins, S., Tramontina, S., Polanczyk, G., Eizirik, M., Swanson, J. M., & Rohde, L. A. (2004). Weekend holidays during methylphenidate use in ADHD children: A randomized clinical trial. *Journal of Child and Adolescent Psychopharmacology, 14* (2), 195-205.

Moll, G. H., Hause, S., Ruther, E., Rothenberger, A., & Huether, G. (2001). Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. *Journal of Child and Adolescent Psychopharmacology, 11* (1), 15-24.

MTA Cooperative Group. (2004). National Institute of Mental Health Multimodal Treatment Study of ADHD follow-up: 24-month outcomes of treatment strategies for attention-deficit/hyperactiity disorder. *Pediatrics, 113*, 754-761.

Mulder, A., Nordquist, R., Orgut, O., & Pennartz, C. (2003). Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. *Behavioural Brain Research, 146*, 77-88.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.).

Norrholm, S. D., Bibb, J. A., Nestler, E. J., Ouimet, C. C., Taylor, J. R., & Greengard, P. (2003). Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience, 116*, 19-22.

Ongur, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex, 10*, 206-219.

Peters, Y., O'Donnell, P., & Carelli, R. (2005). Prefrontal cortical cell firing during maintenance, extinction, and reinstatement of goal-directed behavior for natural reward. *Synapse, 56*, 74-83.

Pickel, V. M., Joh, T. H., Field, P. M., Becker, C. G., & Reis, D. J. (1975). Cellular localization of tyrosine hydroxylase by immunohistochemistry. *The Journal of Histochemistry and Cytochemistry, 23*, 1-12.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Reviews, 25*, 192-216.

Prosser, B., & Reid, R. (1999). Psycholstimulant use for children with ADHD in Australia. *Journal of Emotional and Behavioral Disorders, 7* (2), 110-117.

Prosser, B., & Reid, R. (2009). Changes in use of psychostimulant medication for ADHD in South Australia (1990-2006). *Australian and New Zealand Journal of Psychiatry, 43*, 340-347.

Renaud, B., Joh, T. H., & Reis, D. J. (1979). An abnormal regulation of tyrosine hydroxylase restricted to one catecholamine nucleus in the brain stem of spontaneously hypertensive rats. *Brain Research, 173*, 164-167.

Robinson, T. E., Gorny, G., Mitton, E., & Kolb, B. (2001). Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse, 39*, 257-266.

Robinson, T. E., & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *The Journal of Neuroscience, 17* (21), 8491-8497.

Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience, 11*, 1598-1604.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology, 47*, 33-46.

Rosenberg, D. R., & Lewis, D. A. (1995). Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. *The Journal of Comparative Neurology, 358*, 383-400.

Russell, V. A. (2002). Hypodopaminergic and hypernoradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder - the spontaneously hypertensive rat. *Behavioural Brain Research, 130*, 191-196.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C. L., & Taljaard, J. J. F. (1995). Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of attention-deficit hyperactivity disorder - The spontaneously hypertensive rat. *Behavioural Brain Research, 676*, 343-351.

Sagvolden, T., Metzger, M. A., Schiorbeck, H. K., Rugland, A.-L., Spinnangr, I., & Sagvolden, G. (1992). The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): Changed reactivity to reinforcers and to psychomotor stimulants. *Behavioral and Neural Biology, 58*, 103-112.

Sawyer, M. G., Rey, J. M., Graetz, B. W., Clark, J. J., & Baghurst, P. A. (2002). Use of medication by young people with attention-deficit/hyperactivity disorder. *Medical Journal of Australia, 177*, 21-25.

Swanson, J. M., & Volkow, N. D. (2001). Pharmacokinetic and pharmacodynamic properties of methylphenidate in humans. In M. V. Solanto, A. F. T. Arnsten & F. X. Castellanos (Eds.), *Stimulant drugs and ADHD: Basic and clinical neuroscience* (pp. 259-282). New York: Oxford University Press.

Unger, W. G., Terenghi, G., Zhang, S.-Q., & Polak, J. M. (1988). Alteration in the histochemical presence of tyrosine hydroxylase and CGRP-immunoreactivities in the eye following chronic sympathetic or sensory denervation. *Current Eye Research, 7*, 761-769.

Urbanavicius, J., Ferrerira, M., Costa, G., Abin-Carriquiry, J. A., Wonnacoot, S., & Dajas, F. (2007). Nicotine induces tyrosine hydroxylase plasticity in the neurodegenerating striatum. *Journal of Neurochemistry, 102*, 723-730.

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience, 1*, 191-198.

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse, 51*, 32-58.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Franceschi, D., Maynard, L., et al. (2002). Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeutic implications. *Synapse, 43*, 181-187.

Walton, M. E., Bannerman, D. M., Alterescu, K., & Rushworth, M. F. S. (2003). Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. *Journal of Neuroscience, 23* (16), 6475-6479.

Zito, J. M., Safer, D. J., dosReis, S., Gardner, J. F., Boles, M., & Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. *JAMA, 283* (8), 1025-1030.

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Chapter 6

The role of catecholamine receptors in impulsivity as mediated by the prefrontal cortex.

6.1 Introduction

Impulsivity is characteristic of many disorders, such as Attention Deficit/Hyperactivity Disorder (ADHD), substance abuse, conduct disorder, and schizophrenia (American Psychiatric Association, 2000). Impulsivity can be broadly defined as action without consideration of the consequences. It can be divided into 2 categories: impulsive action which is the inability to inhibit a motor response; and impulsive choice which is the preference of a smaller reward delivered immediately over a larger reward delivered after a delay (Winstanley, Eagle, & Robbins, 2006). Delayed reinforcement (DR) paradigms are employed in laboratories as a measure of impulsive choice (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Evenden & Ryan, 1996; Mobini et al., 2002).

Impulsivity is one of the higher level cognitive functions controlled by the PFC and symptoms of ADHD are associated with neurotransmitter dysfunction of this region (Arnsten, 2009; Levy, 2008). To treat these symptoms, children are commonly prescribed psychostimulants which increase the neurotransmission of the catecholamines dopamine (DA) and noradrenaline (NA) within the PFC (Berridge et al., 2006).

The state of arousal of the subject is related to catecholamine levels in the PFC. Activation of DA neurons occurs in response to expected rewards (Schultz, 1998), while NA neuronal activation corresponds with the subjects' interest for the stimuli (Solanto, 1998). An inverted U represents the dose/response relationship between DA and NA, and PFC function. Optimal PFC function requires moderate levels of DA and NA (Arnsten, 2006, 2009). During periods

of fatigue there is insufficient catecholamine stimulation, while excessive DA and NA levels are evident during periods of stress (Deutch, Clark, & Roth, 1990; Foote, Aston-Jones, & Bloom, 1980). Either situation of too little or too much DA and NA is detrimental to PFC functioning. The involvement of specific receptor types is important in PFC function. Under optimal conditions in the PFC, moderate levels of NA activate the α_{2A} receptors and moderate levels of DA activate the D1 receptor (Arnsten, 1997). NA stimulation of the α_{2A} receptors enhances PFC function by strengthening appropriate neural networks, increasing the 'signal'. In contrast, D1 receptor stimulation enhances PFC function by weakening inappropriate neural connections, decreasing 'noise'. Excessive NA levels in the PFC engage lower-affinity α_1 receptors, which suppresses PFC activation (Birnbaum et al., 2004). High levels of DA in the PFC result in excessive D1 stimulation which suppresses PFC activation by weakening too many neural connections (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007).

Psychostimulant treatment has been shown to affect neural plasticity in the PFC subregions of the orbitofrontal cortex (OFC), and the prelimbic (PrL) and infralimbic (IL) cortices collectively referred to as the medial PFC (mPFC; Crombag, Gorny, Li, Kolb, & Robinson, 2005; Robinson & Kolb, 2004). However the direct involvement of DA and NA in psychostimulant induced plasticity is not clear. Previously in this thesis it was reported that chronic treatment with the psychostimulant methylphenidate (MPH) during adolescence increased impulsivity in the adult non-ADHD Wistar-Kyoto rat (WKY, Chapter 4) and interfered with the normal trajectory of development that occurred following aging and experience of the catecholamine fibres of the IL (Chapter 5). Together, these findings suggest that alterations in catecholamine levels in the PFC during treatment may have produced neural plastic changes that could be responsible for the increased impulsivity observed in Chapter 4.

Previous investigations into the role of DA and NA in impulsivity have employed systemic administration of specific agonists and antagonists. Van Gaalen and colleagues (2006) used a DR paradigm to assess the effect of various drugs on impulsive choice. They found that high doses of the DA D1 receptor antagonist SCH 23390, increased impulsive choice, while the DA D2 receptor antagonist eticlopride had no effect, suggesting the D2 receptor does not mediate impulsive choice. Wade and colleagues (2000) reported that the DA D2 receptor antagonist raclopride decreased the value of a reward, an aspect of impulsive choice, while SCH 23390 was without effect on this task, suggesting the antagonism of the D2 but not D1 receptor is involved in increased impulsivity. Together these findings demonstrate the distinct roles of D1 and D2 receptors in impulsivity, dependent upon the behavioural paradigm used. Van Gaalen and colleagues (2006) also reported that NA was involved in impulsive choice as they found that high doses of the NA α_2 receptor agonists clonidine increased impulsive choice, while the NA α_1 receptor phenylephrine did not alter impulsive choice. The α_{2A} receptor agonist guanfacine has been shown to reduce motor inhibition, a measure of impulsive action (Milstein, Lehmann, Theobald, Dalley, & Robbins, 2007; Sagvolden, 2006). Similarly, systemic administration of atomoxetine, a NA transport inhibitor, decreased impulsive choice in a DR paradigm (Robinson et al., 2008). These studies using receptor selective compounds support a role for DA and NA in impulsivity, however, the systemic administration of the compounds does not allow for any conclusions to be drawn about the role of DA and NA in impulsivity directly mediated by the PFC or PFC sub-regions.

Evidence for the involvement of sub-regions of the PFC in impulsivity has been provided by lesion studies. Lesion studies have produced inconsistent findings with regard to the role of the OFC in responding to DR measures of impulsivity. Mobini and colleagues (2002) reported that lesions of the OFC increased impulsive choice while Winstanley and colleagues (2004) found a reduction in impulsive choice of OFC-lesioned rats. Possible explanations for the

inconsistency include whether training on the operant task was conducted prior to or following lesion surgery and differences in the magnitude of the large reward. Using a DR paradigm that allowed for distinction between value of the reward and sensitivity to delay in rats, Kheramin and colleagues (2004) found that dopaminergic lesions of the OFC produced aversion to increasing delays, which was offset by the increased value attributed to the reward. However, lesions of the mPFC have been reported to have no influence on delay-specific effects on impulsive choice (Cardinal et al., 2001). While lesion studies provide important information about which regions are involved in which behaviours, it is also important to consider the role of specific neurotransmitter receptors in mediating these behaviours.

To date there have been no studies that have investigated the effect of local PFC infusions of DA antagonists or NA agonists on impulsivity. The effects of local infusions of DA antagonists have been assessed on other PFC mediated tasks such as contingency degradation and set-shifting. Infusions of the nonselective D1/D2 receptor antagonist *cis*-(z)-flupenthixol dihydrochloride into the mPFC have been shown to disrupt goal-directed behaviours, as assessed using contingency degradation (Naneix, Marchand, Di Scala, Pape, & Coutureau, 2009). Behavioural flexibility, assessed using a set-shifting task, has also been found to deteriorate following blockade of D1 and D2 receptors in the mPFC and only D1, not D2 receptors in the OFC (Winter, Diekmann, & Schwabe, 2009). Whilst not manipulating DA receptors, levels of DA and it's metabolite DOPAC were measured in the mPFC and OFC of rats performing a DR task (Winstanley, Theobald, Dalley, Cardinal, & Robbins, 2006). It was found that task specific increases in DOPAC levels occurred only in the OFC. Together these results highlight the importance of DA in PFC mediated tasks.

A limited amount of research has been conducted looking at the effects of local PFC infusions

of NA on cognition. The research that has been conducted has focused on working memory ability, which is mediated by the PFC. Stimulation of the α_1 receptor in the PFC has been found to impair working memory function in rats (Arnsten, Mathew, Ubriani, Taylor, & Li, 1999) and monkeys (Mao, Arnsten, & Li, 1999). However, improvements in working memory have been found following stimulation of α_2 receptors in both rats (Birnbaum, Podell, & Arnsten, 2000; Carlson, Tanila, Rama, Mecke, & Pertovaara, 1992) and monkeys (Mao et al., 1999). These studies suggest that α_1 and α_2 receptors have opposing roles in working memory function mediated by the PFC. It is therefore feasible to anticipate that they may have opposing roles in other PFC mediated tasks, such as impulsive choice.

Methylphenidate administration is thought to enhance DA and NA neurotransmission at the level of the PFC for treatment of impulsive symptoms of ADHD (Berridge et al., 2006; Greenhill et al., 2002). However from Chapter 4, it was shown that chronic treatment with MPH increased impulsive choice in 'misdiagnosed' WKY rat controls. This may implicate a change in catecholamine control of the PFC produced by chronic MPH exposure. Furthermore, results from Chapter 5 suggest that chronic MPH treatment in the 'misdiagnosed' WKY rats may interfere with the normal maturation of the catecholamine system in the PFC. The present study was conducted to investigate the neural mechanisms that may potentially underlie the increased impulsivity following chronic MPH treatment reported in Chapter 4 of this thesis. The aim of this study was to measure changes in impulsivity following local infusions of DA receptor antagonists or NA receptor agonists into different regions of the PFC – the mPFC and OFC. Based on the inverted-U catecholamine theory of PFC function, it is anticipated that blockade of the DA D1 receptor will increase impulsivity with little to no effect of DA D2 antagonism, while stimulation of the α_1 and α_2 receptors will increase and decrease impulsivity, respectively. Results from the lesions studies (Cardinal et al., 2001; Kheramin et al., 2004; Mobini et al., 2002; Winstanley et al., 2004)

suggest that the specific effect of the DA receptor antagonists and NA receptor agonists will be most pronounced in the OFC compared to the mPFC.

6.2 Materials and Method

6.2.1 Subjects

Sixty male WKY rats were obtained from the Animal Resources Centre (Canning Vale, WA, Australia). Upon arrival in the laboratory, the rats were housed individually in an opaque, plastic cage (60 x 21.5 x 36 cm, length x height x width) containing sawdust, a block of wood and shredded paper covered with a raised wire mesh roof (27cm total height). The animal holding room was held at a constant temperature of 21˚C. Rats were housed on a reverse light/dark cycle (lights on at 1800 hours until 0600 hours) and experiments were conducted during the rats' active (dark) cycle. At the time of surgery, the rats mean (SEM) weight was 289 (± 2.06) grams, they had been handled daily for one week and were experimentally naïve. The rats were allowed free access to water and standard laboratory rat chow, except during the behavioural task at which time they had their food restricted to maintain their body weight at approximately 85% of their free feeding weight.

The study was conducted with the approval of the Macquarie University Animal Ethics Committee (reference numbers ARA 2009/001 and 2010/001) and followed the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 2004).

6.2.2 Surgical procedure

Each rat had intracranial surgery under anaesthesia induced by isoflurane in oxygen (2L/min), for the bilateral implantation of 10 mm guide cannulae (26 Ga) into the medial prefrontal cortex (mPFC) or the lateral orbiotofrontal cortex (OFC). These brain regions have been

shown to be integral in modulating goal-directed behaviours, disinhibition and impulsivity. The rat was placed in the stereotaxic apparatus (Kopf Instruments, Tujunga, CA), nose bar set at -3.3 mm. Using co-ordinates obtained from the rat brain atlas of Paxinos and Watson (1998), guide cannulae were placed bilaterally into either the OFC (AP: +3.5; ML: ± 2.5; DV: -3.2); or mPFC (AP: +3.2; ML: ± 1; DV: -3.0). The cannulae remained in place by the application of cranioplastic cement around the cannulae and 4 machine screws implanted in the skull. Small stylets the length of the cannulae (wire 33 Ga thickness) were placed in each cannulae to keep them clean and clear. The stylets were removed immediately prior to intracranial microinjections and were replaced once the bilateral microinjection was complete. The rats had seven days of recovery prior to commencement of operant training.

6.2.3 Drugs

All drugs were obtained from Sigma (St Louis, Missouri, USA). SCH23390 was dissolved in distilled water and raclopride, phenylephrine and guanfacine were dissolved in 0.9% NaCl.

6.2.4 Delayed reinforcement (DR) task

The DR task used in this experiment was based on the DR task described in more detail in Pardey et al., (2009, Chapter 2). Twenty-four hours prior to the commencement of the task the rats were placed on food restriction. To perform the task, the rats were placed in operant conditioning chambers in which the test wall contained two cue lights above two levers, on either side of a food magazine. The food reinforcer used was 45 mg Noyes Precision Pellets, Formula A (Research Diets, Inc., New Brunswick, NJ, USA).

During the training phase the rats were trained to lever press for a food reinforcer. Following training, the delay phase commenced in which increasing delays were introduced between depression of the lever associated with the large reinforcer and delivery of the reinforcer. The

delay phase continued until the rats' behaviour had stabilised and they chose the delayed lever approximately 50% of the time. At which point, the test phase commenced in which microinjections were administered immediately prior to their daily session. Restabilisation of the rats' lever selection was required prior to the administration of the next drug dose.

Training phase: The rats were placed in the chambers once per day. During the training phase, the rats learnt that depression of one lever delivered a small (one pellet) immediate reinforcer, while pressing the other lever resulted in a large (five pellets) reinforcer delivered after a delay of two seconds. During the delay, the cue light above the lever flashed. The daily session was complete when the rat had made 30 successful responses, or 30 minutes had expired. The criteria required to move onto the delay phase was 30 successful responses within 30 minutes across three consecutive days.

Delay phase: On each day of the delay phase, the rats received a reminder trial to begin the session which was identical to the training session. Following these reminder trials, the delayed trials began. In these trials the rats were allowed to choose which lever they pressed and therefore which reinforcement they received. The reinforcer associated with the small immediate lever remained constant throughout the delay phase. If the rats chose the large delayed lever, they received five pellets, however the delay between their response and reinforcer delivery increased during each delay session. Over subsequent days in the delay phase, the delay interval became progressively larger as shown in Table 1. As with the training phase, during the delay the cue light flashed until the reinforcer was delivered. Each of the delay durations were experienced six times before proceeding to the longer delay for that particular delay level. The rats initially experienced delay level 1. To progress to delay level 2, the rats must choose the delayed lever at least 25 times so they experience all delay durations for delay level 1. If the rats did not reach this criterion, delay level 1 was repeated.

The rats progressed through the delay levels based on these criteria. Once the rats displayed stable responding at one delay level (between 13 and 19 selections on the same delay level, across three consecutive days), this delay level was maintained for infusion test sessions. The daily session was complete when the rat had made 30 successful choices, or the session timed out.

Table 1. Delay duration (seconds) before reinforcer delivery and time out for each delay level.

Delay Level	Delay duration in seconds (each experienced 6 times)	Time out (mins)
1	0, 1, 2, 3, 4	45
2	0, 2, 4, 6, 8	45
3	0, 5, 10, 15, 20	45
4	0, 10, 20, 40, 60	45
5	0, 20, 40, 60, 80	45
6	0, 30, 60, 90, 120	45
7	0, 50, 100, 150, 200	75
8	0, 100, 200, 300, 400	100

Testing Phase: Each test day session used the same procedure as the delay phase, except the session did not have time restrictions. As the sessions did not time out, the rats had to make 30 choices to complete the task. This ensured that the task was measuring the drugs' effect on impulsive choice, not on locomotor activity. On the first test day the rats received a sham microinjection immediately prior to being placed in the chambers. The sham microinjection involved removing the stylets from the cannulaes, swabbing the surface of the cranioplastic cement with 70% ethanol, inserting and removing 11 mm microinjectors (33 Ga) into each cannulae, then replacing the stylets. Following this the rats completed their daily session using the same delay level with which they met criteria. If the sham microinjection did not alter the number of delayed lever choices made (± 1) from the day prior, the rats received the first bilateral microinjection the following day. If the sham microinjection altered the rats

responses compared to the prior day, then the rats was required to press the delayed lever the same number of times (± 2) on two consecutive days on the same delay level, prior to their first bilateral microinjection to ensure stable response rates.

On each test day of the DR task, rats received bilateral microinjections of either DA antagonists or NA agonists into their mPFC or OFC. Different groups of rats were used for each neurotransmitter such that group 1 (mPFC, n = 15; OFC, n = 15) received DA antagonists and group 2 (mPFC, n = 15; OFC, n = 15) received NA agonists.

Rats that received DA antagonists were injected with each of the doses outlined in Table 2, following a latin square design. DA antagonist doses into the mPFC were given in a volume of 0.5 µl and doses into the OFC were given in a volume of 0.3 µl. Doses were based on work by Winter and colleagues (2009) assessing DA receptor involvement in behavioural flexibility mediated by the mPFC and OFC.

Rats that received NA agonists were injected with each of the doses outlined in Table 3, following a latin square design. NA agonist doses were given in a volume of 0.5 µl in the mPFC and OFC. Phenylephrine doses were based on the findings of Arnsten and colleagues (1999) assessing the effect of the NA α_1 agonist in the PFC on working spatial memory. Guanfacine doses were based on research by Ji and colleagues (2008) assessing the role of the NA α_2 agonist on electrophysiological responses in the mPFC.

Table 2. Dopamine (DA) antagonist doses injected into either region of interest.

DA receptor antagonist	Drug	Dose
Vehicle	Distilled water (VEH)	0 μg/ per side
DA D1 receptor antagonist	SCH23390 (SCH3)	3 μg/ per side
DA D2 receptor antagonist	Raclopride (RAC3)	3 μg/ per side
	Raclopride (RACH6)	6 μg/ per side

Table 3. Noradrenaline (NA) agonist doses injected into either region of interest.

NA receptor agonist	Drug	Dose
Vehicle	Saline (VEH)	0 μg/ per side
NA α_1 receptor agonist	Phenylephrine (PHENL.1)	0.1 μg/ per side
	Phenylephrine (PHENH.3)	0.3 μg/ per side
NA α_2 receptor agonist	Guanfacine (GUANL1)	1 μg/ per side
	Guanfacine (GUANH3)	3 μg/ per side

A microinjection involved removing the stylets from the cannulaes, swabbing the surface of the cranioplastic cement with 70% ethanol, and inserting 11 mm microinjectors into each cannulae. The prescribed dose was administered over a period of one minute via a Hamilton syringe (1 μl) and infusion pump (kdScientific, Holliston, Massachusetts, USA). The injectors remained in place for 30 secs following dosing to allow infusion of the total drug dose. The injectors were then removed and the stylets replaced. The rats were then immediately placed in the chambers and the difference between the number of delayed lever choices on the test day and the day prior quantified the effect of each dose.

The rats had a minimum of 48 hours between each dose. During this time, the rats experienced daily sessions as per the delay phase to ensure their baseline responding had not

changed due to the most recent microinjection. In order to receive the next microinjection, the rats had to exhibit stable delayed lever selection (± 2) on two consecutive days of the same delay level. The delay level was adjusted where necessary to facilitate this.

The rats were placed back on *ad libitum* access to food following the daily session after their final dose. One week after completion of the task, the rats were euthanized via intracardial perfusion with 100 mLs of saline followed by 100 mLs of 10% formaldehyde in saline. Their brains were removed and sectioned on a cryostat to confirm cannulae placement.

6.2.5 Statistical Analyses

Analyses were conducted using analysis of variance (ANOVA). The General Linear Model was used with Greenhouse-Geisser Epsilon (G-G) adjustments for the univariate statistics reported when the assumption of sphericity was violated. Analyses were conducted separately for the DA treatment and NA treatment. Within each treatment, separate repeated measures analysis were conducted on the difference between the delayed reinforcers attained on a treatment day and delayed reinforcers attained on the day immediately prior, for each region of interest. For the rats treated with DA antagonists there were 4 levels of the within subjects factor, 'treatment' (VEH, SCH3, RAC3, and RACH6) for each region. For the rats treated with NA agonists there were 5 levels of the within subjects factor 'treatment' (VEH, PHENL.1, PHENH.3, GUANL1, and GUANH3) for each region. Planned contrasts were conducted to compare the effect of each drug to VEH within each region of interest using a Fischer's least squared difference adjustment.

6.3 Results

6.3.1 Effect of dopamine antagonists on delayed reinforcer choice

The timeline for the completion of the DR task when the rats were locally treated in either the

PFC or OFC with the DA antagonists SCH23390 and raclopride is illustrated in Table 4. Of the rats that received bilateral PFC microinjections, one was lost as his behaviour failed to stabilise following sham and two were lost due to blocked cannulae. In total, six rats were removed from the analysis due to misplacement of one or both of their cannulae. As a result, the PFC and OFC groups each had 10 rats, see cannulae placement in Figure 1.

Table 4. Number of days to complete each phase of the Delayed Reinforcement (DR) Task for rats receiving microinjections of dopamine antagonists via intracranial cannulae in either the medial prefrontal cortex (mPFC) or lateral orbitofrontal cortex (OFC), mean ± SEM.

DR phase	mPFC (mean ± SEM)	OFC (mean ± SEM)
Training	8.13 ± 0.09	9.08 ± 0.702
Delay stabilisation	11.25 ± 1.03	11.85 ± 0.95
Restabilise after sham	3.92 ± 1.27	5.25 ± 1.06
Restabilise after 1st injection	5.38 ± 1.11	5.42 ± 1
Restabilise after 2nd injection	3.61 ± 0.4	6.5 ± 0.78
Restabilise after 3rd injection	4.38 ± 0.53	5.25 ± 1
Total	37 ± 1.65	43.58 ± 1.1

Bilateral microinjections of DA antagonists into the mPFC resulted in a significant main effect of treatment, F (3, 27) = 4.850, p = 0.008, illustrated in Figure 2. Planned contrasts revealed that compared to VEH, both SCH3 (p = 0.002) and RACH6 (p = 0.014) significantly increased impulsivity as indicated by a decrease in delayed lever choice.

Bilateral microinjections of DA antagonists into the OFC resulted in a significant main effect of treatment, F (3, 27) = 4.419, p = 0.012, illustrated in Figure 2. Planned contrasts revealed that RAC3 (p = 0.011) and RACH6 (p = 0.025) significantly decreased delayed lever choice compared to VEH, indicating an increase in impulsivity.

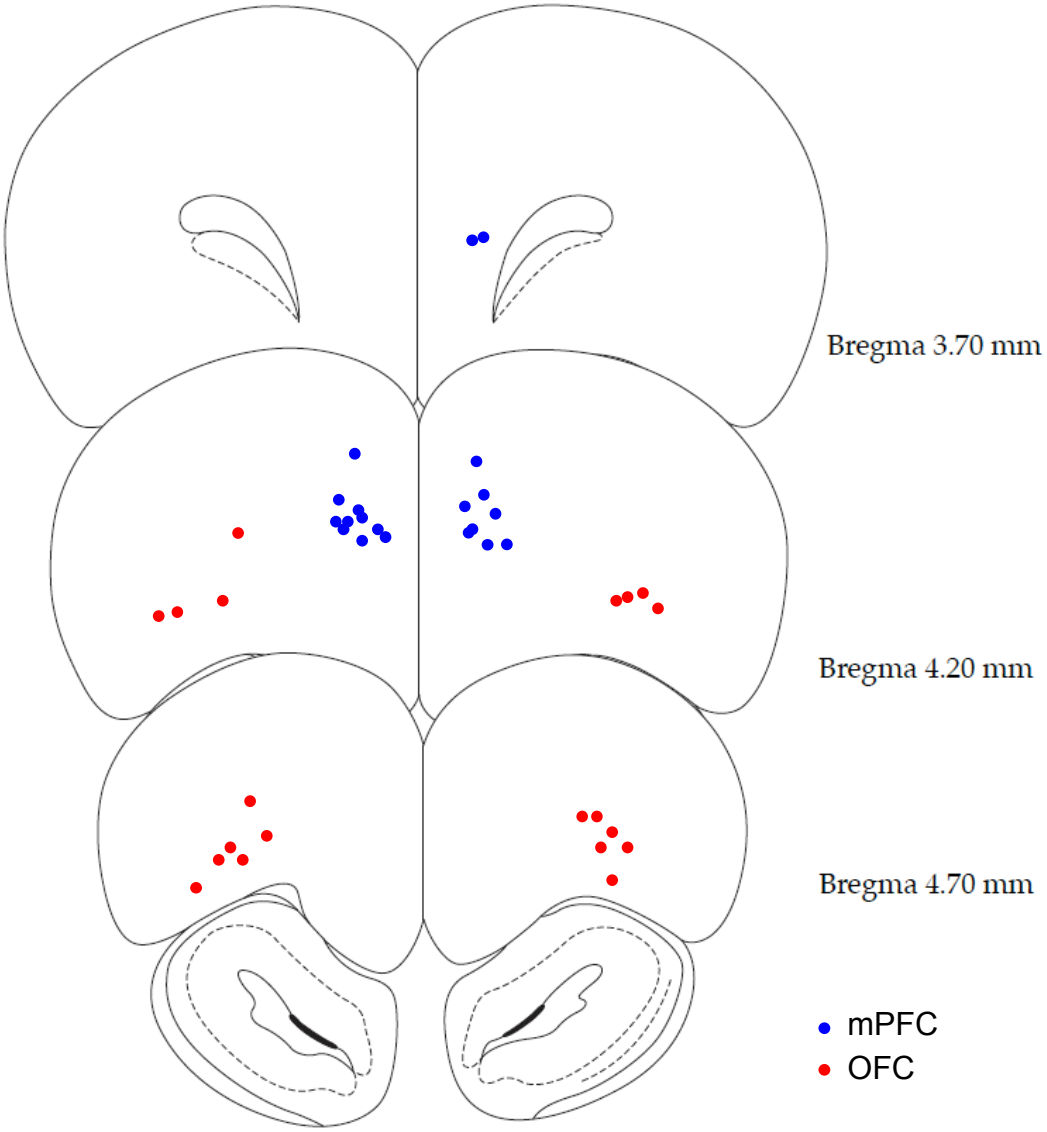

Figure 1. Schematic of cannulae placement for rats injected with dopamine antagonists in the medial prefrontal cortex (mPFC, blue) or the lateral orbitofrontal cortex (OFC, red).

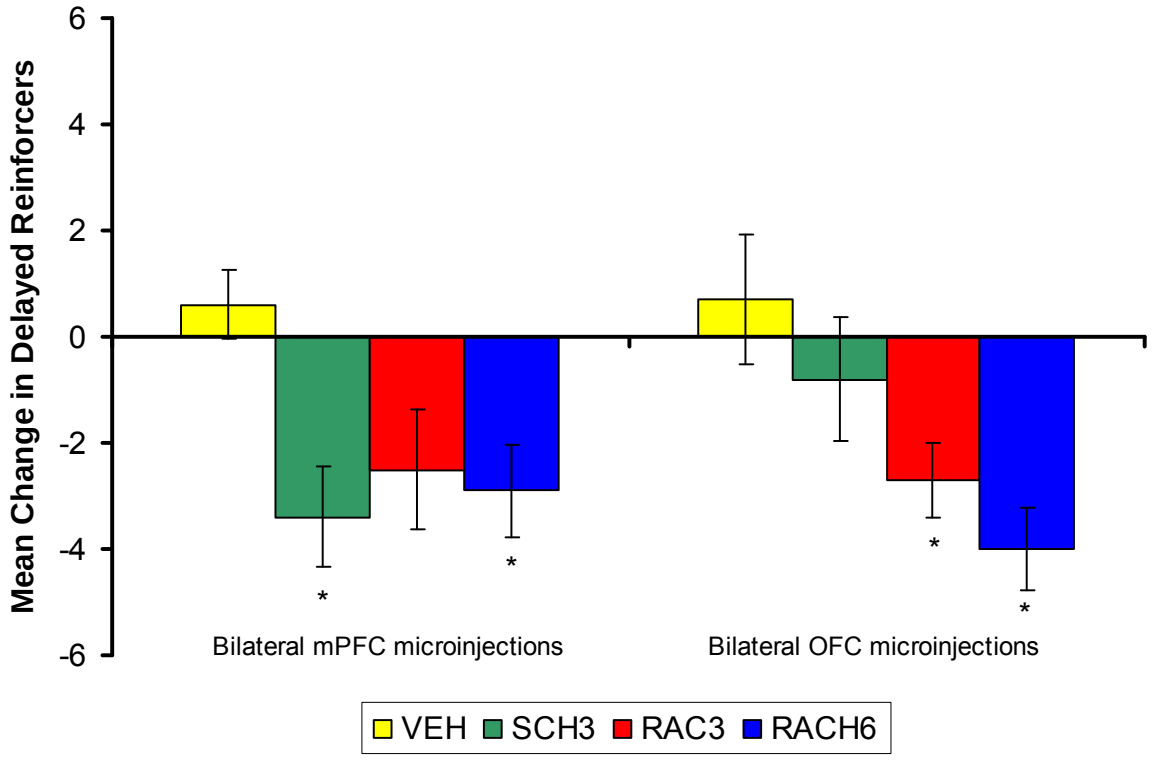

Figure 2. The mean (SEM) difference between the delayed reinforcers attained on a treatment day and delayed reinforcers attained on the day immediately prior, for each region of interest. One group of rats received bilateral microinjections into their medial prefrontal cortex (mPFC, n = 10) and the second group of rats received bilateral microinjections into their lateral orbiotofrontal cortex (OFC, n = 10). Drug doses administered were distilled water (VEH), 3 µg/ per side of SCH23390 (SCH3), 3 µg/ per side of raclopride (RAC3), and 6 µg/ per side of raclopride (RACH6). * Significant difference between the changes in delayed reinforcers for the treatment compared to vehicle, $p < 0.05$.

6.3.2 Effect of noradrenaline agonists on delayed reinforcer choice

The timeline for the completion of the DR task when the rats were locally treated in either the mPFC or OFC with the NA agonists phenylephrine and guanfacine is illustrated in Table 5. Due to cannulae misplacement, 7 rats were removed from the analysis. As a result there were 12 rats that received bilateral mPFC microinjections and 11 rats that received bilateral OFC microinjections, see cannulae placement in Figure 3.

Table 5. Number of days to complete each phase of the Delayed Reinforcement (DR) Task for rats receiving microinjections of noradrenaline agonists via intracranial cannulae in either the medial prefrontal cortex (mPFC) or lateral orbitofrontal cortex (OFC), mean ± SEM.

DR phase	mPFC (mean ± SEM)	OFC (mean ± SEM)
Training	7.8 ± 0.34	7.07 ± 0.23
Delay stabilisation	9.8 ± 0.42	10.33 ± 0.72
Restabilise after sham	3.33 ± 0.82	3.87 ± 0.48
Restabilise after 1st injection	3.6 ± 0.29	3.93 ± 0.34
Restabilise after 2nd injection	3.87 ± 0.31	3.67 ± 0.23
Restabilise after 3rd injection	3.93 ± 0.43	6.47 ± 0.97
Restabilise after 4th injection	3.6 ± 0.21	3.67 ± 0.23
Total	35.93 ± 1.28	39 ± 1.46

The repeated measures analysis assessing the effect of NA agonists on delayed lever choice revealed no main effect of treatment in either the mPFC, F (4, 44) = 0.886, $p > 0.05$, or the OFC, F (4, 40) = 0.518, $p > 0.05$, illustrated in Figure 4. This indicates that stimulation of NA α_1 and α_2 receptors in these regions does not alter impulsive choice.

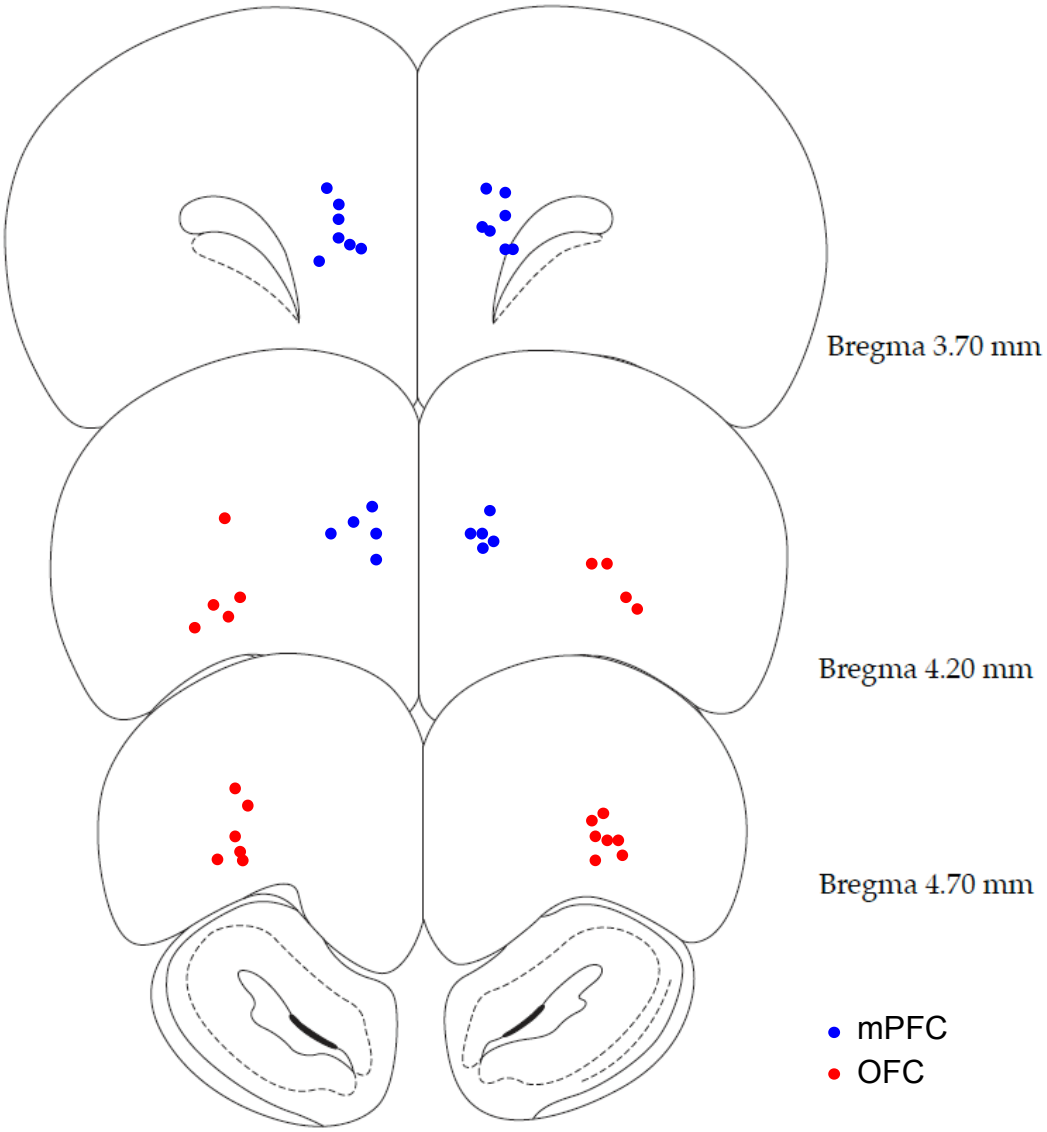

Figure 3. Schematic of cannulae placement for rats injected with noradrenaline agonists in the medial prefrontal cortex (mPFC, blue) or the lateral orbitofrontal cortex (OFC, red).

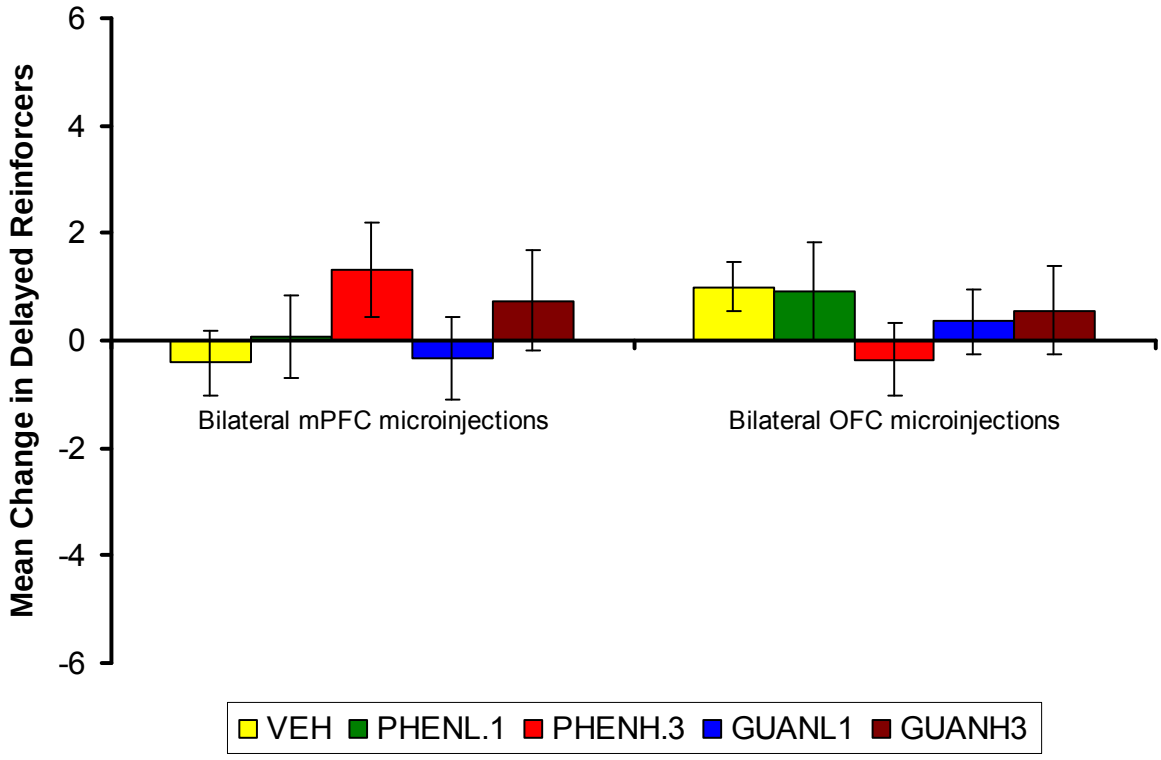

Figure 4. The mean (SEM) difference between the delayed reinforcers attained on a treatment day and delayed reinforcers attained on the day immediately prior, for each region of interest. Rats received bilateral microinjections into either the medial prefrontal cortex (mPFC, n = 12) or the lateral orbiotofrontal cortex (OFC, n = 11). Drug doses administered were saline (VEH), 0.1 µg/ per side of phenylephrine (PHENL.1), 0.3 µg/ per side of phenylephrine (PHENH.3), 1 µg/ per side of guanfacine (GUANL1), and 3 µg/ per side of guanfacine (GUANH3).

6.4 Discussion

The current study was conducted to assess the role of DA and NA receptors in impulsivity as mediated by two sub-regions of the PFC. The results indicate that blockade of the DA D1 and D2 receptors differentially increases impulsive choice, depending upon their location in the PFC. However, NA agonism had no effect on impulsive choice in either brain region. Antagonism of either the DA D1 or the D2 receptor type in the mPFC increased impulsivity. This effect was only observed with higher doses of the D2 receptor antagonist, raclopride,

suggesting that greater inhibition of D2 receptors in the mPFC is required to increase impulsivity. With respect to responding following infusion into the OFC, only D2 receptor antagonism increased impulsivity, with no effect of the local infusion of the D1 antagonist SCH 23390.

In line with previous research reporting increased impulsive choice of OFC lesioned rats (Mobini et al., 2002), the current study observed elevated impulsive choice following blockade of the D2 receptors in the OFC. The involvement of both D1 and D2 receptors in impulsivity is also consistent with reports of increased impulsivity with systemic administration of D1 (van Gaalen et al., 2006) and D2 receptor antagonists (Wade et al., 2000). However, the results obtained through local infusions in the current study are inconsistent with previous lesion studies reporting no effect of mPFC lesion on impulsivity (Cardinal et al., 2001) and those reporting *reduced* impulsivity following OFC lesions (Winstanley et al., 2004). A likely explanation for this disparity is that the previous studies employed excitiotoxic lesions to assess the role of these PFC regions on impulsivity. Such lesions are non-specific and eliminate all neurons within the region. In contrast, the present study was specifically looking at the modulation of the dopaminergic system within these regions. As such, the methodology of the current study maintained the structural integrity of the PFC regions and assessed the impact of transient alterations in specific receptor functions on PFC mediated impulsive choice.

An unexpected finding of the present study was the lack of effect of the NA receptor agonists in either region of the PFC. This finding is inconsistent with previous research reporting increased impulsivity following systemic administration of the α_2 receptor agonist clonidine (van Gaalen et al., 2006). Task differences are an unlikely explanation for these inconsistent results as very similar DR tasks were employed in both studies. The differences in these

results may be due to altered receptor selectivity, as guanfacine is highly selective for the α_{2A} receptor (Arnsten, Steere, & Hunt, 1996). Activation of the α_{2A} receptor occurs during periods of optimal PFC functioning, with stimulation of the α_{2A} receptor strengthening appropriate connections and increasing the 'signal' (Li, Mao, Wang, & Mei, 1999; Wang et al., 2007). As clonidine stimulates both α_2 and imidazoline receptors (van Zwieten, 1999), the increased impulsivity observed by van Gaalen and colleagues (2006) may have resulted from stimulation of the imidazoline receptors. Moreover, clonidine has been shown to inhibit DA turnover (Sica, 2007), a property not observed with guanfacine. Therefore, the impact of clonidine on DA turnover is an alternate explanation for van Gaalen's (2006) findings. Such an interpretation is consistent with the integral role of DA in impulsivity observed in the current study. Further research is necessary to elucidate this hypothesis.

Although lesions of the mPFC result in deficits of motor inhibition, the results of the current study can not be attributed to such a deficit as the rats were not required to delay or inhibit responses. The two levers do not require a different response, but they are associated with different outcomes. Therefore the increased impulsivity following infusion of DA antagonists into the mPFC reflects altered choice/decision making. The results of this study are unable to determine whether the drug administration produced altered sensitivity to the increasing delay or the value of the reward. Previous research has shown that the mPFC influences the rats sensitivity to delay (Izaki, Fujiwara, & Akema, 2007). However, altered DA levels in the OFC influence how much value is attributed to a reward, and therefore whether that particular reward is worth waiting for (Kheramin et al., 2004). Based on this previous research (Izaki et al., 2007; Kheramin et al., 2004), it could be postulated that DA antagonism in the mPFC is associated with delay aversion, while in the OFC DA antagonism is associated with decreased value of the reward.

Previous findings of this thesis indicate that chronic MPH treatment during adolescence increases impulsivity in adulthood (Chapter 4) and alters the maturation of the catecholamine system (Chapter 5). The results of this study indicate that this long-term effect of MPH treatment is most likely due to changes in DA and not NA neurotransmission. This theory is consistent with van Gaalen's (2006) study. They found that blocking the DA transporter with GBR 12909 had the same effect on impulsivity as MPH, while inhibition of the NA transporter with desipramine did not mirror the effect of MPH on impulsivity. Furthermore, the involvement of the DA D2 receptor in impulsivity induced by chronic MPH treatment is consistent with the finding of upregulation in DA D2 receptor density and function after long-term MPH administration (Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 2000; Thanos, Michaelides, Benveniste, Wang, & Volkow, 2007). While it is reported that MPH increases catecholamine levels by blocking both DA and NA transporters (Gatley, Pan, Chen, Chaturvedi, & Ding, 1996; Kuczenski & Segal, 1997; Volkow et al., 2002), the current and previous findings together suggest that MPH predominately influences impulsivity through the dopaminergic system.

Consistent with the results of the current study, DA dysfunction within the PFC has been reported in disorders in which impulsivity is a feature. Impulsivity is an important feature of vulnerability to substance use and can contribute to relapse to drug-seeking (Jentsch & Taylor, 1999; Moeller et al., 2001; Volkow & Fowler, 2000). A significant neurobiological mechanism underlying substance use and addiction is reduced DA function in the PFC (Koob & Volkow, 2010; Volkow, Fowler, Wang, Baler, & Telang, 2009). Deficits in PFC DA function have also been reported in schizophrenia and ADHD (Russell, 2002; Volk & Lewis, 2010), disorders that are also characterised by impulsive behaviours (American Psychiatric Association, 2000; Kaladjian, Jeanningros, Azorin, Anton, & Mazzola-Pomietto, 2011; Nolan, D'Angelo, & Hoptman, 2010).

It is well described that catecholamines have different functions within the brain. The DA system regulates responses to reinforcement (Schultz, 1998). The role of DA has been proposed to be integral in "fine tuning", or adjusting the 'noise' of the neural signal based on task demands (Arnsten, 2007). Low levels of D1 stimulation would be optimal for a low level task, allowing for broad tuning, while in a more demanding high level cognitive task requiring narrow tuning, higher levels of D1 receptor stimulation would be appropriate. This suggests that some PFC tasks may be more sensitive to alterations in DA levels than others. The DA depleted induced impulsivity in the current study is consistent with Arnsten's (2007) hypothesis of DA's involvement in 'fine tuning' PFC neurons. Persistent high levels of tuning are integral for overcoming distractors (Miller, Erickson, & Desimone, 1996) and inhibiting inappropriate behaviours (Funahashi, Chafee, & Goldman-Rakic, 1993). The inhibition of DA receptors in the current study may have increased the 'noise' relative to the signal and therefore altered the rats' responses to reinforcement. On the other hand, the NA system mediates perception and interest in the stimuli (Solanto, 1998). Consistent with the role of NA in the maintenance of interest, well trained rats performing a five-choice serial reaction time task show no marked increase in NA efflux in the PFC (Dalley et al., 2001). The rats in the current study were very well trained by the time the drugs were administered. Therefore, based on Dalley and colleagues (2001) findings, NA levels may not have been fluctuating as a result of task completion. This provides a possible explanation why stimulating NA receptors had no effect on task performance, as the task had become independent of NA efflux. The role of DA and NA in impulsivity as measured by the DR task in the current study is consistent with the different functions of these catecholamine systems within the brain.

The current study has shown that DA receptors play a distinct role in impulsivity depending upon their location in the PFC. This is the first study to investigate the role of DA and NA receptors located in the OFC and mPFC in impulsivity. The results highlight the importance

of DA receptor function in impulsive choice and suggest future directions for treatments of disorders with impulsivity as a core feature. Future research would benefit from assessing impulsivity using multiple tasks, including those assessing impulsive action, to distinguish between the role of these neurotransmitters in the aversion to delay and sensitivity to reward.

6.5 References

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision ed.). Washington, DC: American Psychiatric Association.

Arnsten, A. F. T. (1997). Catecholamine regulation of the prefrontal cortex. *Journal of Psychopharmacology, 11*, 151-162.

Arnsten, A. F. T. (2006). Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology, 31*, 2376-2383.

Arnsten, A. F. T. (2007). Catecholamine and second messenger influences on prefrontal cortical networks of "representational knowledge": A rational bridge between genetics and the symptoms of mental illness. *Cerebral Cortex, 17* (Supplemental 1), i6-i15.

Arnsten, A. F. T. (2009). Towards a new understanding of attention-deficit hyperactivity disorder pathophysiology. *CNS Drugs, 23 (Supplemental 1)*, 33-41.

Arnsten, A. F. T., Mathew, R., Ubriani, R., Taylor, J. R., & Li, B.-M. (1999). α_1 Noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. *Biological Psychiatry, 45*, 26-31.

Arnsten, A. F. T., Steere, J. C., & Hunt, R. D. (1996). The contribution of α_2-noradrenergic mechanisms to prefrontal cortical cognitive function. *Archives of General Psychiatry, 53*, 448-455.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F. T., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60*, 1111-1120.

Birnbaum, S. G., Podell, D. M., & Arnsten, A. F. T. (2000). Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug, FG7142, in rats. *Pharmacology, Biochemistry and Behavior, 67*, 397-403.

Birnbaum, S. G., Yuan, P. X., Wang, M., Vijayraghavan, S., Bloom, A. K., Davis, D. J., et al. (2004). Protein Kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science, 306* (5697), 882-884.

Cardinal, R. N., Pennicott, D. R., Sugathapala, C. L., Robbins, T. W., & Everitt, B. J. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science, 292*, 2499-2501.

Carlson, S., Tanila, H., Rama, P., Mecke, E., & Pertovaara, A. (1992). Effects of medetomidine, and α-2 adrenoceptor agonist, and atipamezole, and α-2 antagonist, on spatial memory performance in adult and aged rats. *Behavioral and Neural Biology, 58*, 113-119.

Crombag, H. S., Gorny, G., Li, Y., Kolb, B., & Robinson, T. E. (2005). Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. *Cerebral Cortex, 15*, 341-348.

Dalley, J. W., McGaughy, J., O'Connell, M. T., Cardinal, R. N., Levita, L., & Robbins, T. W. (2001). Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. *Journal of Neuroscience, 21*, 4908-4914.

Deutch, A. Y., Clark, W. A., & Roth, R. H. (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Research, 521*, 311-315.

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*, 161-170.

Foote, S. L., Aston-Jones, G., & Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal.

Proceedings of the National Academy of Sciences of the United States of America, 77, 3033-3037.

Funahashi, S., Chafee, M. V., & Goldman-Rakic, P. S. (1993). Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. *Nature, 365*, 753-756.

Gatley, S. J., Pan, D., Chen, R., Chaturvedi, G., & Ding, Y.-S. (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sciences, 58* (12), PL 231-239.

Greenhill, L., Beyer, D. H., Finkleson, J., Shaffer, D., Biederman, J., Conners, C. K., et al. (2002). Guidelines and algorithms for the use of methylphenidate in children with attention-deficit/hyperactivity disorder. *Journal of Attention Disorders, 6 (Supplemental 1)*, S89-100.

Izaki, Y., Fujiwara, S.-e., & Akema, T. (2007). Involvement of rat prefrontal cortex in a delayed reinforcement operant task. *Neuroreport, 18* (16), 1687-1690.

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behaviour by reward-related stimuli. *Psychopharmacology (Berl), 146*, 373-390.

Ji, X.-H., Ji, J.-Z., Zhang, H., & Li, B.-M. (2008). Stimulation of α_2 - Adrenoceptors suppresses excitatory synaptic transmission in the medial prefrontal cortex of rat. *Neuropsychopharmacology, 33*, 2263-2271.

Kaladjian, A., Jeanningros, R., Azorin, J. M., Anton, J. L., & Mazzola-Pomietto, P. (2011). Impulsivity and neural correlates of response inhibition in schizophrenia. *Psychological Medicine, 41*, 291-299.

Kheramin, S., Body, S., Ho, M.-Y., Velazquez-Martinez, D. N., Bradshaw, C. M., Szabadi, E., et al. (2004). Effects of orbital prefrontal cortex dopamine depletion on inter-temporal choice: A quantitative analysis. *Psychopharmacology, 175*, 206-214.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology, 35*, 217-238.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: Comparison with amphetamine. *Journal of Neurochemistry, 68*, 2032-2037.

Levy, F. (2008). Pharmacological and therapeutic directions in ADHD: Specificity in the PFC. *Behavioral and Brain Functions, 4*, 12.

Li, B.-M., Mao, Z.-M., Wang, M., & Mei, Z.-T. (1999). Alpha-2 adrenergic modualtion of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology, 21*, 601-610.

Mao, Z.-M., Arnsten, A. F. T., & Li, B.-M. (1999). Local infusion of an α-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. *Biological Psychiatry, 46*, 1259-1265.

Miller, E. K., Erickson, C. A., & Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *Journal of Neuroscience, 16*, 5154-5167.

Milstein, J. A., Lehmann, O., Theobald, D. E., Dalley, J. W., & Robbins, T. W. (2007). Selective depletion of cortical noradrenaline by anti-dopamine beta-hydroxylase-saporin impairs attentional function and enhances the effects of guanfacine in the rat. *Psychopharmacology, 190*, 51-63.

Mobini, S., Body, S., Ho, M.-Y., Bradshaw, C. M., Szabadi, E., Deakin, J. F. W., et al. (2002). Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology, 160*, 290-298.

Moeller, F. G., Dougherty, D. M., Barratt, E. S., Schmitz, J. M., Swann, A. C., & Grabowski, J. (2001). The impact of impulsivity on cocaine use and retention in treatment. *Journal of Substance Abuse Treatment, 21*, 193-198.

Naneix, F., Marchand, A. R., Di Scala, G., Pape, J.-R., & Coutureau, E. (2009). A role for medial prefrontal dopaminergic innervation in instrumental conditioning. *Journal of Neuroscience, 29* (20), 6606-6599.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.).

Nolan, K. A., D'Angelo, D., & Hoptman, M. J. (2010). Self-report and laboratory measures of impulsivity in patients with schizophrenia or schizoaffective disorder and healthy controls. *Psychiatry Research,* doi: 10.1016/j.psychres.2010.10.032 *23*.

Pardey, M. C., Homewood, J., Taylor, A., & Cornish, J. L. (2009). Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods, 176*, 166-171.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxtic coordinates* (4th ed.). New York: Academic Press.

Robinson, E. S. J., Eagle, D. M., Mar, A. C., Bari, A., Banerjee, G., Jiang, X., et al. (2008). Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology, 33*, 1028-1037.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology, 47*, 33-46.

Russell, V. A. (2002). Hypodopaminergic and hypernoradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder - the spontaneously hypertensive rat. *Behavioural Brain Research, 130*, 191-196.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C. L., & Taljaard, J. J. F. (2000). Methylphenidate affects striatal dopamine differently in an animal model for attention-deficit/hyperactivity disorder - The spontaneously hypertensive rat. *Brain Research Bulletin, 53* (2), 187-192.

Sagvolden, T. (2006). The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioral and Brain Functions, 2*, 41.

Schultz, W. (1998). The phasic reward signal of primate dopamine neurons. *Advances in Pharmacology, 42*, 686-690.

Sica, D. A. (2007). Centrally acting antihypertensive agents: An update. *Journal of Clinical Hypertension, 9*, 399-405.

Solanto, M. V. (1998). Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: A review and integration. *Behavioural Brain Research, 94*, 127-152.

Thanos, P. K., Michaelides, M., Benveniste, H., Wang, G. J., & Volkow, N. D. (2007). Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. *Pharmacology Biochemistry and Behavior, 87*, 426-433.

van Gaalen, M. M., van Koten, R., Schoffelmeer, A. N. M., & Vanderschuren, L. J. M. J. (2006). Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biological Psychiatry, 60*, 66-73.

van Zwieten, P. A. (1999). The renaissance of centrally acting antihypertensive drugs. *Journal of Hypertension. Supplement, 17*, S15-S21.

Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., & Arnsten, A. F. T. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nature Neuroscience, 10* (3), 376-384.

Volk, D. W., & Lewis, D. A. (2010). Prefrontal cortical circuits in schizophrenia. *Current Topics in Behavioral Neurosciences, 4*, 485-508.

Volkow, N. D., & Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cerebral Cortex, 10*, 318-325.

Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., & Telang, F. (2009). Imaging dopamine's role in drug abuse and addicition. *Neuropharmacology, 56* (Suppl 1), 3-8.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Franceschi, D., Maynard, L., et al. (2002). Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeutic implications. *Synapse, 43*, 181-187.

Wade, T. R., de Wit, H., & Richards, J. B. (2000). Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology, 150*, 90-101.

Wang, M., Ramos, B., Paspalas, C., Shu, Y., Simen, A., Duque, A., et al. (2007). Alpha2A-adrenoceptor stimulation strengthens working memory networks in inhibiting cAMP-HCN channel signalling in prefrontal cortex. *Cell, 129* (2), 397-410.

Winstanley, C. A., Eagle, D. M., & Robbins, T. W. (2006). Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. *Clinical Psychology Review, 26*, 379-395.

Winstanley, C. A., Theobald, D. E., Cardinal, R. N., & Robbins, T. W. (2004). Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *Journal of Neuroscience, 24* (20), 4718-4722.

Winstanley, C. A., Theobald, D. E., Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2006). Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cerebral Cortex, 16*, 106-114.

Winter, S., Diekmann, M., & Schwabe, K. (2009). Dopamine in the prefrontal cortex regulates rats behavioral flexibility to changing reward value. *Behavioural Brain Research, 198*, 206-213.

Chapter 7

General Discussion

7.1 Major findings

The broad aim of this thesis was to assess the long-term effects of chronic methylphenidate (MPH) treatment in adolescent rats. The strengths of the research were that it utilised a well validated animal model of Attention Deficit/Hyperactivity Disorder (ADHD) and non-ADHD (Chapter 2) and the MPH dosing schedule employed closely approximated clinical dosing regimes (Chapter 3).

A major finding was that chronic MPH treatment to non-ADHD (i.e. misdiagnosed) adolescent rats resulted in increases in impulsivity in adulthood (Chapter 4). The non-ADHD rats that were exposed to MPH during adolescence preferred a small immediate reward to a large reward delivered after a delay and demonstrated increased sensitive to delay in the absence of reinforcement. There were no effects of chronic MPH treatment on impulsivity in adulthood in the ADHD strain. Furthermore, the treatment interfered with the trajectory of maturation of catecholamine projections in the infralimbic (IL) subregion of the prefrontal cortex (PFC) of the non-ADHD rats only (Chapter 5). In the absence of MPH treatment, a reduction in catecholamine positive fibres occurred with age while an increase in the density of fibres was evident following the environmental enriching experience of performing behavioural tasks. These changes in catecholamine density were not evident in the non-ADHD rats that were pretreated with MPH. Together these findings suggest that MPH administration differentially affects non-ADHD (i.e. misdiagnosed) rats, compared to ADHD rats, to produces persistent changes in their cognitive function and neural development.

Another major finding was that dopamine (DA) receptor antagonism had a significant impact

upon impulsivity depending upon their location in subregions of the PFC. Within the medial PFC (mPFC), antagonism of both the DA D1 and D2 receptors increased impulsivity using a delayed reinforcement (DR) task, while within the orbitofrontal cortex (OFC), only blockade of the D2 receptors, but not the D1 receptors, increased impulsivity. Stimulation of noradrenaline (NA) receptors in the OFC and mPFC had no impact upon impulsive choice in this measure of impulsivity (Chapter 6). This is the first study to directly investigate the role of specific catecholamine receptors within well-defined regions of the PFC in impulsivity. The findings of Chapter 6 suggest that alterations in the dopaminergic, but not noradrenergic system, underlie the long-term changes in cognitive function and neural development observed in Chapters 4 and 5.

7.2 Dopamine and noradrenaline in impulsive choice

It has previously been shown that both DA and NA modulate impulsive choice. For example, the systemic administration of DA D1 and D2 receptor antagonists, or NA α_2 receptor agonists has been shown to increase impulsive choice (van Gaalen, van Koten, Schoffelmeer, & Vanderschuren, 2006; Wade, de Wit, & Richards, 2000), with decreased impulsive choice following systemic blockade of the NA transporters (Robinson et al., 2008).

Previous research has focused on impulsivity mediated by the nucleus accumbens (NAc). Within the NAc clear distinctions between the core and shell subregions have been identified (Zaborszky et al., 1985). Both the core and shell of the NAc are densely innervated by dopaminergic projections from the substantia nigra and ventral tegmental area (VTA; Groenewegen, 2003; Hyman, Malenka, & Nestler, 2006). Additionally, noradrenergic afferents are directed specifically towards the NAc shell, but not the core, from the locus coeruleus (Berridge, Stratford, Foote, & Kelley, 1997; Swanson & Hartman, 1975). The NAc is integral for processing rewarding stimuli (Ikemoto, 2007; Koob, 1992). However the core

of the NAc, but not the shell, has been implicated in impulsivity (Bezzina et al., 2008; Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; da Costa Araujo et al., 2009; Pothuizen, Jongen-Relo, Feldon, & Yee, 2005). In contrast, the shell region of the NAc has been shown to influence unlearned behaviours (Kelley, 1999). This role of the NAc shell is consistent with findings that the NAc shell receives noradrenergic innervation (Berridge et al., 1997) and that well-trained rats performing a task do not show NA efflux (Dalley et al., 2001). Together this suggests that DA within the NAc core is associated with impulsive choice, while NA within the shell is not.

The results of this thesis demonstrate a similar pattern for catecholamines in impulsive choice mediated by the PFC. Antagonism of DA receptors in the PFC induced impulsive choice while stimulation of NA α receptors was without consequence. It is possible that this influence of PFC DA on impulsive choice is driven by the direct and indirect connections of the PFC to the NAc. DA in the PFC regulates glutamatergic efferents to the NAc as well as the VTA, which in turn has significant dopaminergic projections to the NAc (Carr & Sesack, 2000; Sesack & Carr, 2002; Sesack & Pickel, 1992). The altered communication between the PFC and NAc would impact on DA release in the NAc and may change the dynamics of reward signalling and associated impulse control (Schultz, 1998). The research presented in this thesis demonstrates the importance of the functional integrity of DA in impulsive choice mediated by the PFC. In contrast, the noradrenergic system did not have any association with PFC-mediated impulsive choice. Although systemic administration of NA ligands has been shown to impact impulsive choice (Robinson et al., 2008; van Gaalen et al., 2006), stimulation of the NA α receptors within specific regions of the PFC had no impact upon impulsive choice in the current study. Consistent with the role of NA in the maintenance of interest (Solanto, 1998), well trained rats performing a five-choice serial reaction time task show no marked increase in NA efflux in the PFC (Dalley et al., 2001). The rats in the current

study were very well trained prior to drug administration to the PFC. This suggests that at the time of test, the involvement of NA neurotransmission in the PFC for the impulsivity task would be minimal and is highlighted by the lack of effect of NA receptor activation on task performance. Together these findings suggest that impulsive choice mediated by the PFC relies on DA neurotransmission and functions independently of the NA system.

The involvement of DA but not NA in PFC-mediated impulsivity provides additional information with which to interpret the long-term effects of chronic MPH treatment on neural development (Chapter 5). For the measures of neural development, analyses of tyrosine hydroxylase (TH) staining in the PFC were compared in MPH pretreated WKYs following time-periods corresponding to the impulsivity task (short-term group) and the completion of all behavioural tasks (long-term group, behavioural group). As the rats aged, from the point in time equivalent to when they were demonstrating impulsivity to much later in adulthood, the relationship between the density of TH staining following treatment changed. From Figure 2 in Chapter 5, rats in the short-term group that were pretreated with MPH tended to have less TH positive fibres compared to the water treated rats. However this relationship reversed with age such that the MPH pretreated rats in the long-term group tended to have more TH positive fibres compared to their water treated counterparts. As TH immunohistochemistry stains for both DA and NA neuron fibres it is not known whether the changes in TH staining over time following MPH administration reflected alterations to the DA and/or NA systems. However, the results of Chapter 6 do implicate DA and not NA systems in PFC medicated impulsivity suggesting that the changing influence of MPH pretreatment on TH positive fibres was most likely mediated by plasticity of the DA system.

Long-term alterations in DA, but not NA function following MPH exposure are also consistent with the differing ages of development of these neurotransmitter systems. In rats,

NA afferents projecting from the LC achieve their adult pattern of connectivity by PND 7 (Levitt & Moore, 1979). By contrast, there is a continual increase in the density of the dopaminergic innervation to the PFC until early adulthood, with the adult patterns of DA connectivity not achieved until two months after birth (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988). Despite therapeutic doses of MPH increasing extracellular levels of both DA and NA in the PFC (Berridge et al., 2006), the current study was conducted during the vulnerable stage of maturation of the DA and not NA cells. Chronic MPH administration during this stage may have predominantly affected the development of the DA system to alter PFC function and subsequent behaviours.

7.3 Postulated mechanism for the long-term effect of chronic MPH treatment

The action of MPH is similar to cocaine in that they both block DA and NA transporters, increasing synaptic concentrations of these neurotransmitters (Berridge et al., 2006; Kuczenski & Segal, 1997). Previous research has demonstrated PFC dysfunction following chronic cocaine administration, similar to the current findings. In monkeys and rats, chronic treatment with cocaine has been shown to impair the PFC mediated task of reversal learning in adulthood (Jenstch, Olausson, De La Garza, & Taylor, 2002; Schoenbaum, Saddoris, Ramus, Shaham, & Setlow, 2004). Impairments in reversal learning have been associated with impulsivity (Jentsch & Taylor, 1999). As there is limited research which investigates the underlying mechanisms of chronic MPH treatment, previous studies which assess the neurobiological underpinnings of cocaine sensitisation may provide appropriate comparisons. It is important to note that the method of administration employed in cocaine sensitisation studies differs significantly from current study, and therefore has reduced therapeutic relevance. While the majority of studies investigating behavioural sensitisation to cocaine have focused on the regions of the NAc and VTA (Kalivas, Pierce, Cornish, & Sorg, 1998; Robinson & Berridge, 1993), this discussion will focus on the effect of cocaine administration

on PFC function to relate to the findings of this thesis.

Sensitisation is the term used to describe augmentation of a drug response with repeated administration (Kalivas et al., 1998). Acute doses of cocaine have shown an increase in DA concentration in the mPFC (Sorg, Davidson, Kalivas, & Prasad, 1997; Sorg & Kalivas, 1993). While repeated exposure to intermittently administered cocaine significantly enhanced cocaine-induced DA efflux in the NAc (Kalivas et al., 1998), repeated cocaine administration significantly attenuated cocaine-induced DA transmission in the mPFC (Sorg et al., 1997). Cocaine sensitisation has also been associated with reduced functioning of postsynaptic DA D2 receptors in the mPFC (Steketee, 2003). Under normal circumstances, stimulation of mPFC DA D2 receptors has been reported to increase the inhibitory neurotransmitter GABA (Grobin & Deutch, 1998), indirectly inhibiting output pyramidal neurons. DA D2 receptor stimulation also directly inhibits these pyramidal neurons (Law-Tho, Hirsch, & Crepel, 1994). Together these studies show that activation of DA D2 receptors produce direct and indirect inhibitory output from the mPFC to the NAc. When DA D2 receptor function is reduced, in the case of cocaine sensitisation, there is a decrease in the inhibition of the pyramidal neurons, resulting in an increase in excitatory output to the NAc which augments the behavioural response to the drug. Together these studies suggest that repeated exposure to cocaine attenuates dopamine release in the mPFC and modifies D2 receptor function in this area.

The findings of this thesis similarly implicate alterations in D2 receptor functioning following chronic MPH treatment. Chronic exposure to MPH treatment during development resulted in impulsivity in adulthood. In addition, using an identical behavioural measure, it was demonstrated that similar increases in impulsivity resulted from antagonism of DA D2 receptors in the mPFC and OFC. These data are in line with the findings of upregulation of striatal DA D2 receptor density and function after long-term MPH administration (Russell, de

Villiers, Sagvolden, Lamm, & Taljaard, 2000; Thanos, Michaelides, Benveniste, Wang, & Volkow, 2007b). Taken together the data suggest that chronic MPH treatment alters DA D2 receptor function, in a similar manner to that reported after repeated cocaine administration. An additional finding of the set of experiments conducted in Chapter 6 was the increase in impulsivity following DA D1 receptor antagonism in the mPFC, but not the OFC. This is also consistent with findings reporting regional specificity in the role of the D1 receptor in cocaine sensitisation. D1 receptor stimulation in the ventral mPFC had no impact upon cocaine sensitisation (Beyer & Steketee, 2002), while activation of these receptors in the dorsal mPFC blocked the sensitised response (Sorg, Li, & Wu, 2001). Similar regional specific involvement of D1 receptors was observed in impulsivity in the experiments conducted in Chapter 6 and suggests that alterations of D1 receptors within specific subregions of the PFC may also be responsible for the impulsivity induced by chronic MPH treatment (Chapter 4). Together these findings highlight the alterations in the dopaminergic system that follow MPH exposure.

Cocaine also increases extracellular levels of NA in the PFC (Florin, Kuczenski, & Segal, 1994). Despite the acute affect cocaine has on PFC NA levels, research has demonstrated that NA is not involved in cocaine sensitisation (Vanderschuren, Beemster, & Schoffelmeer, 2003). Furthermore, the DA transporter but not the NA transporter, has been shown to be integral in the development of cocaine sensitisation (Hall et al., 2009; Mead, Rocha, Donovan, & Katz, 2002) and the increase in dendritic spine density in the NAc associated with cocaine sensitisation (Martin et al., 2010). The limited involvement of PFC NA following chronic cocaine administration, together with the current finding that stimulation of NA α receptors did not influence impulsivity, further suggests that chronic MPH treatment did not produce a long-term increase in impulsivity via the noradrenergic system.

Specific alterations in dopaminergic functioning following chronic MPH treatment may

provide an explanation for why deficits in adulthood were only found in the WKY and not the SHR strain. Similar to children with ADHD, SHRs have been found to have deficient DA systems (Leo et al., 2003; Russell, Sagvolden, & Johansen, 2005a). Therefore, the hypofunctioning dopaminergic systems may affect the ability for chronic MPH treatment to alter long-term PFC function in this strain.

It is important to keep in mind that as an animal model of ADHD, MPH treatment is therapeutic for the SHRs, however this has been debated. MPH acts in the short-term to normalise the SHRs deficient DA system (Russell et al., 2005a). However, when MPH is administered to a non-ADHD (i.e. misdiagnosed) rat it inappropriately activates the DA system. The results of this thesis suggest that the inappropriate activation of the DA system in the non-ADHD rats produced long-term deficits in impulsivity and interfered with the normal developmental trajectory of the catecholamine fibres within the IL region of the PFC. Such findings hold significant implications for children misdiagnosed with ADHD. These data suggest that should misdiagnosed children receive MPH treatment, they may experience long-term deficits in cognitive and neural development.

MPH sensitisation has not yet been confirmed using a therapeutically relevant method of drug administration. The mechanism postulated above is based upon cocaine sensitisation studies as there is limited research investigating the underlying mechanism of chronic MPH treatment. However, the methods of drug administration differ between the previous research and current study which greatly impacts the pharmacokinetics of the drug, as discussed in Chapter 3. Future research employing a more therapeutically relevant drug administration procedure, such as the method developed in this thesis, would elucidate the mechanisms underlying MPH sensitisation.

7.4 Potential implications for ADHD diagnosis

It is not the aim of this thesis to comment if Ritalin® should be prescribed to children. Indeed, Ritalin® does have a place in treating ADHD (Greenhill et al., 2002; Solanto et al., 2009). However, the findings of the research presented in this thesis argue for a more stringent diagnosis of ADHD. Previous research has shown that higher cerebrospinal fluid concentrations of the DA metabolite, homovanillic acid (HVA), were associated with greater behavioural improvement, while low concentrations of HVA were associated with worsening behaviour ratings following MPH treatment in children with ADHD (Castellanos, Elia et al., 1996). Therefore, measuring levels of HVA in cerebrospinal fluid could potentially be used to predict whether an individual would respond favourably to MPH treatment, i.e. show a reduction in behavioural symptoms following MPH treatment. However, a lumbar puncture is required to conduct such an assessment in children which is inappropriate given the potential risk and cost associated with the procedure.

In the absence of definitive physiological determinants (Wallis, 2010a), perhaps the more restrictive criteria of the ICD 10 should be employed. The ICD 10 criterion generally identifies patients with the more severe symptomatology of the ADHD-Combined subtype, whilst the ADHD-Predominantly Inattentive (ADHD-I) and ADHD-Predominantly Hyperactive/Impulsive subtypes are not diagnosed under ICD 10 criteria. While prescription of MPH is restricted to Paediatricians and Psychiatrists in some states in Australia, this is not a national policy. Despite these restrictions, subjective interpretations of the DSM-IV diagnostic criteria are the main determiners of diagnosis, and therefore employing the more stringent ICD 10 diagnostic criteria may be more appropriate.

7.5 Implications for nonmedical use of MPH

The findings of this thesis not only have implications for adolescents who are misdiagnosed

with ADHD, but also for those who misuse MPH and/or take it illicitly. Prescription stimulants are commonly misused (Kollins, 2008) and MPH has acquired many street names including “Vitamin R” and “the smart drug” (Kollins, MacDonald, & Rush, 2001). The nonmedical to medical ratio for stimulant use is much higher than that for opiates, despite more opiate prescriptions per se (McCabe, Teter, & Boyd, 2006). Research identifying the motives for nonmedical use of stimulants indicates that stimulant misuse among college students is more common for ‘performance enhancement’, with fewer students reportedly using prescription stimulants to ‘get high’ (Teter, McCabe, LaGrange, Cranford, & Boyd, 2006). Analysis of the American Association of Poison Control Centers Toxic Exposure Surveillance System identified 759 cases of children between the ages of 10 and 19 years that had abused MPH between 1993-1999 (Klein-Schwartz & McGrath, 2003). Almost half of the identified cases involved children 10 to 14 years of age.

While research has shown an acute dose of MPH can increase concentration and attention in people without ADHD, the results of this thesis suggest that these people who do not have ADHD and are misusing MPH to enhance their concentration and attention, may be placing themselves at risk of long-term neural consequences. Although the current findings were the result of a relatively chronic treatment period (4 weeks), previous preclinical research has shown memory impairments following shorter periods of MPH administered in high doses (Heyser, Pelletier, & Ferris, 2004).

An important consideration in the long-term effects of chronic MPH use is age at the time of treatment. Different long-term consequences have been reported for rats chronically treated with MPH in either early or late adolescence (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002). Although the adult brain is considered to have reached maturity, there is evidence that chronic MPH treatment can produce long-term effects on neural processing. For

example, spatial memory impairments have been reported three weeks after cessation of chronic MPH treatment during adulthood in rats (LeBlanc-Duchin & Taukulis, 2009) and the development of sensitisation and tolerance to the drug have been reported following chronic MPH administration to adult rats (Crawford, McDougall, Meier, Collins, & Watson, 1998; Kuczenski & Segal, 2001a; Yang, Amini, Swann, & Dafny, 2003b; Yang, Swann, & Dafny, 2010). Different methodologies employed in previous studies which assess a variety of age ranges limit the conclusions that can be made. The long-term neural and behavioural effects of chronic MPH treatment at different stages in the lifespan are yet to be systematically assessed and are a challenge for future research.

7.6 Potential influence of MPH on substance abuse

There is much concern about psychostimulant treatment during adolescence leading to future substance abuse (Kollins et al., 2001; Vitiello, 2001). Rats will readily self-administer MPH (Botly, Burton, Rizos, & Fletcher, 2008), demonstrating the abuse potential of MPH administration. Furthermore, repeated administration of MPH can produce behavioural sensitisation (Crawford et al., 1998; Gaytan, Yang, Swann, & Dafny, 2000; Kuczenski & Segal, 2001a; Yang et al., 2003b; Yang, Swann, & Dafny, 2006a; Yang et al., 2010) or tolerance at high doses (Yang et al., 2003b; Yang et al., 2010), where the processes of sensitisation and/or tolerance are thought to contribute to substance abuse and dependence (Dafny & Yang, 2006).

Results of earlier research assessing cross-sensitisation following chronic MPH pre-treatment have been mixed. Cross-sensitisation is a phenomenon in which repeated administration of one drug augments the response to an alternate drug, demonstrating that similar neurobiological systems have been affected. The majority of research has found that pre-exposure to MPH during adolescence results in reduced rewarding effects, and in some

instances aversion, to cocaine in adulthood as measured in a conditioned place preference (CPP) paradigm (Andersen et al., 2002; Augustyniak, Kourrich, Rezazadeh, Stewart, & Arvanitogiannis, 2006; Carlezon, Mague, & Andersen, 2003; Mague, Andersen, & Carlezon, 2005), while other research has found enhanced sensitivity to cocaine in adulthood (Brandon, Marinelli, Baker, & White, 2001). More recently, Griggs and colleagues (2010) demonstrated that twice daily administration of MPH over four weeks during adolescence increased acquisition of cocaine self-administration, in line with previous findings assessing cocaine sensitivity via self-administration (Brandon et al., 2001). A possible explanation for the contrasting findings of cocaine sensitivity following pre-exposure to MPH involves the route of cocaine administration. In CCP paradigms, the drug was delivered by the experimenter via intraperitoneal (i.p) injection, while self-administration procedures are initiated by the rat and deliver the drug intravenously (i.v.). Plasma levels rise significantly faster following i.v. administration, increasing the abuse potential of the drug (Volkow & Swanson, 2003).

Following abrupt cessation of chronic psychostimulant treatment, a withdrawal period ensues. Withdrawal is associated with negative emotional states such as dysphoria, anxiety and irritability (Jupp & Lawerence, 2010). These symptoms of withdrawal are thought to provide negative reinforcement and therefore induce the desire to take the drug to alleviate these symptoms (Koob & Le Moal, 1997; Weiss et al., 2001). Previously, long-term depressive and anxiety-like behaviours, in addition to altered stress responses have been reported in rats chronically treated with MPH throughout adolescence (Bolanos, Barrot, Berton, Wallace-Black, & Nestler, 2003; Bolanos et al., 2008; Carlezon et al., 2003), although not consistently reported (Gray et al., 2007). It is possible that the increases in negative affect that occur following chronic MPH treatment may enhance the desire for future drug use in an attempt to alleviate these negative emotional symptoms (Koob & Le Moal, 1997; Weiss et al., 2001).

Impulsivity is also an important feature of vulnerability to substance use and can contribute to drug-seeking behaviour (Jentsch & Taylor, 1999; Moeller et al., 2001; Volkow & Fowler, 2000). Persistent drug-taking can be viewed as the preference for immediate gratification with little regard for future consequences, a very similar concept to the preference for a small yet immediate reinforcer of heightened impulsive choice. Alcohol dependent individuals have significantly higher trait impulsivity and respond impulsively on cognitive tasks compared to healthy controls (Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009a, 2009b). Preclinical research has also demonstrated that high levels of impulsive choice are associated with increased levels of alcohol consumption and faster acquisition of cocaine self-administration (Perry, Larson, German, Madden, & Carroll, 2005; Perry, Nelson, & Carroll, 2008; Poulos, Le, & Parker, 1995). Furthermore, rodents genetically bred to be high alcohol consumers, but are alcohol naïve, have been shown to exhibit increased impulsive choice (Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008), suggesting that elevated impulsivity could be a predisposing vulnerability factor for high alcohol preference (Winstanley, Olausson, Taylor, & Jentsch, 2010).

A role for impulsivity in driving and maintaining drug dependence is of considerable concern given the findings of this thesis. The increased impulsivity that occurred following inappropriate MPH treatment may increase the potential of future substance abuse among incorrectly diagnosed populations. Together, research suggests that exposure to MPH during adolescence may produce altered psychological (Bolanos et al., 2003; Bolanos et al., 2008; Carlezon et al., 2003) and cognitive functioning (Chapter 4) that may increase the risk of future substance abuse. Support for an increased risk of substance abuse occurring due to MPH preexposure has been demonstrated by the increased sensitivity to and acquisition of cocaine self-administration following chronic MPH administration (Brandon et al., 2001; Griggs et al., 2010).

It is important to note that the significant effects of chronic MPH administration reported in this thesis did not translate to an animal model of ADHD, the SHR. This suggests that the increased risk factors for substance abuse may be restricted to individuals who are misdiagnosed and treated for ADHD or those who misuse prescription MPH. Further investigations with appropriate animal models of ADHD are required to address this issue.

7.7 Methodological limitations

There are some methodological features of the thesis which may limit the conclusions. Firstly, the results from Chapter 6 demonstrate that the impulsivity task employed throughout this thesis is heavily dependent upon DA functioning. Therefore, the use of this task limits the conclusions that can be drawn about the effect on NA of long-term neurobiological alterations of chronic MPH treatment. NA influences behaviour in situations that are novel or non-routine (Aston-Jones & Cohen, 2005) and has been associated in the mediation of impulsive actions (Milstein, Lehmann, Theobald, Dalley, & Robbins, 2007; Sagvolden, 2006). Although previous research has demonstrated that the acute effects on impulsivity of MPH treatment are replicated by blockade of DA but not NA transporters (van Gaalen et al., 2006), future research would benefit from assessing long-term MPH effects on both impulsive choice and impulsive actions, the latter of which is NA dependent.

Secondly, the water deprivation method used to administer MPH throughout this thesis may have impacted upon the pharmacokinetics of the drug. However this is unlikely due to similar levels of behavioural activation following MPH administration using the present water restriction method (Chapter 3) that have previously been obtained following MPH administration via gavage (Gerasimov et al., 2000). Measuring the plasma concentrations of MPH following administration using the present water restriction method would confirm the appropriateness of this method of administration.

Thirdly, it has recently been reported that there are sub-strains within the WKY strain (Sagvolden et al., 2009). The origin of the WKY sub-strain employed throughout this thesis is from Charles River Laboratories, USA. This sub-strain, known as WKY/NCrl, has been suggested as an animal model for ADHD-I subtype (Sagvolden, DasBanerjee, Zhang-James, Middleton, & Faraone, 2008). However, as measures of attention were not employed in this thesis, the results can not address this claim. In the tasks as they were employed in this laboratory, the SHR demonstrated hyperactivity, impulsivity and increased sensitivity to reinforcer delay, compared to the WKY/NCrl. Therefore, this WKY sub-strain was considered appropriate to use as a control. Furthermore, the use of the WKY as the single control strain has been criticised (Alsop, 2007; Drolet, Proulx, Pearson, Rochford, & Deschepper, 2002). However, similar levels of behavioural activation were observed following oral administration of MPH in Chapter 3 of this thesis and the afore mentioned gavage study which was conducted in Sprague-Dawley (SD) rats (Gerasimov et al., 2000). This suggests that the behavioural pharmacology of MPH in WKY is similar to a common control strain (SD), indicating that this WKY sub-strain was an appropriate control.

Finally, although the majority of the results of this thesis pertain to the WKY, some caution should be employed in the interpretation of the results of the SHRs. The MPH dose administered in this thesis was determined by a pilot study (Chapter 3), together with previous research which assessed blood plasma levels after oral administration of MPH (Berridge et al., 2006; Kuczenski & Segal, 2002). While the dose administered to WKYs in the pilot study (Chapter 3) produced similar locomotor activation to an equivalent dose administered to SD rats, the dose may not have been appropriate for the SHR. Additionally, SHRs develop hypertension, and as such have a symptom that is not characteristic of ADHD. However, the majority of the experiments in this thesis were conducted during the age of 4 to 12 weeks, prior to the onset of hypertension in the SHR (Russell, 2002) and the ADHD symptoms

expressed by the SHR have been found to be independent of hypertension (Kantak et al., 2008). Despite the potential confounding influence of hypertension, the SHR remains a valid animal model of ADHD.

7.8 Areas for future research

The findings of this thesis provide sound bases for various future studies and a selection of the studies of interest are discussed below. The current findings can not determine whether the long-term increase in impulsivity following MPH treatment is a transient or persistent effect, as impulsivity was assessed at one point in time following treatment. Future research could address the persistence of this effect by conducting multiple assessments of impulsivity following cessation of chronic MPH treatment.

Further investigations of the effect of chronic MPH treatment on alternate brain regions could also be fruitful. One possible region of interest is the comparison of medial and lateral dorsal striatum. The dorsal striatum has been implicated in instrumental learning with dissociable roles of the medial and lateral regions (Balleine, Liljeholm, & Ostlund, 2009). The dorsomedial striatum is associated with goal-directed learning, while the dorsolateral striatum is responsible for habitual or procedural learning (Balleine et al., 2009). Habitual actions have been associated with psychostimulant abuse (Nelson & Killcross, 2006). Furthermore, NAc dysfunction has also been shown to be an integral component of psychostimulant abuse and addiction (Cornish & Kalivas, 2000; Di Chiara, 2002). Therefore investigations of the effect of MPH in these regions may elucidate the increased substance abuse risk following chronic MPH treatment.

Additional neurobiological underpinnings of chronic MPH treatment are worthy of further investigation. Glutamate interacts with DA and is integral in regulating output from the

pyramidal neurons in mPFC (Steketee, 2003). Previously, it has been shown that the morphology of the PFC pyramidal neurons have been altered following amphetamine administration (Crombag, Gorny, Li, Kolb, & Robinson, 2005; Heijtz, Kolb, & Forssberg, 2003). Therefore, investigation of the effect of chronic MPH treatment on the glutamatergic system would be warranted, particularly given its role in behavioural sensitisation to psychostimulants (Kalivas, 1995). Additionally, research assessing levels of proteins such as of calcium/calmodulin-dependent protein kinase II (CaMKII) following chronic MPH treatment may also be may be helpful in elucidating the occurrence of neurodevelopmental changes, as levels of CaMKII levels have been suggested to reflect synaptic plasticity (Kennedy, 2000).

It would be particularly interesting to investigate the effect of chronic MPH administration on gene expression, in particular to establish if MPH, like other psychostimulants, possesses the ability to turn genes on or off (LaPlant & Nestler, 2010). If genetic adaptations were evident, the question of transfer of that adaptation to offspring becomes an important avenue of investigation. The heritability of a psychostimulant induced genetic adaptation may predispose the offspring to a variety of maladaptive mood and behavioural disorders including substance abuse and dependence, ADHD, conduct disorder, bipolar disorder, anxiety and depression. The impact of psychostimulants on the heritability of gene expression is a broad area of study which presents an exciting challenge for future research.

7.9 Concluding comments

Development of the adolescent brain is very protracted. The lengthy window of development increases the vulnerability of neurocircuits to early adverse experiences which may result in long lasting cognitive and neurochemical alterations. The current findings demonstrated that inappropriate chronic MPH administration during adolescence induced impulsivity in

adulthood, interfered with the development of the catecholamine projections within the PFC and the mechanism underlying these effects was likely attributable to alterations in the DA, but not NA system. The data reported here suggest that treatment with psychostimulants for childhood disorders such as ADHD should be undertaken cautiously. This thesis identified detrimental effects of inappropriate treatment for ADHD and highlights the need for more stringent diagnostic criteria for this disorder.

Chapter 8

References

Achenbach, T. M., & Edelbrock, C. S. (1991). *Child Behavior Checklist*. Burlington, VT: University Associates in Psychiatry.

Adler, R. (1995). Stimulant treatment for ADHD: a comment. *Australian Prescriber, 18*, 64.

Adriani, W., Canese, R., Podo, F., & Laviola, G. (2007). ^{1}H MRS-detectable metabolic brain changes and reduced impulsive behavior in adult rats exposed to methylphenidate during adolescence. *Neurotoxicology and Teratology, 29*, 116-125.

Adriani, W., & Laviola, G. (2003). Elevated levels of impulsivity and reduced place conditioning with *d*-Amphetamine: Two behavioral features of adolescence in mice. *Behavioral Neuroscience, 117* (4), 695-703.

Adriani, W., & Laviola, G. (2004). Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology, 15*, 341-352.

Aghajanian, G. K., & Bloom, F. E. (1967). The formation of synaptic junctions in developing rat brain: A quantitative electron microscopic study. *Brain Research, 6*, 716-727.

Ahmann, P. A., Waltonen, S. J., Theye, F. W., Olson, K. A., & Van Erem, A. J. (1993). Placebo-controlled evaluation of Ritalin side effects. *Pediatrics, 91*, 1101-1106.

Albayrak, O., Friedel, S., Schimmelmann, B. G., Hinney, A., & Hebebrand, J. (2008). Genetic aspects in attention-deficit/hyperactivity disorder. *Journal of Neural Transmission, 115*, 305-315.

Alper, R. H., Demarest, K. T., & Moore, K. E. (1980). Dehydration selectively increases dopamine synthesis in tuberohypophyseal dopaminergic neurons. *Neuroendocrinology, 31*, 112-115.

Alsop, B. (2007). Problems with spontaneously hypertensive rats (SHR) as a model of

Attention-Deficit/Hyperactivity Disorder (AD/HD). *Journal of Neuroscience Methods, 162*, 42-48.

American Psychiatric Association. (1968). *Diagnostic and Statistical Manual of Mental Disorders* (2nd ed.). Washington, DC: American Psychiatric Association.

American Psychiatric Association. (1980). *Diagnostic and Statistical Manual of Mental Disorders* (3rd ed.). Washington, DC: American Psychiatric Association.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, Text Revision ed.). Washington, DC: American Psychiatric Association.

Andersen, S. L. (2002). Changes in the second messenger cyclic AMP during development may underlie motoric symptoms in attention deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research, 130*, 197-201.

Andersen, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews, 27*, 3-18.

Andersen, S. L., Arvanitogiannis, A., Pliakas, A. M., LeBlanc, C., & Carlezon, W. A. (2002). Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nature Neuroscience, 5* (1), 13-14.

Anderson, P. (2002). Assessment and development of executive function during childhood. *Child Neuropsychology, 8*, 71-82.

Angold, A., Erkanli, A., Egger, H. L., & Costello, E. J. (2000). Stimulant treatment for children: A community perspective. *Journal of the American Academy of Child and Adolescent Psychiatry, 39* (8), 975-984.

Aoki, C., Go, C.-G., Venkatesan, C., & Kurose, H. (1994). Perkaryal and synaptic localization of α_{2A}-adrenergic receptor-like immunoreactivity. *Brain Research, 650*, 181-204.

Aoyama, T., Kotaki, H., & Iga, T. (1990). Dose-dependent kinetics of methylphenidate enantiomers after oral administration of racemic methylphenidate to rats. *Journal of*

Pharmacobio-dynamics, 13, 647-652.

Arnsten, A. F. T. (1997). Catecholamine regulation of the prefrontal cortex. *Journal of Psychopharmacology, 11*, 151-162.

Arnsten, A. F. T. (2006). Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology, 31*, 2376-2383.

Arnsten, A. F. T. (2007). Catecholamine and second messenger influences on prefrontal cortical networks of "representational knowledge": A rational bridge between genetics and the symptoms of mental illness. *Cerebral Cortex, 17* (Supplemental 1), i6-i15.

Arnsten, A. F. T. (2009). Towards a new understanding of attention-deficit hyperactivity disorder pathophysiology. *CNS Drugs, 23 (Supplemental 1)*, 33-41.

Arnsten, A. F. T., & Dudley, A. G. (2005). Methylphenidate improves prefrontal cortical cognitive function through α_2 adrenoreceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. *Behavioral and Brain Functions, 1*, 1-9.

Arnsten, A. F. T., & Li, B. M. (2005). Neurobiology of executive functions: Catecholamine influences on prefrontal cortical functions. *Biological Psychiatry, 57*, 1377-1384.

Arnsten, A. F. T., Mathew, R., Ubriani, R., Taylor, J. R., & Li, B.-M. (1999). α_1 Noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. *Biological Psychiatry, 45*, 26-31.

Arnsten, A. F. T., Steere, J. C., & Hunt, R. D. (1996). The contribution of α_2-noradrenergic mechanisms to prefrontal cortical cognitive function. *Archives of General Psychiatry, 53*, 448-455.

Askenasy, E. P., Taber, K. H., Yang, P., & Dafny, N. (2007). Methylphenidate (Ritalin): Behavioral studies in the rat. *International Journal of Neuroscience, 117* (6), 757-794.

Aston-Jones, G., & Cohen, J. D. (2005). Adaptive gain and the role of the locus coeruleus norepinephrine system in optimal performance. *Journal of Comparative Neurology,*

493, 99-110.

Augustyniak, P. N., Kourrich, S., Rezazadeh, S. M., Stewart, J., & Arvanitogiannis, A. (2006). Differential behavioral and neurochemical effects of cocaine after early exposure to methylphenidate in an animal model of attention deficit hyperactivity disorder. *Behavioural Brain Research, 167*, 379-382.

Baddeley, A. D., & Hitch, G. J. (1974). Working memory. In G. H. Bower (Ed.), *The psychology of learning and motivation: Advances in research and theory* (Vol. 8, pp. 647-667). New York: Academic Press.

Balcombe, J. P., Barnard, N. D., & Sandusky, C. (2004). Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science, 43*, 42-51.

Balleine, B. W., Liljeholm, M., & Ostlund, S. B. (2009). The integrative function of the basal ganglia in instrumental conditioning. *Behavioural Brain Research, 199*, 43-52.

Barbaresi, W. J., Katusic, S. K., Colligan, R. C., Weaver, A. L., Leibson, C. L., & Jacobsen, S. J. (2006). Long-term stimulant medication treatment of Attention-Deficit/Hyperactivity Disorder: Results from a population-based study. *Developmental and Behavioral Pediatrics, 27* (1), 1-10.

Barkley, R. A. (1990). *Attention deficit hyperactivity disorder: A hand-book for diagnosis and treatment*. New York: Guilford Press.

Barkley, R. A. (1999). Response inhibition in attention-deficit hyperactivity disorder. *Mental Retardation and Developmental Disabilities, 5*, 177-184.

Barkley, R. A. (2005). *Attention deficit hyperactivity disorder: A hand-book for diagnosis and treatment* (3rd ed.). New York: Guilford Press.

Barkley, R. A., McMurray, M. B., Edelbrock, C. S., & Robbins, K. (1990). Side effects of methylphenidate in children with attention deficit hyperactivity disorder: A systemic, placebo-controlled evaluation. *Pediatrics, 86*, 184-192.

Barone, S., Das, K. P., Lassiter, L., & White, L. D. (2000). Vulnerable processes of nervous

system development: A review of markers and methods. *NeuroToxicology, 21*, 15-36.

Bayer, S. A., Altman, J., Russo, R. j., & Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *NeuroToxicology, 14*, 83-144.

Becker, M. G., Isaac, W., & Hynd, G. W. (1987). Neuropsychological development of nonverbal behaviors attributed to "frontal lobe" functioning. *Developmental Neuropsychology, 3*, 275-298.

Berbatis, C. G., Sunderland, V. B., & Bulsara, M. (2002). Licit psychostimulant consumption in Australia, 1984-2000: International and jurisdictional comparison. *The Medical Journal of Australia, 177*, 539-543.

Berger-Sweeney, J., & Hohmann, C. F. (1997). Behavioral consequences of abnormal cortical development: Insights into developmental disabilities. *Behavioural Brain Research, 86*, 121-142.

Berger, B., Verney, C., Febvret, A., Vigny, A., & Helle, K. B. (1985). Postnatal ontogenesis of the dopaminergic innervation in the rat anterior cingulate cortex (area 24). Immunocytochemical and catecholamine fluorescence histochemical analysis. *Brain Research, 353*, 31-47.

Berger, D., & Sagvolden, T. (1998). Sex differences in operant discrimination behaviour in an animal model of Attention-Deficit Hyperactivity Disorder. *Behavioural Brain Research, 94*, 73-82.

Berridge, C. W. (2008). Noradrenergic modulation of arousal. *Brain Research Reviews, 58*, 1-17.

Berridge, C. W., & Devilbiss, D. M. (2008). Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biological Psychiatry, 64*, 626-635.

Berridge, C. W., & Devilbiss, D. M. (2010). Psychostimulant as cognitive enhancers: The

prefrontal cortex, catecholamines, and attention-deficit/hyperactivity disorder. *Biological Psychiatry,* doi: 10.1016/j.biopsych.2010.06.023 *In Press*.

Berridge, C. W., Devilbiss, D. M., Andrzejewski, M. E., Arnsten, A. F. T., Kelley, A. E., Schmeichel, B., et al. (2006). Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biological Psychiatry, 60*, 1111-1120.

Berridge, C. W., Stratford, T. L., Foote, S. L., & Kelley, A. E. (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse, 27*, 230-241.

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews, 42*, 33-84.

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

Bethancourt, J. A., Camarena, Z. Z., & Britton, G. B. (2009). Exposure to oral methylphenidate from adolescence through young adulthood produces transient effects on hippocampal-sensitive memory in rats. *Behavioural Brain Research, 202*, 50-57.

Beyer, C. E., & Steketee, J. D. (2002). Cocaine sensitization: Modulation by dopamine D_2 receptors in the rat medial prefrontal cortex. *Cerebral Cortex, 12*, 526-535.

Bezzina, G., Body, S., Cheung, T. H., Hampson, C. L., Bradshaw, C. M., Szabadi, E., et al. (2008). Effect of disconnecting the orbital prefrontal cortex from the nucleus accumbens core on inter-temporal choice behaviour: a quantitative analysis. *Behavioural Brain Research, 191*, 272-279.

Biederman, J., & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet, 366*, 237-248.

Biederman, J., Faraone, S. V., Keenan, K, Knee, D, & Tsuang, M. T. (1990). Family-genetic

and psychosocial risk factors in DSM-III Attention-Deficit Disorder. *Journal of American Academy of Child and Adolescent Psychiatry, 29*, 526-533.

Birnbaum, S. G., Podell, D. M., & Arnsten, A. F. T. (2000). Noradrenergic alpha-2 receptor agonists reverse working memory deficits induced by the anxiogenic drug, FG7142, in rats. *Pharmacology, Biochemistry and Behavior, 67*, 397-403.

Birnbaum, S. G., Yuan, P. X., Wang, M., Vijayraghavan, S., Bloom, A. K., Davis, D. J., et al. (2004). Protein Kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science, 306* (5697), 882-884.

Bisagno, V., Ferguson, D., & Luine, V. N. (2003). Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. *Pharmacology, Biochemistry and Behavior, 74*, 859-867.

Blakemore, S.-J. (2008). The social brain in adolescence. *Nature Reviews Neuroscience, 9*, 267-277.

Boix, F., Qiao, S.-w., Kolpus, T., & Sagvolden, T. (1998). Chronic L-deprenyl treatment alters brain monoamine levels and reduces impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder. *Behavioural Brain Research, 94*, 153-162.

Bolanos, C. A., Barrot, M., Berton, O., Wallace-Black, D., & Nestler, E. J. (2003). Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biological Psychiatry, 54*, 1317-1329.

Bolanos, C. A., Willey, M. D., Maffeo, M. L., Powers, K. D., Kinka, D. W., Grausam, K. B., et al. (2008). Antidepressant treatment can normalize adult behavioral deficits induced by early-life exposure to methylphenidate. *Biological Psychiatry, 63*, 309-316.

Botly, L. C., Burton, C. L., Rizos, Z., & Fletcher, P. J. (2008). Characterization of methylphenidate self-administration and reinstatement in the rat. *Psychopharmacology (Berl), 199*, 55-66.

Bourgeois, J.-P., Goldman-Rakic, P. S., & Rakic, P. (1994). Synaptogenesis in the prefrontal

cortex of rhesus monkeys. *Cerebral Cortex, 4*, 78-96.

Brandon, C. L., Marinelli, M., Baker, L. K., & White, F. J. (2001). Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology, 25* (5), 651-661.

Braun, J. M., Kahn, R. S., Froehlich, T., Auinger, P., & Lanphear, B. P. (2006). Exposures to environmental toxicants and Attention Deficit Hyperactivity Disorder in U.S. children. *Environmental Health Perspectives, 114*, 1904-1909.

Britton, G. B., Segan, A. T., Sejour, J., & Mancebo, S. E. (2007). Early exposure to methylphenidate increases fear responses in an aversice context in adult rats. *Developmental Psychobiology, 49*, 265-275.

Bucci, D. J., Hopkins, M. E., Keene, C. S., Sharma, M., & Orr, L. E. (2008). Sex differences in learning and inhibition in spontaneously hypertensive rats. *Behavioural Brain Research, 187*, 27-32.

Bull, E., Reavill, C., Hagan, J. J., Overend, P., & Jones, D. N. (2000). Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: Acquisition and performance on the DRL-60s test. *Behavioural Brain Research, 109*, 27-35.

Bymaster, F., Katner, J., Nelson, D., Hemrick-Luecke, S., Threlkeld, P., Heiligenstein, J., et al. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: A potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology, 27*, 699-711.

Cardinal, R. N. (2006). Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks, 19*, 1277-1301.

Cardinal, R. N., Pennicott, D. R., Sugathapala, C. L., Robbins, T. W., & Everitt, B. J. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science, 292*, 2499-2501.

Carlezon, W. A., Mague, S. D., & Andersen, S. L. (2003). Enduring behavioral effects of early exposure to methylphenidate in rats. *Biological Psychiatry, 54*, 1330-1337.

Carlson, S., Tanila, H., Rama, P., Mecke, E., & Pertovaara, A. (1992). Effects of medetomidine, and α-2 adrenoceptor agonist, and atipamezole, and α-2 antagonist, on spatial memory performance in adult and aged rats. *Behavioral and Neural Biology, 58*, 113-119.

Carlson, S. M. (2005). Developmentally sensitive measures of executive function in preschool children. *Developmental Neuropscyhology, 28* (2), 595-616.

Carr, D. B., & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *Journal of Neuroscience, 20*, 3864-3873.

Castellanos, F. X., Elia, J., Kruesi, M. J. P., Marsh, W. L., Gulotta, C. S., Potter, W. Z., et al. (1996a). Cerebrospinal fluid homovanillic acid predicts behavioral responses to stimulant in 47 boys with attention-deficit hyperactivity disorder. *Neuropsychopharmacology, 14*, 125-137.

Castellanos, F. X., Giedd, J. N., Marsh, W. L., Hamburger, S. D., Vaituzis, A. C., Dickstein, D. P., et al. (1996b). Quantitative brain magnetic resonance imaging in Attention-Deficit Hyperactivity Disorder. *Archives of General Psychiatry, 53* (7), 607-616.

Chudasama, Y., Passetti, F., Rhodes, S. E. V., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: Differential effects on selectivity, impulsivity and compulsivity. *Behavioural Brain Research, 146*, 105-119.

Chuhan, Y. S., & Taukulis, H. K. (2006). Impairment of single-trial memory formation by oral methylphenidate in the rat. *Neurobiology of Learning and Memory, 85*, 125-131.

Clements, K. M., & Wainwright, P. E. (2006). Spontaneously hypertensive, Wistar-Kyoto and

Sprague-Dawley rats differ in performance on a win-shift task in the water radial arm maze. *Behavioural Brain Research, 167*, 295-304.

CNN. (2002, 13 March). Do you think ADHD is overdiagnosed? Retrieved 4 January, 2011, from http://edition.cnn.com/POLL/results/84571.html

Coffey, B. J. (1997). Attention-deficit hyperactivity disorder. In J. D. Noshpitz (Ed.), *Handbook of Child and Adolescent Psychiatry* (pp. 456-482). New York: John Wiley & Sons.

Commissaris, R. L., Palmer, A., Neophytou, S., Graham, M., Beckett, S., & Marsden, C. A. (2000). Acoustically elicited behaviours in Lister hooded and Wistar rats. *Physiology and Behavior, 68*, 521-531.

Conners, C. K., Sitarenios, G., Parker, J., & Epstein, J. N. (1998a). Revision and restandardization of the Conners' Parent Rating Scale (CPRS-R): Factor structure, reliability and criterion validity. *Journal of Abnormal Child Psychology, 25*, 257-268.

Conners, C. K., Sitarenios, G., Parker, J., & Epstein, J. N. (1998b). Revision and restandardization of the Conners' Teacher Rating Scale (CTRS-R): Factor structure, reliability and criterion validity. *Journal of Abnormal Child Psychology, 26*, 279-291.

Cook, E. H. J., Stein, M. A., Krasowski, M. D., Cox, N. J.,Olkon, D. M., Kieffer, J. E., et al. (1995). Attention-Deficit Hyperactivity Disorder and the dopamine transporter gene. *American Journal of Human Genetics, 56*, 993-998.

Cornish, J. L., & Kalivas, P. W. (2000). Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *The Journal of Neuroscience, 20*, RC89 (81-85).

Coyle, J. T., & Molliver, M. E. (1977). Major innervation of newborn rat cortex by monoaminergic neurons. *Science, 196*, 444-447.

Crawford, C. A., McDougall, S. A., Meier, T. L., Collins, R. L., & Watson, J. B. (1998). Repeated methylphenidate treatment induces behavioral sensitization and decreases

protein kinase A and dopamine-stimulated adenylyl cyclase activity in the dorsal striatum. *Psychopharmacology, 136*, 34-43.

Crombag, H. S., Gorny, G., Li, Y., Kolb, B., & Robinson, T. E. (2005). Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. *Cerebral Cortex, 15*, 341-348.

Csakvari, E., Kurunczi, A., Hoyk, Z., Gyenes, A., Naftolin, F., & Parducz, A. (2008). Estradiol-induced synaptic remodeling of tyrosine hydroxylase immunopositive neurons in the rat arcuate nucleus. *Endocrinology, 149*, 4137-4141.

da Costa Araujo, S., Body, S., Hampson, C. L., Langley, R. W., Deakin, J. F., Anderson, I. M., et al. (2009). Effects of lesions of the nucleus accumbens core on inter-temporal choice: further observations with an adjusting delay procedure. *Behavioural Brain Research, 202*, 272-277.

Dafny, N., & Yang, P. (2006). The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: A review of its locomotor effects. *Brain Research Bulletin, 68*, 393-405.

Dahlstrom, A., & Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia, 20*, 398-399.

Dalley, J. W., Mar, A. C., Economidou, D., & Robbins, T. W. (2008). Neurobehavioral mechanisms of impulsivity: Fronto-striatal systems and functional neurochemistry. *Pharmacology, Biochemistry and Behavior, 90*, 250-260.

Dalley, J. W., McGaughy, J., O'Connell, M. T., Cardinal, R. N., Levita, L., & Robbins, T. W. (2001). Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. *Journal of Neuroscience, 21*, 4908-4914.

Davids, E., Zhang, K., Tarazi, F. I., & Baldessarini, R. J. (2003). Animal models of Attention-Deficit Hyperactivity Disorder. *Brain Research Reviews, 42*, 1-21.

Day, H. E. W., Campeau, S., Watson, S. J., & Akil, H. (1997). Distribution of α_{1a}-, α_{1b}- and α_{1d}-adrengeric receptor mRNA in the rat brain and spinal cord. *Journal of Chemical Neuroanatomy, 13*, 115-139.

De Brito Gariepy, H., Carayon, P., Ferrari, B., & Couture, R. (2010). Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension. *Neuropeptides, 44*, 191-198.

Deutch, A. Y., Clark, W. A., & Roth, R. H. (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Research, 521*, 311-315.

Dey, S., Mactutus, C. F., Booze, R. M., & Snow, D. M. (2006). Specificity of prenatal cocaine on inhibition of locus coeruleus neurite outgrowth. *Neuroscience, 139*, 899-907.

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behavioural Brain Research, 137*, 75-114.

Diamond, A. (1991). Guidelines from study of brain-behaviour relationships during development. In H. S. Levin, H. M. Eisenderg & A. L. Benton (Eds.), *Frontal lobe function and dysfunction*: Oxford University Press.

Drolet, G., Proulx, K., Pearson, D., Rochford, J., & Deschepper, C. F. (2002). Comparisons of behavioral and neurochemical characteristics between WKY, WKHA, and Wistar Rat Strains. *Neuropsychopharmacology, 27*, 400-409.

Eagle, D. M., Baunez, C., Hutcheson, D. M., Lehmann, O., Shah, A. P., & Robbins, T. W. (2008). Stop-signal reaction-time task performance: Role of prefrontal cortex and subthalamic nucleus. *Cerebral Cortex, 18*, 178-188.

Engert, V., Joober, R., Meaney, M. J., Hellhammer, D. H., & Pruessner, J. C. (2009). Behavioral response to methylphenidate challenge: Influence of early life parental care. *Developmental Psychobiology, 51* (5), 408-416.

Engert, V., & Pruessner, J. C. (2008). Dopaminergic and noradrenergic contributions to functionality in ADHD: The role of methylphenidate. *Current Neuropharmacology, 6*, 322-328.

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: The effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*, 161-170.

Faraone, S. V., & Biederman, J. (1998). Neurobiology of Attention-Deficit Hyperactivity Disorder. *Biological Psychiatry, 44*, 951-958.

Faraone, S. V., Biederman, J., & Mick, E. (2006). The age-dependent decline of attention deficit hyperactivity disorder: A meta-analysis of follow-up studies. *Psychological Medicine, 36*, 159-165.

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology, 48* (3), 589-601.

Floresco, S. B., & Whelan, J. M. (2009). Perturbations in different forms of cost/benefit decision making induced by repeated amphetamine exposure. *Psychopharmacology, 205*, 189-201.

Florin, S. M., Kuczenski, R., & Segal, D. S. (1994). Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. *Brain Research, 654*, 53-62.

Fluxe, K., Hokfelt, T., Johansson, O., Jonsson, G., Lidbrink, P., & Ljungdahl, A. (1974). The origin of the dopamine nerve terminals in limbic and frontal cortex. Evidence for meso-cortico dopamine neurons. *Brain Research, 82*, 349-355.

Foote, S. L., Aston-Jones, G., & Bloom, F. E. (1980). Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proceedings of the National Academy of Sciences of the United States of America, 77*,

3033-3037.

Fox, A. T., Hand, D. J., & Reilly, M. P. (2008). Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. *Behavioural Brain Research, 187*, 146-152.

Fryer, S. L., McGee, C. L., Matt, G. E., Riley, E. P., & Mattson, S. N. (2007). Evaluation of psychopathological conditions in children with heavy prenatal alcohol exposure. *Pediatrics, 119*, e733-741.

Funahashi, S., Chafee, M. V., & Goldman-Rakic, P. S. (1993). Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. *Nature, 365*, 753-756.

Gan, W.-B., Kwon, E., Feng, G., Sanes, J. R., & Lichtman, J. W. (2003). Synaptic dynamism measured over minutes to months: age-dependent decline in an autonomic ganglion. *Nature Neuroscience, 6*, 956-960.

Gatley, S. J., Pan, D., Chen, R., Chaturvedi, G., & Ding, Y.-S. (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sciences, 58* (12), PL 231-239.

Gaytan, O., Yang, P., Swann, A., & Dafny, N. (2000). Diurnal differences in sensitization to methylphenidate. *Brain Research, 864*, 24-39.

Gerasimov, M., Franceschi, M., Volkow, N. D., Gifford, A., Gatley, S. J., Marsteller, D., et al. (2000). Comparison between intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *The Journal of Pharmacology and Experimental Therapeutics, 295* (1), 51-57.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: A longnitudinal MRI study. *Nature Neuroscience, 2*, 861-863.

Giedd, J. N., Blumenthal, J., Molloy, E., & Castellanos, F. X. (2001). Brain imaging of attention deficit/hyperactivity disorder. *Annals of the New York Academy of Sciences, 931*, 33-49.

Goldman-Rakic, P. S., Lidow, M. S., & Gallager, D. W. (1990). Overlap of dopaminergic, adrenergic, and serotoninergic receptors and complementarity of their subtypes in primate prefrontal cortex. *The Journal of Neuroscience, 10*, 2125-2138.

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: A research note. *Journal of Child Psychology and Psychiatry, 38*, 581-586.

Goodman, R., Ford, T., Richards, H., Gatward, R., & Meltzer, H. (2000). The Development and Well-Being Assessment: Description and initial validation of an integrated assessment of child and adolescent psychopathology. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 41*, 645-655.

Gorska, T., Chojnicka-Gittins, B., Majczynski, H., & Zmysolwski, W. (2009). Recovery of overground locomotion following partial spinal lesions of different extent in the rat. *Behavioural Brain Research, 196*, 286-296.

Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience, 41*, 1-24.

Grace, A. A. (1995). The tonic/phasic model of dopamine system regulation: its relevance for understanding low stimulant abuse can alter basal ganglia function. *Drug and Alcohol Dependence, 37*, 111-129.

Gray, J. D., Punsoni, M., Tabori, N. E., Melton, J. T., Fanslow, V., Ward, M. J., et al. (2007). Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *The Journal of Neuroscience, 27*, 7196-7207.

Greenhill, L., Beyer, D. H., Finkleson, J., Shaffer, D., Biederman, J., Conners, C. K., et al. (2002). Guidelines and algorithms for the use of methylphenidate in children with attention-deficit/hyperactivity disorder. *Journal of Attention Disorders, 6 (Supplemental 1)*, S89-100.

Griggs, R., Weir, C., Wayman, W., & Koeltzow, T. E. (2010). Intermittent methylphenidate during adolescent development produces locomotor hyperactivity and an enhanced response to cocaine compared to continuous treatment in rats. *Pharmacology, Biochemistry and Behavior, 96*, 166-174.

Grobin, A. C., & Deutch, A. Y. (1998). Dopaminergic regulation of extracellular γ-aminobutyric acid levels in the prefrontal cortex of the rat. *Journal of Pharmacology and Experimental Therapeutics, 285*, 350-357.

Groenewegen, H. J. (2003). The basal ganglia and motor control. *Neural Plasticity, 10*, 107-120.

Hall, F. S., Li, X.-F., Randall-Thompson, J., Sora, I., Murphy, D. L., Lesch, K.-P., et al. (2009). Cocaine-conditioned locomotion in dopamine transporter, norepinephrine transporter and serotonin transporter knockout mice. *Neuroscience, 162*, 870-880.

Hand, D. J., Fox, A. T., & Reilly, M. P. (2006). Response acquisition with delayed reinforcement in a rodent model of attention-deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research, 175*, 337-342.

Hand, D. J., Fox, A. T., & Reilly, M. P. (2009). Differential effects of *d*-amphetamine on impulsive choice in spontaneously hypertensive and Wistar-Kyoto rats. *Behavioural Pharmacology, 20*, 549-553.

Handler, M. W., & DuPaul, G. J. (2005). Assessment of ADHD: Differences across psychology specialty areas. *Journal of Attention Disorders, 9* (2), 402-412.

Hannestad, J., Gallezot, J.-D., Planeta-Wilson, B., Lin, S.-F., Williams, W. A., van Dyck, C. H., et al. (2010). Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. *Biological Psychiatry, 68*, 854-860.

Hart, E. L., Lahey, B. B., Loeber, R., Applegate, B., & Frick, P. J. (1995). Developmental change in attention-deficit hyperactivity disorder in boys: a four-year longitudinal study. *Journal of Abnormal Child Psychology, 23*, 729-749.

Hassani, O. K., Cromwell, H. C., & Schultz, W. (2001). Influence of expectation of different rewards on behavior-related neuronal activity in the striatum. *Journal of Neurophysiology, 85*, 2477-2489.

Heijtz, R. D., Kolb, B., & Forssberg, H. (2003). Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? *European Journal of Neuroscience, 18*, 3394-3399.

Heijtz, R. D., Kolb, B., & Forssberg, H. (2007). Motor inhibitory role of dopamine D1 receptors: Implications for ADHD. *Physiology and Behavior, 92*, 155-160.

Heyser, C. J., Pelletier, M., & Ferris, J. S. (2004). The effects of methylphenidate on novel object exploration in weanling and periadolescent rats. *Annals of the New York Academy of Sciences, 1021*, 465-469.

Hieble, J. P., Bylund, D. B., Clarke, D. E., Eikenburg, D. C., Langer, S. Z., Lefkowitz, R. J., et al. (1995). International union of pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: Consensus update. *Pharmacological Reviews, 47*, 267-270.

Hollingworth, S. A., Nissen, L. M., Stathis, S. S., Siskind, D. J., Varghese, J. M. N., & Scott, J. G. (2011). Australian national trends in stimulant dispensing: 2002 - 2009. *Australian and New Zealand Journal of Psychiatry, 45*, 332-336.

Homayoun, H., & Moghaddam, B. (2006). Progression of cellular adaptations in medial prefrontal and orbitofrontal cortex in response to repeated amphetamine. *The Journal of Neuroscience, 26* (31), 8025-8039.

Hughes, J. E., Amyx, H., Howard, J. L., Nanry, K. P., & Pollard, G. T. (1994). Health effects of water restriction to motivate lever-pressing in rats. *Laboratory Animal Science, 44*, 134-140.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Research, 163*, 195-205.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *The Journal of Comparative Neurology, 387*, 167-178.

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: The role of reward-related learning and memory. *Annual Review of Neuroscience, 29*, 565-598.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nuclues accumbens-olfactory tubercle complex. *Brain Research Reviews, 56*, 27-78.

Insel, P. A. (1993). Adrenergic receptors, G proteins, and cell regulation: Implications for aging research. *Experimental Gerontology, 28*, 341-348.

Isquith, P. K., Gioia, G. A., & Espy, K. A. (2004). Executive function in preschool children: Examination through everyday behavior. *Developmental Neuropsychology, 26* (1), 403-422.

Izaki, Y., Fujiwara, S.-e., & Akema, T. (2007). Involvement of rat prefrontal cortex in a delayed reinforcement operant task. *Neuroreport, 18* (16), 1687-1690.

Jackson, D. M., & Westlind-Danielsson, A. (1994). Dopamine receptors: Molecular biology, biochemistry and behavioural aspects. *Pharmacology and Therapeutics, 64*, 291-370.

Jenstch, J. D., Olausson, P., De La Garza, R., & Taylor, J. R. (2002). Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. *Neuropsychopharmacology, 26*, 183-190.

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behaviour by reward-related stimuli. *Psychopharmacology (Berl), 146*, 373-390.

Ji, X.-H., Ji, J.-Z., Zhang, H., & Li, B.-M. (2008). Stimulation of α_2 - Adrenoceptors suppresses excitatory synaptic transmission in the medial prefrontal cortex of rat. *Neuropsychopharmacology, 33*, 2263-2271.

Johansen, E. B., Aase, H., Meyer, A., & Sagvolden, T. (2002). Attention-Deficit/Hyperactivity Disorder (ADHD) behavior explained by dysfunctioning reinforcement and extinction processes. *Behavioural Brain Research, 130*, 37-45.

Johansen, E. B., Killeen, P. R., & Sagvolden, T. (2007). Behavioral variability, elimination of responses, and delay-of-reinforcement gradients in SHR and WKY rats. *Behavioral and Brain Functions, 3*, 60-90.

Johansen, E. B., & Sagvolden, T. (2005a). Behavioral effects of intra-cranial self-stimulation in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 162*, 32-46.

Johansen, E. B., & Sagvolden, T. (2005b). Slower extinction of responses maintained by intra-cranial self-stimulation (ICSS) in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 162*, 22-31.

Johansen, E. B., Sagvolden, T., & Kvande, G. (2005). Effects of delayed reinforcers on the behavior of an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioural Brain Research, 162*, 47-61.

Jones, G. H., Marsden, C. A., & Robbins, T. W. (1991). Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: Neurochemical correlates. *Behavioural Brain Research, 43* (1), 35-50.

Joseph, R. (1999). Environmental influences on neural plasticity, the limbic system, emotional development and attachment: A review. *Child Psychiatry and Human Development, 29*, 189-208.

Jupp, B., & Lawrence, A. J. (2010). New horizons for therapeutics in drug and alcohol abuse. *Pharmacology and Therapeutics, 125*, 138-168.

Kaladjian, A., Jeanningros, R., Azorin, J. M., Anton, J. L., & Mazzola-Pomietto, P. (2011). Impulsivity and neural correlates of response inhibition in schizophrenia. *Psychological Medicine, 41*, 291-299.

Kalivas, P. W. (1995). Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug and Alcohol Dependence, 37*, 95-100.

Kalivas, P. W., Pierce, R. C., Cornish, J. L., & Sorg, B. A. (1998). A role for sensitization in craving and relapse in cocaine addiction. *Journal of Psychopharmacology, 12*, 49-53.

Kalsbeek, A., De Bruin, J. P. C., Feenstra, M. G. P., Matthijssen, M. A. H., & Uylings, H. B. M. (1988a). Neonatal thermal lesions of the mesolimbocortical dopaminergic projection decrease food-hoarding behavior. *Brain Research, 475*, 80-90.

Kalsbeek, A., Voorn, P., Buijs, R. M., Pool, C. W., & Uylings, H. B. M. (1988b). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *The Journal of Comparative Neurology, 269*, 58-72.

Kantak, K. M., Singh, T., Kerstetter, K. A., Dembro, K. A., Mutebi, M. M., Harvey, R. C., et al. (2008). Advancing the spontaneous hypertensive rat model of attention deficit/hyperactivity disorder. *Behavioral Neuroscience, 122* (2), 340-357.

Kasparov, S., & Teschemacher, A. G. (2008). Altered central catecholaminergic transmission and cardio-vascular disease. *Experimental Physiology, 93*, 725-740.

Kebabian, J. W., & Calne, D. B. (1979). Multiple receptors for dopamine. *Nature, 277*, 93-96.

Kelley, A. E. (1999). Neural integrative activities of nucleus acumbens subregions in relation to learning and motivation. *Psychobiology, 27*, 198-213.

Kelley, A. R., Schochet, T., & Landry, C. F. (2004). Risk taking and novelty seeking in adolescence. *Annals of New York Academy of Sciences, 1021*, 27-32.

Kennedy, M. B. (2000). Signal-processing machines at the postsynaptic density. *Science, 290*, 750-754.

Kheramin, S., Body, S., Ho, M.-Y., Velazquez-Martinez, D. N., Bradshaw, C. M., Szabadi, E., et al. (2004). Effects of orbital prefrontal cortex dopamine depletion on inter-temporal choice: A quantitative analysis. *Psychopharmacology, 175*, 206-214.

Kiss, A., Jezova, D., & Aguilera, G. (1994). Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Research, 663*, 84-92.

Kiyatkin, E. A., & Rebec, G. V. (1996). Dopaminergic modulation of glutamate-induced excitations of neurons in the neostriatum and nucleus accumbens of awake, unrestrained rats. *Journal of Neurophysiology, 75* (142-153).

Klein-Schwartz, W., & McGrath, J. (2003). Poison Centers' experience with methylphenidate abuse in pre-teen and adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*, 288-294.

Klein, A., Wessolleck, J., Papazogluo, A., Metz, G. A., & Nikkhah, G. (2009). Walking pattern analysis after unilateral 6-OHDA lesion and transplantation of foetal dopaminergic progenitor cells in rats. *Behavioural Brain Research, 199*, 317-325.

Kolb, B., Gibb, R., & Gorny, G. (2003a). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory, 79*, 1-10.

Kolb, B., Gorny, G., Li, Y., Samaha, A.-N., & Robinson, T. E. (2003b). Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 10523-10528.

Kollins, S. H. (2008). ADHD, substance use disorders, and psychostimulant treatment: Current literature and treatment guidelines. *Journal of Attention Disorders, 12*, 115-125.

Kollins, S. H., MacDonald, E. K., & Rush, C. R. (2001). Assessing the abuse potential of methylphenidate in nonhuman and human subjects: A review. *Pharmacology Biochemistry and Behavior, 68*, 611-627.

Koob, G. F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward

pathways. *Trends in Pharmacological Sciences, 13*, 177-184.

Koob, G. F., & Le Moal, M. (1997). Drug abuse: Hedonic homeostatic dysregulation. *Science, 278*, 52-58.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology, 35*, 217-238.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: Comparison with amphetamine. *Journal of Neurochemistry, 68*, 2032-2037.

Kuczenski, R., & Segal, D. S. (2001). Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: Relative roles of dopamine and norepinephrine. *The Journal of Pharmacology and Experimental Therapeutics, 296*, 876-883.

Kuczenski, R., & Segal, D. S. (2002). Exposure of adolescent rats to oral methylphenidate: Preferential effects on extracellular noreprinephrine and absence of sensitization and cross-sensitization to methamphetamine. *The Journal of Neuroscience, 22* (16), 7264-7271.

Lagace, D. C., Yee, J. K., Bolanos, C. A., & Eisch, A. J. (2006). Juvenile administration of methylphenidate attenuates adult hippocampal neurogenesis. *Biological Psychiatry, 60*, 1121-1130.

LaPlant, Q., & Nestler, E. J. (2010). CRACKing the histone code: Cocaine's effects on chromatin structure and function. *Hormones and Behavior,* doi: 10.1016/j.yhbeh.2010.05.015.

Laviola, G., Macri, S., Morley-Fletcher, S., & Adriani, W. (2003). Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews, 27*, 19-31.

Law-Tho, D., Hirsch, J. C., & Crepel, F. (1994). Dopamine modulation of synaptic

transmission in rat prefrontal cortex: An in vitro electrophysiological study. *Neuroscience Research, 21*, 151-160.

Lawrence, A. J., Luty, J., Bogdan, N. A., Sahakian, B. J., & Clark, L. (2009a). Impulsivity and response inhibition in alcohol dependence and problem gambling. *Psychopharmacology, 207*, 163-172.

Lawrence, A. J., Luty, J., Bogdan, N. A., Sahakian, B. J., & Clark, L. (2009b). Problem gamblers share deficits in impulsive decision-making with alcohol-dependent individuals. *Addiction, 104*, 1006-1015.

LeBlanc-Duchin, D., & Taukulis, H. K. (2004). Behavioral reactivity to a noradrenergic challenge after chronic oral methylphenidate (Ritalin) in rats. *Pharmacology, Biochemistry and Behavior, 79*, 641-649.

LeBlanc-Duchin, D., & Taukulis, H. K. (2007). Chronic oral methylphenidate administration to periadolescent rats yields prolonged impairment of memory for objects. *Neurobiology of Learning and Memory, 88*, 312-320.

LeBlanc-Duchin, D., & Taukulis, H. K. (2009). Chronic oral methylphenidate induces post-treatment impairment in recognition and spatial memory in adult rats. *Neurobiology of Learning and Memory, 91*, 218-225.

Lee, K.-W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., & Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 3399-3404.

Leo, D., Sorrentino, E., Volpicelli, F., Eyman, M., Greco, D., Viggiano, D., et al. (2003). Altered midbrain dopaminergic neurotransmission during development in an animal model of ADHD. *Neuroscience and Biobehavioral Reviews, 27*, 661-669.

Leonard, B. E., McCartan, D., White, J., & King, D. J. (2004). Methylphenidate: A review of its neuropharmacological, neuropsychological and adverse clinical effects. *Human*

Psychopharmacology: Clinical and Experimental, 19, 151-180.

Levitt, M., Spector, S., Sjoerdsma, S., & Udenfriend, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *The Journal of Pharmacology and Experimental Therapeutics, 148*, 1-7.

Levitt, P., & Moore, R. Y. (1979). Development of the noradrenergic innervation of neocortex. *Brain Research, 162*, 243-259.

Levy, F. (2008). Pharmacological and therapeutic directions in ADHD: Specificity in the PFC. *Behavioral and Brain Functions, 4*, 12.

Li, B.-M., Mao, Z.-M., Wang, M., & Mei, Z.-T. (1999). Alpha-2 adrenergic modualtion of prefrontal cortical neuronal activity related to spatial working memory in monkeys. *Neuropsychopharmacology, 21*, 601-610.

Li, Y., & Kauer, J. A. (2004). Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. *Synapse, 51* (1), 1-10.

Lidow, M. S., Goldman-Rakic, P. S., Gallager, D. W., & Rakic, P. (1991). Distribution of dopaminergic receptors in the primate cerebral cortex: Quantitative autoradiographic analysis using [^{3}H]raclopride, [^{3}H]spiperone and [^{3}H]SCH23390. *Neuroscience, 40*, 657-671.

MacDonald, E., Kobilka, B. K., & Scheinin, M. (1997). Gene targeting - homing in on α_2-adrenoceptor-subtype function. *Trends in Pharmacological Sciences, 18*, 211-219.

Mague, S. D., Andersen, S. L., & Carlezon, W. A. (2005). Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. *Biological Psychiatry, 57*, 120-125.

Mao, Z.-M., Arnsten, A. F. T., & Li, B.-M. (1999). Local infusion of an α-1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. *Biological Psychiatry, 46*, 1259-1265.

Marsteller, D. A., Gerasimov, M. R., Schiffer, W. K., Geiger, J. M., Barnett, C. R., Borg, J. S., et al. (2002). Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. *Neuropsychopharmacology, 27*, 163-170.

Martin, B. J., Naughton, B. J., Thirtamara-Rajamani, K., Yoon, D. J., Han, D. D., Devries, A. C., et al. (2010). Dopamine transporter inhibition is necessary for cocaine-induced increases in dendritic spine density in the nucleus accumbens. *Synapse,* doi: 10.1002/syn.20865.

Martins, S., Tramontina, S., Polanczyk, G., Eizirik, M., Swanson, J. M., & Rohde, L. A. (2004). Weekend holidays during methylphenidate use in ADHD children: A randomized clinical trial. *Journal of Child and Adolescent Psychopharmacology, 14* (2), 195-205.

McCabe, S. E., Teter, C. J., & Boyd, C. J. (2006). Medical use, illicit use, and diversion of abusable prescription drugs. *Journal of American College Health, 54*, 269-278.

McClure, S. J., & Robinson, P. J. (1996). Dynamin, endocytosis and intracellular signalling (Review). *Molecular Membrane Biology, 13*, 189-215.

McGraw, D. P. (2004). Parenting with Pills. Retrieved 4 January, 2011, from http://www.drphil.com/slideshows/slideshow/1978/?id=1978&showID=371

McGregor, I. S. (1996). Using Strawberry Tree Workbench Mac and Workbench PC for data acquisition and control in the animal learning laboratory. *Behavior Research Methods, Instruments and Computers, 28*, 38-48.

Mead, A. N., Rocha, B. A., Donovan, D. M., & Katz, J. L. (2002). Intravenous cocaine induced-activity and behavioural sensitization in norepinephrine-, but not dopamine-transporter knockout mice. *European Journal of Neuroscience, 15*, 514-520.

Miller, E. K., Erickson, C. A., & Desimone, R. (1996). Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *Journal of Neuroscience, 16*, 5154-5167.

Millichap, J. G. (2008). Etiologic classification of Attention-Deficit/Hyperactivity Disorder. *Pediatrics, 121*, e358-365.

Milstein, J. A., Lehmann, O., Theobald, D. E., Dalley, J. W., & Robbins, T. W. (2007). Selective depletion of cortical noradrenaline by anti-dopamine beta-hydroxylase-saporin impairs attentional function and enhances the effects of guanfacine in the rat. *Psychopharmacology, 190*, 51-63.

Mobini, S., Body, S., Ho, M.-Y., Bradshaw, C. M., Szabadi, E., Deakin, J. F. W., et al. (2002). Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology, 160*, 290-298.

Moeller, F. G., Dougherty, D. M., Barratt, E. S., Schmitz, J. M., Swann, A. C., & Grabowski, J. (2001). The impact of impulsivity on cocaine use and retention in treatment. *Journal of Substance Abuse Treatment, 21*, 193-198.

Moll, G. H., Hause, S., Ruther, E., Rothenberger, A., & Huether, G. (2001). Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. *Journal of Child and Adolescent Psychopharmacology, 11* (1), 15-24.

Mook, D. M., Jeffrey, J., & Neuringer, A. (1993). Spontaneously hypertensive rats (SHR) readily learn to vary but not repeat instrumental responses. *Behavioral and Neural Biology, 59*, 126-135.

MTA Cooperative Group. (2004). National Institute of Mental Health Multimodal Treatment Study of ADHD follow-up: 24-month outcomes of treatment strategies for attention-deficit/hyperactiity disorder. *Pediatrics, 113*, 754-761.

Mulder, A., Nordquist, R., Orgut, O., & Pennartz, C. (2003). Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. *Behavioural Brain Research, 146*, 77-88.

Murphy, S. H., Smith, P., Shaivitz, A. B., Rossberg, M. I., & Hurn, P. D. (2001). The effect of

brief halothane anesthesia during daily gavage on complications and body weight in rats. *Contemporary Topics in Laboratory Animal Science, 40*, 9-12.

Myers, M. M., Musty, R. E., & Hendley, E. D. (1982). Attenuation of hyperactivity in the Spontaneously Hypertensive rat by amphetamine. *Behavioral and Neural Biology, 34*, 42-54.

Naneix, F., Marchand, A. R., Di Scala, G., Pape, J.-R., & Coutureau, E. (2009). A role for medial prefrontal dopaminergic innervation in instrumental conditioning. *Journal of Neuroscience, 29* (20), 6606-6599.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes* (7th ed.).

Neave, N., Lloyd, S., Sahgal, A., & Aggleton, J. P. (1994). Lack of effect of lesions in the anterior cingulate cortex and retrosplenial cortex on certain tests of spatial memory in the rat. *Behavioural Brain Research, 65*, 89-101.

Nelson, A., & Killcross, S. (2006). Amphetamine exposure enhances habit formation. *Journal of Neuroscience, 26*, 3805-3812.

Nigg, J. T., & Breslau, N. (2007). Prenatal smoking exposure, low birth weight, and disruptive behavior disorders. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*, 362-369.

Nolan, K. A., D'Angelo, D., & Hoptman, M. J. (2010). Self-report and laboratory measures of impulsivity in patients with schizophrenia or schizoaffective disorder and healthy controls. *Psychiatry Research,* doi: 10.1016/j.psychres.2010.10.032 *23*.

Norrholm, S. D., Bibb, J. A., Nestler, E. J., Ouimet, C. C., Taylor, J. R., & Greengard, P. (2003). Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience, 116*, 19-22.

Oberlin, B. G., & Grahame, N. J. (2009). High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task.

Alcoholism: Clinical and Experimental Research, 33, 1294-1303.

Ongur, D., & Price, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cerebral Cortex, 10*, 206-219.

Ordway, G. A., O'Donnell, J. M., & Frazer, A. (1987). Effects of clenbuterol on central *Beta*-1 and *Beta*-2 adrenergic receptors of the rat. *The Journal of Pharmacology and Experimental Therapeutics, 241*, 187-195.

Pardey, M. C., Homewood, J., Taylor, A., & Cornish, J. L. (2009). Re-evaluation of an animal model for ADHD using a free-operant choice task. *Journal of Neuroscience Methods, 176*, 166-171.

Patrick, K. S., Ellington, K. R., & Breese, G. R. (1984). Distribution of methylphenidate and *p*-hydroxymethylphenidate in rats. *The Journal of Pharmacology and Experimental Therapeutics, 231* (1), 61-65.

Paus, T., Zijdenbos, A., Worsley, K., Collins, D. L., Blumenthal, J., Giedd, J. N., et al. (1999). Structural maturation of neural pathways in children and adolescents: In vivo study. *Science, 283*, 1908-1911.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxtic coordinates* (4th ed.). New York: Academic Press.

Perry, G. M. L., Sagvolden, T., & Faraone, S. V. (2010a). Intra-individual variability in genetic and environmental models of attention-deficit/hyperactivity disorder. *American Journal of Medical Genetics Part B, 153B*, 1094-1101.

Perry, G. M. L., Sagvolden, T., & Faraone, S. V. (2010b). Intraindividual variability (IIV) in an animal model of ADHD - the Spontaneously Hypertensive Rat. *Behavioral and Brain Functions, 6*, 56-60.

Perry, J. L., Larson, E. B., German, J. P., Madden, G. J., & Carroll, M. E. (2005). Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. *Psychopharmacology (Berl), 178*, 193-201.

Perry, J. L., Nelson, S. E., & Carroll, M. E. (2008). Impulsive choice as a predictor of acquisition of IV cocaine self-administration and reinstatement of cocaine-seeking behavior in male and female rats. *Clinical Psychopharmacology, 16*, 165-177.

Peters, Y., O'Donnell, P., & Carelli, R. (2005). Prefrontal cortical cell firing during maintenance, extinction, and reinstatement of goal-directed behavior for natural reward. *Synapse, 56*, 74-83.

Pickel, V. M., Joh, T. H., Field, P. M., Becker, C. G., & Reis, D. J. (1975). Cellular localization of tyrosine hydroxylase by immunohistochemistry. *The Journal of Histochemistry and Cytochemistry, 23*, 1-12.

Pierce, R. C., & Kalivas, P. W. (1997). A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. *Brain Research Reviews, 25*, 192-216.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biological Psychiatry, 57*, 1385-1390.

Polanczyk, G., de Lime, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: A systematic review and metaregression analysis. *American Journal of Psychiatry, 164*, 942-948.

Pothuizen, H. H., Jongen-Relo, A. L., Feldon, J., & Yee, B. K. (2005). Double dissociation of the effects of selective nucleus accumbens core and shell lesions on impulsive-choice behaviour and salience learning in rats. *European Journal of Neuroscience, 22*, 2605-2616.

Potter, L. (1967). Role of intraneuronal vesicles in the synthesis, storage, and release of noradrenaline. *Circulation Research, 21* (6 (Supplemental III)), 13-24.

Poucet, B. (1990). A further characterization of the spatial problem-solving deficit induced by lesions of the medial frontal cortex in the rat. *Behavioural Brain Research, 41*, 229-237.

Poulos, C. X., Le, A. D., & Parker, J. L. (1995). Impulsivity predicts individual susceptibility to high levels of alcohol self-administration. *Behavioural Pharmacology, 6*, 810-814.

Preen, D. B., Calver, J., Sanfilippo, F. M., Bulsara, M., & Holman, C. D. J. (2007). Patterns of psychostimulant prescribing to children with ADHD in Western Australia: Variations in age, gender, medication type and dose prescribed. *Australian and New Zealand Journal of Public Health, 31*, 120-126.

Prosser, B., & Reid, R. (1999). Psycholstimulant use for children with ADHD in Australia. *Journal of Emotional and Behavioral Disorders, 7* (2), 110-117.

Prosser, B., & Reid, R. (2009). Changes in use of psychostimulant medication for ADHD in South Australia (1990-2006). *Australian and New Zealand Journal of Psychiatry, 43*, 340-347.

Ramos, B., & Arnsten, A. F. T. (2007). Adrenergic pharmacology and cognition: Focus on the prefrontal cortex. *Pharmacology and Therapeutics, 113*, 523-536.

Ramos, B., Stark, D., Verduzco, L., van Dyck, C. H., & Arnsten, A. F. T. (2006). α2A-adrenoceptor stimulation improves prefrontal cortical regulation of behaviour through inhibition of cAMP signaling in aging animals. *Learning and Memory, 13*, 770-776.

Rapport, M. D., & Moffitt, C. (2002). Attention deficit/hyperactivity disorder and methylphenidate: A review of height/weight, cardiovascular, and somatic complaint side effects. *Clinical Psychology Review, 22*, 1107-1131.

Renaud, B., Joh, T. H., & Reis, D. J. (1979). An abnormal regulation of tyrosine hydroxylase restricted to one catecholamine nucleus in the brain stem of spontaneously hypertensive rats. *Brain Research, 173*, 164-167.

Reynolds, C. R., & Kamphaus, R. W. (1992). *Behavior Assessment Schedule for Children*. Circle Pines, MN: American Guidance Service.

Rice, D., & Barone Jr., S. (2000). Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environmental Health*

Perspectives, 108 (supplement 3), 511-533.

Robinson, E. S. J., Eagle, D. M., Mar, S. C., Bari, A., Banerjee, G., Jiang, W., et al. (2008). Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology, 33*, 1028-1037.

Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews, 11*, 157-198.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews, 18*, 247-291.

Robinson, T. E., Gorny, G., Mitton, E., & Kolb, B. (2001). Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse, 39*, 257-266.

Robinson, T. E., & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *The Journal of Neuroscience, 17* (21), 8491-8497.

Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *European Journal of Neuroscience, 11*, 1598-1604.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology, 47*, 33-46.

Rodriguez, A., & Bohlin, G. (2005). Are maternal smoking and stress during pregnancy related to ADHD symptoms in children? *Journal of Child Psychology and Psychiatry and Allied Disciplines, 46*, 246-254.

Rodriguez, J. S., Morris, S. M., Hotchkiss, C. E., Doerge, D. R., Allen, R. R., Mattison, D. R., et al. (2010). The effect of chronic methylphenidate administration on operant test battery performance in juvenile rhesus monkeys. *Neurotoxicology and Teratology, 32*,

142-151.

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cerebral Cortex, 10*, 284-294.

Rosenberg, D. R., & Lewis, D. A. (1995). Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. *The Journal of Comparative Neurology, 358*, 383-400.

Rossi, A. F., Bichot, N. P., Desimone, R., & Ungerleider, L. G. (2007). Top-down attentional deficits in Macaques with lesions of lateral prefrontal cortex. *The Journal of Neuroscience, 27* (42), 11306-11314.

The Royal Australasian College of Physicians. (2009). *Draft Australian Guidelines on Attention Deficit Hyperacitivty Disorder (ADHD).*

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S. C. R., Simmons, A., et al. (1999). Hypofrontality in Attention Deficit Hyperactivity Disorder during higher-order motor control: A study with functional MRI. *The American Journal of Psychiatry, 156* (6), 891-896.

Russell, V. A. (2002). Hypodopaminergic and hypernoradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder - the spontaneously hypertensive rat. *Behavioural Brain Research, 130*, 191-196.

Russell, V. A., Allie, S., & Wiggins, T. (2000a). Increased noradrenergic activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder - the spontaneously hypertensive rat. *Behavioural Brain Research, 117*, 69-74.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C. L., & Taljaard, J. J. F. (1995). Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of attention-deficit hyperactivity disorder - The spontaneously hypertensive rat. *Behavioural Brain Research, 676*, 343-351.

Russell, V. A., de Villiers, A. S., Sagvolden, T., Lamm, M. C. L., & Taljaard, J. J. F. (2000b). Methylphenidate affects striatal dopamine differently in an animal model for attention-

deficit/hyperactivity disorder - The spontaneously hypertensive rat. *Brain Research Bulletin, 53* (2), 187-192.

Russell, V. A., Sagvolden, T., & Johansen, E. B. (2005). Animal models of attention-deficit/hyperactivity disorder. *Behavioral and Brain Functions, 1*, 9.

Russell, V. A., & Wiggins, T. (2000). Increased glutamate-stimulated norepinephrine release from prefrontal cortex slices of spontaneously hypertensive rats. *Metabolic Brain Disease, 15*, 297-304.

Sagvolden, T. (2000). Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of Attention-Deficit/Hyperactivity Disorder (AD/HD). *Neuroscience and Biobehavioral Reviews, 24*, 31-39.

Sagvolden, T. (2006). The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of Attention-Deficit/Hyperactivity Disorder (ADHD). *Behavioral and Brain Functions, 2*, 41.

Sagvolden, T., Aase, H., Zeiner, P., & Berger, D. (1998). Altered reinforcement mechanisms in Attention-Deficit/Hyperactivity Disorder. *Behavioural Brain Research, 94*, 61-71.

Sagvolden, T., DasBanerjee, T., Zhang-James, Y., Middleton, F. A., & Faraone, S. V. (2008). Behavioral and genetic evidence for a novel animal model of attention-deficit/hyperactivity disorder predominantly inattentive subtype. *Behavioral and Brain Functions, 4*, 56.

Sagvolden, T., Johansen, E. B., Aase, H., & Russell, V. A. (2005). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behavioral and Brain Sciences, 28*, 397-419.

Sagvolden, T., Johansen, E. B., Woien, G., Walaas, S. I., Storm-Mathisen, J., Bergersen, L. H., et al. (2009). The spontaneously hypertensive rat model of ADHD - The importance of selecting the appropriate reference strain. *Neuropharmacology, 57*, 619-

626.

Sagvolden, T., Metzger, M. A., Schiorbeck, H. K., Rugland, A.-L., Spinnangr, I., & Sagvolden, G. (1992). The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): Changed reactivity to reinforcers and to psychomotor stimulants. *Behavioral and Neural Biology, 58*, 103-112.

Sagvolden, T., Petterson, M. B., & Larsen, M. C. (1993). Spontaneously hypertensive rats (SHR) as a putative animal model of childhood hyperkinesis: SHR behavior compared to four other rat strains. *Physiology and Behavior, 54* (6), 1047-1055.

Sagvolden, T., Russell, V. A., Aase, H., Johansen, E. B., & Farshbaf, M. (2005). Rodent models of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57*, 1239-1247.

Sawyer, M. G., Rey, J. M., Graetz, B. W., Clark, J. J., & Baghurst, P. A. (2002). Use of medication by young people with attention-deficit/hyperactivity disorder. *Medical Journal of Australia, 177*, 21-25.

Scherer, E. B. S., da Cunha, M. J., Matte, C., Schmitz, F., Netto, C. A., & Wyse, A. T. S. (2010). Methylphenidate affects memory, brain-derived neurotrophic factor immunocontent and brain acetylcholinesterase activity in the rat. *Neurobiology of Learning and Memory, 94*, 247-253.

Schoenbaum, G., Saddoris, M. P., Ramus, S. J., Shaham, Y., & Setlow, B. (2004). Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. *European Journal of Neuroscience, 19*, 1997-2002.

Schultz, W. (1998). The phasic reward signal of primate dopamine neurons. *Advances in Pharmacology, 42*, 686-690.

Sciutto, M. J., & Eisenberg, M. (2007). Evaluating the evidence for and against the overdiagnosis of ADHD. *Journal of Attention Disorders, 11* (2), 106-113.

Seeman, P., & Madras, B. (1998). Anti-hyperactivity medication: methylphenidate and

amphetamine. *Molecular Psychiatry, 3*, 386-396.

Seeman, P., & Madras, B. (2002). Methylphenidate elevates resting dopamine which lowers the impulse-triggered release of dopamine: a hypothesis. *Behavioural Brain Research, 130*, 79-83.

Sesack, S. R., & Carr, D. B. (2002). Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. *Physiology and Behavior, 77* (513-517).

Sesack, S. R., & Pickel, V. M. (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *Journal of Comparative Neurology, 320* (145-160).

Sica, D. A. (2007). Centrally acting antihypertensive agents: An update. *Journal of Clinical Hypertension, 9*, 399-405.

Skounti, M., Phialithis, A., & Galanakis, E. (2007). Variations in prevalence of attention deficit hyperactivity disorder worldwide. *European Journal of Pediatrics, 166*, 117-123.

Smelter, R. W., Rasch, B. W., Fleming, J., Nazos, P., & Baranowski, S. (1996). Is attention deficit disorder becoming a desired diagnosis? *Phi Delta Kappan, 77*, 429-432.

Solanto, M. V. (1998). Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: A review and integration. *Behavioural Brain Research, 94*, 127-152.

Solanto, M. V., & Alvir, J. (2009). Reliability of DSM-IV symptom ratings of ADHD: Implications for DSM-V. *Journal of Attention Disorders, 13* (2), 107-116.

Solanto, M. V., Newcorn, J., Vail, L., Gilbert, S., Ivanov, I., & Lara, R. (2009). Stimulant drug response in the predominantly inattentive and combined subtypes of attention-deficit/hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology, 19*, 663-671.

Sonuga-Barke, E. J. S., Taylor, E., Sembi, S., & Smith, J. (1992). Hyperactivity and delay aversion I: the effect of delay on choice. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 33*, 387-398.

Sorg, B. A., Davidson, D. L., Kalivas, P. W., & Prasad, B. M. (1997). Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. *Journal of Pharmacology and Experimental Therapeutics, 281*, 54-61.

Sorg, B. A., & Kalivas, P. W. (1993). Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. *Neuroscience, 53*, 695-703.

Sorg, B. A., Li, N., & Wu, W. R. (2001). Dopamine D1 receptor activation in the medial prefrontal cortex prevents the expression of cocaine sensitization. *Journal of Pharmacology and Experimental Therapeutics, 297*, 501-508.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Jerigan, T. L., & Toga, A. W. (1999). *In vivo* evidence for post-adolescent brain maturation in frontal and striatal regions. *Nature Neuroscience, 2*, 859-861.

Sowell, E. R., Thompson, P. M., Tessner, K. D., & Toga, A. W. (2001). Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation. *The Journal of Neuroscience, 21*, 8819-8829.

Steketee, J. D. (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Research Reviews, 41*, 203-228.

Stoof, J. C., & Kebabian, J. W. (1981). Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature, 294*, 366-368.

Strauss, A., & Lehtinen, L. (1947). *Psychopathology and education of the brain-injured child*. New York: Grune and Stratton.

Sutherland, K. R., Alsop, B., McNaughton, N., Hyland, B. I., Tripp, G., & Wickens, J. R.

(2009). Sensitivity to delay of reinforcement in two animal models of attention deficit hyperactivity disorder (ADHD). *Behavioural Brain Research, 205*, 372-376.

Swanson, C. J., Heath, S., Stratford, T. R., & Kelley, A. E. (1997). Differential behavioral responses to dopaminergic stimulation of nucleus accumbens subregions in the rat. *Pharmacology, Biochemistry and Behavior, 58*, 933-945.

Swanson, J. (1992). *School-based assessments and interventions for ADD students*. Irvine, CA: K.C. Publications.

Swanson, J. M., & Volkow, N. D. (2001). Pharmacokinetic and pharmacodynamic properties of methylphenidate in humans. In M. V. Solanto, A. F. T. Arnsten & F. X. Castellanos (Eds.), *Stimulant drugs and ADHD: Basic and clinical neuroscience* (pp. 259-282). New York: Oxford University Press.

Swanson, J. M., & Volkow, N. D. (2002). Pharmacokinetic and pharmacodynamic properties of stimulants: implications for the design of new treatments for ADHD. *Behavioural Brain Research, 130*, 73-78.

Swanson, L. W., & Hartman, B. K. (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their afferent connections in the rat utilizing dopamine-B-hydroxylase as a marker. *Journal of Comparative Neurology, 163*, 467-487.

Sydney Morning Herald. (2010, 18 August). ADHD diagnosed by mistake in young. Retrieved 21 February, 2011, from http://www.smh.com.au/lifestyle/wellbeing/adhd-diagnosed-by-mistake-in-young-20100817-128ma.html

Tannock, R. (1998). Attention Deficit Hyperactiivty Disorder: advances in cognitive, neurobiological and genetic research. *Journal of Child Psychology and Psychiatry, 39*, 65-99.

Taylor, C. L., Latimer, M. P., & Winn, P. (2003). Impaired delayed spatial win-shift behaviour on the eight arm radial maze following excitotoxic lesions of the medial

prefrontal cortex in the rat. *Behavioural Brain Research, 147*, 107-114.

Taylor, E. (1994). Syndromes of attention deficit and over activity. In M. Rutter, E. Taylor & L. Hersov (Eds.), *Child and Adolescent Psychiatry* (3rd ed., pp. 285-307). London: Blackwell Science.

Teter, C. J., McCabe, S. E., LaGrange, K., Cranford, J. A., & Boyd, C. J. (2006). Illicit use of specific prescription stimulants among college students: Prevalence, motives, and routes of administration. *Pharmacotherapy, 26*, 1501-1510.

Thanos, P. K., Ivanov, I., Robinson, J. K., Michaelides, M., Wang, G.-J., Swanson, J. M., et al. (2010). Dissociation between spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats in baseline performance and methylphenidate response on measures of attention, impulsivity and hyperactivity in a Visual Stimulus Position Discrimination Task. *Pharmacology, Biochemistry and Behavior, 94*, 374-379.

Thanos, P. K., Michaelides, M., Benveniste, H., Wang, G. J., & Volkow, N. D. (2007). Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. *Pharmacology, Biochemistry and Behavior, 87*, 426-433.

Thapar, A., Fowler, T., Rice, F., Scourfield, J., van den Bree, M., Thomas, H., et al. (2003). Maternal smoking during pregnancy and Attention Deficit Hyperactivity Disorder symptoms in offspring. *American Journal of Psychiatry, 160*, 1985-1990.

Thapar, A., Holmes, J., Poulton, K., & Harrington, R. (1999). Genetic basis of attention deficit and hyperactivity. *The British Journal of Psychiatry, 174*, 105-111.

Torres-Reveron, A., Gray, J. D., Melton, J. T., Punsoni, M., Tabori, N. E., Ward, M. J., et al. (2009). Early postnatal exposure to methylphenidate alters stress reactivity and increases hippocampal ectopic granule cells in adult rats. *Brain Research Bulletin, 78*, 175-181.

Tripp, G., & Wickens, J. R. (2009). Neurobiology of ADHD. *Neuropharmacology, 57*, 579-

589.

Tully, K., & Bolshakov, V. Y. (2010). Emotional enhancement of memory: how norepinephrine enables synaptic plasticity. *Molecular Brain, 3*, 15.

Unger, W. G., Terenghi, G., Zhang, S.-Q., & Polak, J. M. (1988). Alteration in the histochemical presence of tyrosine hydroxylase and CGRP-immunoreactivities in the eye following chronic sympathetic or sensory denervation. *Current Eye Research, 7*, 761-769.

Urbanavicius, J., Ferrerira, M., Costa, G., Abin-Carriquiry, J. A., Wonnacoot, S., & Dajas, F. (2007). Nicotine induces tyrosine hydroxylase plasticity in the neurodegenerating striatum. *Journal of Neurochemistry, 102*, 723-730.

van den Bergh, F. S., Bloemarts, E., Chan, J. S. W., Groenink, L., Olivier, B., & Oosting, R. S. (2006). Spontaneously hypertensive rats do not predict symptoms of Attention-Deficit Hyperactivity Disorder. *Pharmacology, Biochemistry and Behavior, 83*, 380-390.

van Gaalen, M. M., van Koten, R., Schoffelmeer, A. N. M., & Vanderschuren, L. J. M. J. (2006). Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biological Psychiatry, 60*, 66-73.

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience, 1*, 191-198.

Van Tol, H. H. M., Bunzow, J. R., Guan, H.-C., Sunahara, R. K., Seeman, P., Niznik, H. B., et al. (1991). Cloning of the gene for a human D_4 receptor with high affinity for the antipsychotic clozapine. *Nature, 350*, 610-614.

van Zwieten, P. A. (1999). The renaissance of centrally acting antihypertensive drugs. *Journal of Hypertension. Supplement, 17*, S15-S21.

Vanderschuren, L. J. M. J., Beemster, P., & Schoffelmeer, A. N. M. (2003). On the role of noradrenaline in psychostimulant-induced psychomotor activity and sensitization.

Psychopharmacology, 169, 176-185.

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse, 51*, 32-58.

Vijayraghavan, S., Wang, M., Birnbaum, S. G., Williams, G. V., & Arnsten, A. F. T. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nature Neuroscience, 10* (3), 376-384.

Vitiello, B. (2001). Long-term effects of stimulant medications on the brain: Possible relevance to the treatment of attention deficit hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology, 11*, 25-34.

Volk, D. W., & Lewis, D. A. (2010). Prefrontal cortical circuits in schizophrenia. *Current Topics in Behavioral Neurosciences, 4*, 485-508.

Volkow, N. D., & Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cerebral Cortex, 10*, 318-325.

Volkow, N. D., Fowler, J. S., Wang, G.-J., Ding, Y.-S., & Gatley, S. J. (2002a). Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: Results from imaging studies. *European Neuropsychopharmacology, 12*, 557-566.

Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., & Telang, F. (2009). Imaging dopamine's role in drug abuse and addicition. *Neuropharmacology, 56* (Suppl 1), 3-8.

Volkow, N. D., & Swanson, J. M. (2003). Variables that affect the clinical use and abuse of methylphenidate in the treatment of ADHD. *American Journal of Psychiatry, 160*, 1909-1918.

Volkow, N. D., Wang, G.-J., Fowler, J. S., & Ding, Y.-S. (2005). Imaging the effects of methylphenidate on brain dopamine: New model on its therapeutic actions for Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57*, 1410-1415.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Angrist, B., Hitzemann, R., et al. (1997). Effects of methylphenidate on regional brain glucose metabolsim in humans:

Relationship to dopamine D_2 receptors. *American Journal of Psychiatry, 154* (1), 50-55.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Franceschi, D., Maynard, L., et al. (2002b). Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeutic implications. *Synapse, 43*, 181-187.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Gerasimov, M., Maynard, L., et al. (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *The Journal of Neuroscience, 21*, RC121.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Jayne, M., Franceschi, D., et al. (2002c). "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. *Synapse, 44*, 175-180.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Telang, F., Maynard, L., Logan, J., et al. (2004). Evidence that methylphenidate enhances the saliency of a mathematical task by increasing dopamine in the human brain. *American Journal of Psychiatry, 161* (7), 1173-1180.

Wade, T. R., de Wit, H., & Richards, J. B. (2000). Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology, 150*, 90-101.

Wallis, D. (2010). The search for biomarkers for attention deficit/hyperactivity disorder. *Drug News and Perspectives, 23*, 438-449.

Walton, M. E., Bannerman, D. M., Alterescu, K., & Rushworth, M. F. S. (2003). Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. *Journal of Neuroscience, 23* (16), 6475-6479.

Wang, M., Ramos, B., Paspalas, C., Shu, Y., Simen, A., Duque, A., et al. (2007). Alpha2A-adrenoceptor stimulation strengthens working memory networks in inhibiting cAMP-HCN channel signalling in prefrontal cortex. *Cell, 129* (2), 397-410.

Wargin, W., Patrick, K. S., Kilts, C., Gualtieri, C. T., Ellington, K. R., Mueller, R. A., et al. (1983). Pharmacokinetics of methylphenidate in man, rat and monkey. *The Journal of Pharmacology and Experimental Therapeutics, 226* (2), 382-386.

Weiss, F., Ciccocioppo, R., Parsons, L. H., Katner, S., Liu, X., Zorrilla, E. P., et al. (2001). Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress and conditioning factors. *Annals of the New York Academy of Sciences, 937*, 1-26.

Wender, P. H. (1973). Minimal brain dysfunction in children. Diagnosis and management. *Pediatric Clinics of North America, 20*, 187-202.

Wheeler, T. L., Eppolito, A. K., Smith, L. N., Huff, T. B., & Smith, R. F. (2007). A novel method for oral stimulant administration in the neonate rat and similar species. *Journal of Neuroscience Methods, 159*, 282-285.

Wilens, T. E. (2008). Effects of methylphenidate on the catecholaminergic system in Attention-Deficit/Hyperactivity Disorder. *Journal of Clinical Psychopharmacology, 28* (3), S46-S53.

Wilhelm, C. J., & Mitchell, S. H. (2008). Rats bred for high alcohol drinking are more sensitive to delayed and probabilistic outcomes. *Genes, Brain, and Behavior, 7*, 705-713.

Winstanley, C. A., Eagle, D. M., & Robbins, T. W. (2006). Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. *Clinical Psychology Review, 26*, 379-395.

Winstanley, C. A., Olausson, P., Taylor, J. R., & Jentsch, J. D. (2010). Insight into the relationship between impulsivity and substance abuse from studies using animal models. *Alcoholism: Clinical and Experimental Research, 34*, 1306-1318.

Winstanley, C. A., Theobald, D. E., Cardinal, R. N., & Robbins, T. W. (2004). Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *Journal of Neuroscience, 24* (20), 4718-4722.

Winstanley, C. A., Theobald, D. E., Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2006). Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cerebral Cortex, 16*, 106-114.

Winter, S., Diekmann, M., & Schwabe, K. (2009). Dopamine in the prefrontal cortex regulates rats behavioral flexibility to changing reward value. *Behavioural Brain Research, 198*, 206-213.

Wolraich, M. L., & Doffing, M. A. (2004). Pharmacokinetic considerations in the treatment of Attention-Deficit/Hyperactivity Disorder with methylphenidate. *CNS Drugs, 18* (4), 243-250.

Wolraich, M. L., Hannah, J. N., Pinnock, T. Y., Baumgaertel, A., & Brown, J. (1996). Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a county-wide sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 35*, 319-324.

World Health Organization. (1992). *International Classification of Diseases (ICD-10)* (10th ed.). Geneva: World Health Organization.

Wurtman, R. J., & Fernstrom, J. D. (1975). Control of brain monoamine synthesis by diet and plasma amino acids. *The American Journal of Clinical Nutrition, 28*, 638-647.

Yang, P. B., Amini, B., Swann, A. C., & Dafny, N. (2003a). Strain differences in the behavioral responses of male rats to chronically administered methylphendiate. *Brain Research, 971*, 139-152.

Yang, P. B., Amini, B., Swann, A. C., & Dafny, N. (2003b). Strain differences in the behavioral responses of male rats to chronically administered methylphenidate. *Brain Research, 971*, 139-152.

Yang, P. B., Swann, A., & Dafny, N. (2006). Chronic methylphenidate modulates locomotor activity and sensory evoked responses in the VTA and NAc of freely behaving rats.

Neuropharmacology, 51, 546-556.

Yang, P. B., Swann, A. C., & Dafny, N. (2010). Psychostimulants given in adolescence modulate their effects in adulthood using the open field and the wheel-running assays. *Brain Research Bulletin, 82*, 208-217.

Zaborszky, L., Alheid, G. F., Beinfeld, M. C., Eiden, L. E., Heimer, L., & Palkovits, M. (1985). Cholecystokinin innervation of the ventral stiatum: a morphological and radioimmunological study. *Neuroscience, 14*, 427-453.

Zandbelt, B. B., & Vink, M. (2010). On the role of the striatum in response inhibition. *PLoS one, 5* (11), e13848.

Zelazo, P. D., & Muller, U. (2002). Executive function in typical and atypical development. In U. Goswami (Ed.), *Blackwell handbook of childhood cognitive development*. Oxford: Blackwell.

Zito, J. M., Safer, D. J., dosReis, S., Gardner, J. F., Boles, M., & Lynch, F. (2000). Trends in the prescribing of psychotropic medications to preschoolers. *JAMA, 283* (8), 1025-1030.

MACQUARIE UNIVERSITY ~ SYDNEY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2006/019 - 3
Original Approval Duration: 1 September 2006 to 31 August 2008 (24 months)
Extension of approval: to 31 August 2009 (additional 12 months)

To: Mrs Margery Pardey (nee Aylett) **(PI)**
Department of Psychology
Macquarie University
Phone: (02) 9428 5060
Fax: (02) 9850 7759
Email: maylett@maccs.mq.edu.au

Associate Investigator (s)
Dr Jennifer Cornish Phone: (02) 9850 9467

Other participants
Dr Kelly Clements Phone: 0413348373
Mr Wayne McTegg Phone: (02) 9850 7757
Ms Niree Krachshaar

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: "EFFECTS OF ADHD MEDICATION ON CONGITIVE AND NEURAL DEVELOPMENT FOLLOWING MISDIAGNOSIS IN ADOLESCENCE: AN ANIMAL MODEL"

Type of animal research and description of project:
Research (Neurophysiology / Pharmacology) – Attention Deficit Hyperactive Disorder (ADHD). The aim of this study is to determine what effect ADHD medication has on brain development when administered to subjects who do not have ADHD. (1) Rats are purchased from an external source, flown to the central animal facility and housed in room 142 (F9A). Rats will be housed in standard tubs (59.7 x 36.8 x 18.0 cm) containing 2 rats per tub. Rats will have *ad libitum* access to food and water. (2) Rats are given 7 days to acclimate to their new environment. (3) Rats given 7 days of handling, tagging (texta marks on tails) and weighing. (4) To habituate them for the experiments, the rats will be given saline via gavage for 3 days. Then the rats will receive methylphenidate (MPH) 10mg/kg orally via gavage. (5) Cognitive and behavioural testing will be conducted using delayed reinforcement task (DRT), radial arm maze and cross arm maze. (6) Following data collection the rat will be euthanased by CO2 or decapitation. (7) Histological analysis will then be performed in changes in neuron growth and dendritic branching, thought to be associated with cognitive disorders in ADHD.

Species of animal: Spontaneous Hypertensive and Wistar Kyoto strain male rats

Number: total of 192 rats over three years

Location: Central Animal House Facility, Macquarie University, NSW 2109.

Amendments considered by the AEC during last period:
1. Addition of Ms Niree Kraushaar 2. Reseacher's name from Aylett to Pardey 3. Water reward in maze 4. Method of drug administration to oral with prior water deprivation 5. Twice daily drug administration 6. Method of euthanasia to Lethabarb followed by cardiac perfusion with saline and formalin or rapid decapitation 7. Extension of approval to a total of 3 years 8. Euthanasia of a group of animals after drug administration without cognitive testing

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions: N/A

(This authority has been issued as the above condition (s) has been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **25 August 2008** to **31 August 2009**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a final report at the end of this period.**

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Date: 5-9-08

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY

AEC Reference No: **2009/001**

Full Approval Duration: 01 APRIL 2009 TO 31 MARCH 2010 (12 months)

To: Mrs Margery Pardey
Dept of Psychology
Macquarie University
Phone: 0410 643 182
Email: maylett@maccs.mq.edu.au

Associate Investigator(s)
Dr J Cornish Phone: (02) 9850 9467
Dr J Homewood Phone: (02) 9850 6215

Animal Technician
Mr W McTegg Phone: (02) 9850 7757

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: THE ROLE OF DOPAMINE RECEPTORS IN IMPULSIVITY MEDIATED BY THE PREFRONTAL CORTEX

Type of animal research and description of project: Research (Neurophysiology / Pharmacology) – This study aims to investigate the neurobiological mechanisms underlying long-term cognitive deficit. Rats will undergo a modified Delayed Reinforcement Test as described in the approved protocol application including a training phase, delay phase and testing phase. Rats will be deeply anaesthetised at end with pentobarbitone sodium and will undergo intracardiac perfusion-fixation (PBS followed by paraformadehyde 4%). Brains will be removed for verification of injection sites.

Species of animal: *Rattus novegicus* (Wistar Kyoto) male approx 1 month at commencement 100-200 gm

Number: 32 rats per year for one year

Location: Central Animal House Facility, Macquarie University, NSW 2109.

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions: N/A

(This authority has been issued as the above condition (s) has been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **01 APRIL 2009 to 31 MARCH 2010**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a FINAL report at the end of this period.**

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Date: 2.4.09

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2010/001 **Date of expiry: 30 April 2011**

Full Approval Duration: 1 May 2010 to 30 April 2011 (12 months)

Principal Investigator:
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Jennifer.cornish@mq.edu.au

Associate Investigators:
Mrs Margery Pardey (02) 8084 9460
0410 643 182
Dr Judi Homewood (02) 9850 6215
(02) 9411 1041

Animal Technician:
Mr Wayne McTegg (02) 9850 7757

The above-named are authorised by:

MACQUARIE UNIVERSITY AEC to conduct the following research:

Title of the project: The role of noradrenaline receptors in impulsivity mediated by the prefrontal cortex

Type of animal research and description of project:
Research (Behavioural / Pharmacology) – this project aims to determine the role of noradrenaline receptors in the prefrontal and orbitofrontal cortices on impulsive choice.
After standard housing in the CAHF, rats are given 7 days acclimation and 7 days handling, tagging (texta) and weighing, then undergo surgery for implantation of bilateral intracranial microinjection cannulae. Rats recover for 5-7 days then undergo the Delayed Reinforcement Task. At completion of experiments, rats are anaesthetised with pentobarb sodium then undergo intracardiac perfusion-fixation and removal of brains for verification of microinjection sites.

All procedures must be performed in accordance with the AEC approved protocol.

					Numbers approved				
Species	**Strain**	**Sex**	**Weight**	**Age**	**Year 1**	**Year 2**	**Year 3**	**Total**	**Supplier/ Source**
Rat	WKY	Male	100–200g	1 month at commencement	32			32	ARC, Perth
				TOTAL				**32**	

Location of research: Room 142, Building F9A, Macquarie University NSW

Approval is subject to compliance with the following conditions:

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **1 May 2010 to 30 April 2011**, unless suspended, cancelled or surrendered, **and is contingent upon receipt of a FINAL REPORT at the end of this period.**

Date: 29.4.10

Dr Karolyn White
Director, Research Ethics
Acting Chair AEC

Form C (issued under part IV of the Animal Research Act, 1985)