STUDY OF THE EFFECTS OF METHYLPHENIDATE (RITALIN) IN AN ANIMAL MODEL FOR ATTENTION-DEFICIT HYPERACTIVITY DISORDER – THE SPONTANEOUSLY HYPERTENSIVE RAT

BY GOSIAMEMANG LESEGO SADI LELAKA

Submitted in fulfillment of the requirements for the degree of Master of Science (MSc.) (Med) in the faculty of Health Sciences at the University of Cape Town

August 2004

Supervisors: Associate Professor V.A. Russell

Dr L.A. Kellaway

DEDICATION

This work is dedicated to the loving memory of my mama Selebano Ivy Bogosi, my best friend Florence Bokha Gabatsewe Gare and my niece Naledi "Star" Matong. They saw the work begun but did not live long enough to see it completed. May their souls rest in peace!!

CONTENTS

DECLARATION

I, Gosiamemang Lesego Sadi Lelaka, declare that this dissertation is original, except where acknowledgements indicate otherwise and that neither the whole work nor any part of it has been, or is being, or is to be submitted for another degree in this or another University.

I give permission to the University of Cape Town to reproduce for purposes of research either the whole or any part of the contents of this dissertation.

Signed: Signed by candidate

Date: 03-09-2004.

ACKNOWLEDGEMENTS

The success of this work is owed to the almighty God for his love and sustenance and guidance especially during those times when emotions clouded sound judgement.

My heartfelt gratitude is extended to my supervisors: Professor Vivienne Ann Russell and Dr Laurie Kellaway. Without their guidance and wisdom this work would not have been. I am especially grateful to Prof. Russell for her constant "panel beating" and refining. The less fortunate are those who missed the scrutiny of her eye. I am equally grateful to Prof. Alan St Clair Gibson for those much appreciated constructive criticisms during the lab meetings. I feel greatly honoured to have been associated with researchers of their caliber.

To my fellow lab-mates, Musa Mabandla and Thabelo Khoboko, I just want to say "I couldn't have asked for better company". You were the family I did not have in Cape Town, the brother and sister I could not have traded for silver or gold. Thank you.

My sincere thanks also go to Mr. Harry Hall and Mr. Keith Hall for making the running wheels and the place preference cages respectively and for being there to troubleshoot every now and then. In the same manner I am grateful to Mr. Iekraam Fakier for designing and fitting the motion sensors for the place preference cages and for being there to troubleshoot ever so frequently. Thank you.

I would like to express my gratitude to the University of Cape Town animal unit and to Mr. Basil Sedres and Mrs. Shula Johnson for breeding the rats used in this study. I would also like to thank Mr. Paul Rossouw for always keeping the glassware spotlessly clean.

I owe immeasurable gratitude to the Government of Botswana, Ministry of Health and Kanye College of Nursing for giving me the chance to study and for all the financial support rendered. I owe it all to the Pula nation.

Words fail to express the extent of my gratitude to my family. To my husband I would like to say "thank you for believing in me and allowing yourself to return to 'bachelorhood' to allow me to pursue academic goals. Thank you for being the mother to my otherwise 'motherless' kids especially having to learn to 'breast feed' a six-week-old baby". I couldn't have asked for anything better. To my three little angels (Kabo, Lettie and Atlang) I want to say thank you for allowing yourselves to be temporarily 'motherless' while mum was a student. You are super kids. I am highly indebted to Atlie for forfeiting the right to breast feed to allow mum to study.

I would like to thank Mr. William A. Carlezon, Jr. and Mr. Roy Wise for the help they offered in the construction of the place preference apparatus. It would not have been possible to design without their expert advice.

My sincere thanks go to the University of Cape Town and the Department of Human Biology for giving me a chance to study. To everybody in the university and the Faculty of Health Sciences who contributed in any way to the completion of this work, I am highly grateful. Thank you all.

LIST OF ABBREVIATIONS

[^{3}H]DA	radioactively (tritium) labelled dopamine
[^{3}H]NE	radioactively (tritium) labelled norepinephrine
$^{45}Ca^{2+}$	radioactively labelled calcium
5-HT	serotonin (5-hydroxytryptamine)
6-OHDA	6-hydroxydopamine
ADHD	attention-deficit hyperactivity disorder
ANOVA	analysis of variance
Ca^{2+}	calcium
CaMKII	calmodulin-dependent protein kinase II
CNS	central nervous system
CPP	conditioned place preference
DA	dopamine
D-AMP	D-amphetamine
DAT	dopamine transporter
DS	Drug (paired) Side
EDTA	ethylenediaminetetraacetic acid
IP	intraperitoneal

K^+	potassium
LC	locus coerulus
LE	Long Evans rat
MAO	monoamine oxidase
MFB	medial forebrain bundle
MP	methylphenidate hydrochloride (ritalin)
NAC	nucleus accumbens
NE	norepinephrine
P20 (21-60)	postnatal (post partum) day 20 (21-60)
PET	positron emission tomography
PFC	prefrontal cortex
SD	Sprague- Dawley rat
SEM	standard error of the mean
SHR	spontaneously hypertensive rat
SS	Saline (paired) Side
VTA	ventral tegmental area
WKY	Wistar Kyoto rat

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Attention-deficit hyperactivity disorder (ADHD) is the most prevalent childhood psychiatric disorder where the cardinal symptoms are all behavioural. ADHD children display excessive motor activity, restlessness, impulsive behaviour, poor social adjustments and have problems sustaining attention. Mimicking the symptoms of ADHD is the spontaneously hypertensive rat (SHR) which display hyperactivity, inability to withhold inappropriate responses in behavioural paradigms and poor ability to sustain attention in behavioural tasks. The SHR is therefore regarded as a putative model for the study of ADHD.

The aim of the study was (i) to investigate the behaviour of SHR when allowed free access to a running wheel using Wistar Kyoto rats (WKY) as controls, (ii) to establish whether methylphenidate (MP) administered orally can reduce SHR hyperactivity, (iii) to carry out preliminary experiments to repeat previously reported results on glutamate and potassium stimulated release of dopamine (DA) from nucleus accumbens (NAC) core and shell of SHR and WKY, (iv) to investigate the effect of pre-pubertal exposure to orally administered MP on norepinephrine (NE) release from prefrontal cortex (PFC) slices of five-week-old SHR and WKY and (v) to establish whether pre-adolescent exposure to MP (orally) increases susceptibility of the rats to cocaine-induced place preference in adulthood (P60).

Allowing the rats to voluntarily take MP orally was of prime importance as it closely resembles the normal route of MP administration in ADHD.

Housing SHR and WKY in cages with attached running wheels so that the rats could run voluntarily; we demonstrated that SHR ran more than WKY. When SHR had been treated with orally administered MP at doses of 0.5, 1.0, and 2.0mg/kg for two weeks prior to being placed in cages with attached running wheels, their running activity was significantly reduced ($p < 0.001$). MP dose 2.0mg/kg was the most effective dose in reducing the running of SHR.

In preliminary experiments, using an *in vitro* superfusion technique, Glutamate (1mM) and potassium (25mM) were used to evoke release of [^{3}H]dopamine ([^{3}H]DA) from nucleus accumbens shell and core of four- to six-week old SHR and WKY. No significant difference was observed between the two strains using either glutamate or K^+ stimulation. Core [^{3}H]DA release was significantly greater than shell release for both strains in response to both excitatory glutamate stimulation and depolarisation by K^+.

The current study confirmed previously reported results that preadolescent SHR (aged between four and six weeks) released significantly more glutamate-stimulated [^{3}H]norepinephrine ([^{3}H]NE) from prefrontal cortex slices than WKY ($p < 0.05$). Reproducing previous findings was of importance to demonstrate reproducibility of results and mastery of the technique.

SHR and WKY were pre-treated with 2.0mg/kg methylphenidate orally for two weeks from postnatal day 21. When glutamate (100μM) was used to evoke [^{3}H]NE release from prefrontal cortex slices of these rats at five weeks of age, no significant difference in [3H]NE was observed between MP-treated and vehicle-treated SHR. MP-treated WKY released significantly more [^{3}H]NE than vehicle-treated WKY.

SHR and WKY pre-treated with 2.0mg/kg MP orally from postnatal days 21 to 35 were challenged with 10mg/kg or 20mg/kg cocaine at postnatal day 60. Using a place preference apparatus, both vehicle-treated and MP-treated SHR conditioned to 10mg/kg cocaine for two days did not show an increased preference for the cocaine-paired compartment. On the contrary, both MP and vehicle-treated WKY showed increased preference for the cocaine-paired compartment. A higher dose of cocaine, 20mg/kg produced a significant conditioned place preference in vehicle-treated SHR, WKY and MP-treated WKY but did not produce the same effect on MP-treated SHR.

Conclusions: SHR run more than WKY in freely moving running wheels demonstrating their hyperactivity.

MP reduces voluntary running of SHR in freely moving running wheels showing that MP has ability to reduce hyperactivity in SHR.

Glutamate and potassium stimulated release of [^{3}H]DA from nucleus accumbens core is more than shell for both SHR and WKY, suggesting

possible structural and/or functional heterogeneity between NAC core and NAC shell in these rats

Glutamate stimulated release of [^{3}H]NE from PFC slices of SHR is twice greater than release from PFC slices of age-matched WKY, a possible explanation for the ADHD-like symptoms exhibited by SHR.

Challenged with 10mg/kg cocaine at postnatal day 60, SHR do not show preference for cocaine-paired environment, suggesting that cocaine may not be rewarding to them at this dose.

MP-treated WKY and vehicle-treated WKY showed increased preference for cocaine-paired environment when challenged with 10mg and 20mg/kg cocaine at postnatal day 60, demonstrating that both doses of cocaine are rewarding to them. Pre-treatment with MP did not appear to have any significant effect on promoting or reducing cocaine-induced place preference in these rats

MP-treated SHR do not show increased preference for the cocaine-paired environment when challenged with either 10mg or 20mg/kg cocaine at P60. This finding may suggest that pre-treatment with MP dose of 2.0mg/kg preadolescently had a beneficial effect in reducing the rewarding effects of cocaine in these rats

CHAPTER 1: INTRODUCTION

BACKGROUND TO THE STUDY

Attention-deficit hyperactivity disorder (ADHD) is a childhood neurobehavioral disorder and the most prevalent childhood psychiatric disorder (Castellanos and Tannock, 2002; Moll *et al.*, 2000) occurring in between 3% and 8% of school going children (Machetti *et al.*, 2001), although more permissive statistics render estimates up to 17% (Castellanos and Tannock, 2002). The disorder is characterised by poorly sustained attention, overactivity and impulsiveness (Sagvolden and Seargent, 1998). Children with ADHD have been reported to be easily distractible, show less control in withholding inappropriate responses, are restless, fidgety and display unnecessary body movements (Sagvolden *et al.*, 1998). Some of the problems with sustained attention include the dislike of and failure to attend to details (Taylor, 1998). Actometers fastened on bodies of ADHD patients, ultrasonic beams in rooms and stabilimeter chairs have all recorded high levels of interruptions, suggesting excess movements and increased motor activity (Taylor, 1998). Children with ADHD are at a higher risk of learning disabilities, social maladaptations and conduct disorder (Wilens *et al.*, 2003).

ADHD research has been hampered by confusion over nomenclature and diagnostic criteria (Castellanos and Tannock, 2002). The cardinal symptoms of ADHD are behavioural and neurobiological studies on this disorder are interpreted on the basis of the behavioural output. On this note Castellanos and Tannock, (2002) argued that though

symptom scales have been clinically useful, they still pose problems of bias and subjectivity. Compounding the problems of bias and subjectivity, Kehoe, (2001, Editorial) mentioned that there are those who believe that ADHD does not exist as a true disorder and those who on the other hand are too quick to make the diagnosis without an adequate patient workup.

Behaviour is an output from interaction of several neural circuits carrying information from different parts of the cortex and limbic structures to the striatum for integration and transmission back to the cortex through the output nuclei of basal ganglia and thalamus (Parkinson *et al.*, 2000; Lou *et al.*, 1998; Russell, 2003). These brain areas are innervated mainly by dopamine (DA) and norepinephrine (NE) neurons (Russell, 2003; Solanto, 1998). The ascending DA systems consist of nigrostriatal neurons that project from the substantia nigra to the striatum (caudate and putamen) and the mesocorticolimbic projection from the ventral tegmental area (VTA) in the midbrain to the limbic structures including the nucleus accumbens (NAC) and to the cortex including the prefrontal cortex (PFC) (Kandel *et al.*, 2000; Solanto, 1998). See figure 1.

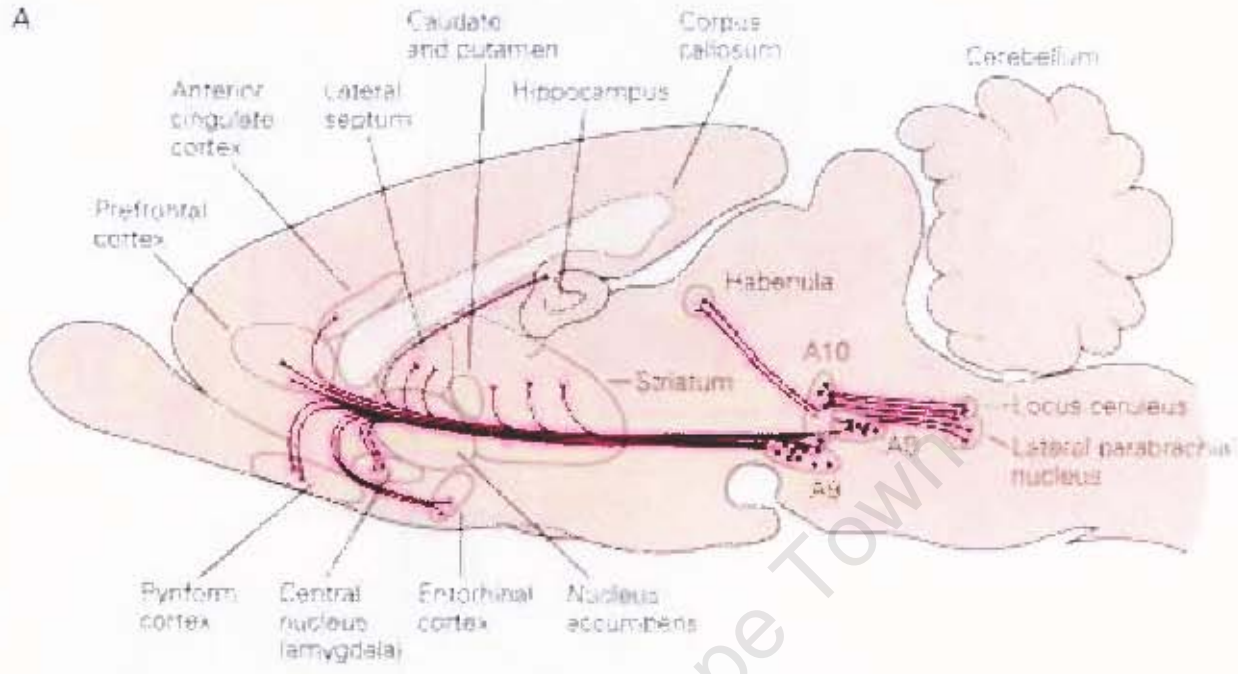


Figure 1. Schematic illustration of major DA neurons and their projections.

The NE system is more widespread than DA system and innervates more terminal areas throughout the brain. The main NE system is the dorsal noradrenergic bundle originating from the locus coeruleus (LC). It projects rostrally to the medial forebrain bundle (MFB) and limbic system (Solanto, 1998). NE projections are denser in the PFC and cingulate gyrus (Solanto 1998). See Figure 2.

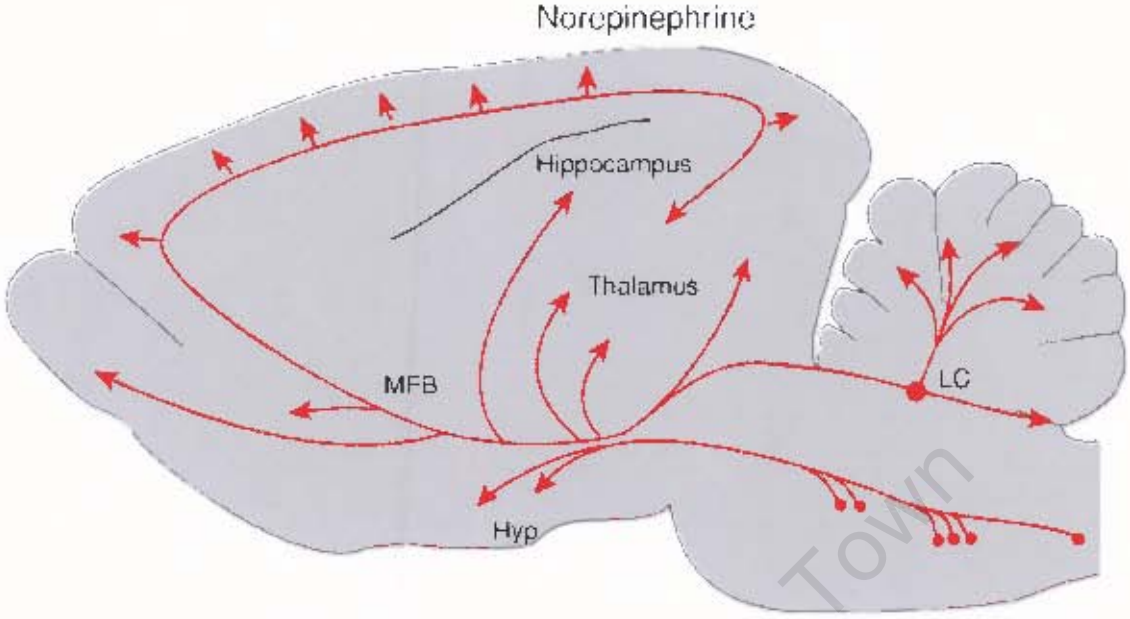


Figure 2. Schematic diagram of the major NE neurons and their projections.

Legend:

Hyp hypothalamus

MFB medial forebrain bundle

FC frontal cortex

Diagram adopted from Zigmond *et al.*, (1999) (Page 208)

The PFC receives DA and NE projections from the cell bodies in the brain stem. Both DA and NE are important in the functioning of the PFC (Arnstern, 1998; Solanto, 1998). The PFC is important in cognition, learning (Kandel *et al.*, 2000; Arnsten, 1998; Martini, 1998) and abstract intellectual functions such as predicting future consequences of events (Martini, 1998), inhibition of inappropriate responses and execution of organised behaviour (Arnsten, 1998). Arnsten, (1998) reports that lesions to the PFC have been

behaviour (Arnsten, 1998). Arnsten, (1998) reports that lesions to the PFC have been shown to result in impaired ability to sustain attention, and impaired ability to inhibit processing of irrelevant information. On the same note, depletion of DA and NE by the infusion of the neurotoxin 6-hydroxydopamine (6-OHDA), which targets mainly catecholamine terminals, resulted in impaired spatial working-memory task to the same level as lesions to the PFC, emphasizing the importance of both catecholamines and PFC to attentional process (Arnsten, 1998). See illustrations of projections DA and NE neurons in figure 1 and Figure 2.

Reviewing the neurophysiology of NE and DA, Solanto, (1998) reported that neurons from LC of a rat demonstrated selective response to a novel stimulus, which diminished with repetition of the stimulus. NE neurons also enhance signal-to-noise ratio helping to filter out irrelevant stimuli (Arnsten, 1998; Solanto, 1998). Low levels of NE (and low activity of LC neurons) have been shown to result in decreased vigilance and drowsiness (Arnsten, 1998; Solanto, 1998). Increased firing rate of LC neurons and consequently high levels of NE have been shown to result in impaired vigilance, hyperarousal, agitated behaviour, higher frequency of false alarms and increased response of LC neurons to distracters in monkeys (Anston-Jones *et al.*, 1991; Rajkowski *et al.*, 1994; Rajkowski *et al.*, 1997 in Solanto, 1998; Anston-Jones *et al.*, 1994), consequently the animal becomes more alert to environmental cues and disruptive stimuli (Arnsten, 1998). Both reviews by Arnstern and Solanto agree that high as well as low levels of NE impair PFC function and attentional regulation. DA enhances PFC working-memory function through D-1 receptor

stimulation while NE enhances PFC function through post-synaptic α-2 receptor stimulation (Arnsten, 1998).

In view of the contribution and influence of the catecholamines, NE and DA to the proper functioning of the PFC and their importance to attentional process there is a general consensus that ADHD symptoms are a result of dysfunction of the PFC (Arnsten, 1998). Poor sustained attention and distractibility in rat models of the disorder have been linked to increased NE release from PFC of these rats (Russell and Wiggins, 2000; Russell *et al.*, 2000).

Dopamine neurons fire in response to stimuli associated with reward and reinforcement of appropriate behaviour and regulating motor output and tonic readiness to respond (Arnsten, 1998; Hyman and Malenka, 2001; Solanto, 1998). As with NE, insufficient or excess of DA can result in ADHD-like symptoms (Arnsten, 1998). It is believed that the somewhat "off the mark" behaviour of the children with ADHD is a result of deficiency in DA-mediated reinforcement mechanisms (Sagvolden *et al.*, 1998).

Mesolimbic DA neurons in the ventral tegmental area of the midbrain that project to the NAC and other forebrain areas such as the dorsal striatum play an essential role in the reinforcement of appropriate behaviour (Kandel *et al.*, 2000; Arnsten, 1998; Hyman and Malenka, 2001) and reward (Kandel *et al.*, 2000, Arnsten, 1998; Spanagel and Weiss, 1999; Hyman and Malenka, 2001). These neurons are also targets for addictive drugs (Spanagel and Weiss, 1999; Hyman and Malenka, 2001).

The NAC receives inputs from the cortex, hippocampus and amygdala and sends its outputs to the pallidum and substantia nigra and is postulated to be an interface between the limbic and motor areas of the brain (Kandel *et al.*, 2000; Mongenson *et al.*, 1980; Russell, 2000). Addictive drugs increase levels of DA in the NAC synaptic cleft and actions that lead to this increase of DA in the brain's reward circuit tend to be repeated (Arnsten, 1998; Hyman and Malenka, 2001).

The NAC is divided into two functionally heterogeneous structures, the shell and the core (Jongen-relo *et al.*, 2002). The shell has strong connections to the limbic system (Kandel *et al.*, 2000) and is associated more with motivational behaviours (Jongen-relo *et al.*, 2002; Kandel *et al.*, 2000). The core region has been referred to as the "motor sector of the NAC" because of its close involvement with and resemblance to the dorsal striatum (Jongen-relo *et al.*, 2002). Core-lesioned animals have demonstrated increased locomotor activity relative to shell-lesioned animals, suggesting impairment in motor functions (Jongen-relo *et al.*, 2002).

The most effective and most frequently prescribed treatment for ADHD is methylphenidate (MP) (Volkow *et al.*, 2002; Sproson, *et al.*, 2001; Solanto, 1998). Treatment with MP has shown improvement in both the cognitive and behavioural aspects for most children with ADHD (Volkow *et al.*, 2002; Barkely *et al.*, 2003; Sproson, *et al.*, 2001; Solanto, 1998). In a review of the neuropsychopharmarcological effects of stimulant drugs on ADHD, Solanto (1998) integrates findings from studies which report improved performances in accuracy on choice reaction time, increased

index of discrimination between target and non-target stimuli, focused attention, increased selective attention, and sustained attention in continuous performance test. The improvement in working-memory is reportedly long-term. Kramer *et al.*, (2001) found that MP enhanced the ability to rapidly and accurately coordinate performance of multiple tasks, enabling rapid and accurate switching between tasks and to selectively focus attention on the currently relevant response set.

Treatment of childhood ADHD symptoms with MP is done over a number of years and may extend into adolescence (Solanto, 1998). Despite this chronic administration of the drug, Solanto, (1998), states that there are no reports of development of drug tolerance or adverse withdrawal effects.

The reported benefits of treating children with ADHD with MP, a psychostimulant, have not done much to alleviate public concerns about the drug possibly predisposing children to drug and substance abuse later in life (Barkely *et al.*, 2003). Some of the concerns stem from the fact that MP is a stimulant like cocaine and may possess the same addictive potentials as cocaine (Barkely *et al.*, 2003). Addiction may result from repetitive drug taking. Hyman and Malenka (2001) define addiction as "a persistent state in which compulsive drug use escapes control, even when serious negative consequences ensue." It is from this understanding that concerns about repetitive use of psychostimulants to treat ADHD arise. Addictive drugs are rewarding and reinforcing i.e. the brain interprets their effects as positive and behaviour associated with them tends to be repeated (Hyman and Malenka, 2001).

Psychostimulants such as methylphenidate and cocaine act by blocking the dopamine transporter (DAT) (Spanagel and Weiss, 1999). This inhibits reuptake and raises levels of dopamine (DA) in the areas innervated by DA neurons such as the PFC and striatum [caudate putamen and NAC]; prolonging the time DA remains in the synaptic cleft and extracellullar fluid (Sproson, *et al.*, 2001; Kandel *et al.*, 2000; Keating *et al.*, 2001; Spanagel and Wiss, 1999; Solanto, 1998). They also block norepinephrine (NE) re-uptake (Sproson, *et al.*, 2001; Kandel *et al.*, 2000; Keating *et al.*, 2001; Volkow *et al.*, 2001). Unlike cocaine however, MP has little affinity for the serotonin transporter (Izenwasser *et al.*, 1999; Pierce and Kalvis 1997). MP also enters and clears from the brain more slowly than cocaine (Barkely *et al.*, 2003) and may be less addictive.

MP and D-amphetamine (D-AMP), another psychostimulant used in the treatment of ADHD, do not directly stimulate catecholaminergic receptors but facilitate the action of both NE and DA (Solanto, 1998). In addition to reuptake inhibition and facilitation of catecholamine release, these drugs also inhibit the catabolic action of monoamine oxidase (MAO) (Solanto, 1998). Lacroix and Ferron, (1988) reported that MP produced a reduction in firing rate of LC that was dose-dependent. Chronic administration of MP has reportedly resulted in down-regulation of α-1 and α-2 norepinephrine receptors (Mathieu *et al.*, (1989) in Solanto, 1998). Ilgin *et al.*, (2001) reported that non-drug treated children with ADHD had higher D-2 receptor availability in the striatum which is down-regulated to near normal values following MP therapy. Some evidence suggests possible involvement of serotonin in the therapeutic effficacy of stimulants in the treatment of ADHD but MP at therapeutically relevant doses have produced no increase in

extracellular concentrations of this transmitter (Kuczenski and Segal 2001) suggesting that this psychostimulant may not be acting on serotonin transporter.

Repeated psychostimulant use may lead to subsequent sensitisation in rats (Kuczenski and Segal, 2001; Brandon *et al.*, 2001; Meririnne *et al.*, 2001) and humans (Robinson and Berridge, 2000). It is important to determine whether sensitisation develops to the dose of MP administered to the spontaneously hypertensive rats (SHR) and the control Wistar Kyoto (WKY) as sensitisation is believed to play a role in the development of drug-craving and drug abuse in rats (Brandon *et al.*, 2001) and humans (Robinson and Berridge, 2000). Evidence from literature suggests that exposure to MP (0.31, 0.62, 1.25, 5 or 20 mg/kg) causes sensitisation to the drug in adult male Han:Wistar rats (Meririnne *et al.*, 2001) and repeated low dose methylphenidate (2 mg/kg intraperitoneal) increased susceptibility to cocaine self-administration in adult rats exposed to MP during adolescence (Brandon *et al.*, 2001). However, there is evidence that early exposure (pre-adolescence) to MP (2.0mg/kg) in rats causes decreased responsiveness to the rewarding effects of cocaine and increased aversion to the drug in adulthood (Anderson *et al.*, 2002).

There is no common ground for evidence from laboratory experiments on whether MP predisposes its users to substance abuse disorders. As Gerasimov *et al.*, (2000) pointed out; interpretation of preclinical data done with MP is complicated not only by interspecies differences but also by route of drug administration. ADHD children take MP orally but many researchers give the drug intravenously or intraperinoteally. Taken

orally, MP is absorbed slowly through the gastrointestinal tract and is metabolised to a greater extent to ritalinic acid, a substance that has negligible psychostimulant properties (Gerasimov *et al.*, 2000). Other factors making preclinical data on MP difficult to interpret include doses used in studies, time of administration of the drug, age at which administration of the drug starts, experimental animals used and experimental techniques involved (Volkow and Insel, 2003, Editorial; Kuczenski and Segal, 2001). As Volkow and Insel, (2003), commented in their editorial, some researchers use very high doses which have questionable relevance to clinical treatment. Administration of the drug to the rats during the light phase of their activity cycle (period of general inactivity or sleep) has been equated to treating children at night which may not be equivalent to treating children during the day. The relevance of treating animals at adulthood when human patients are mainly preadolescents and the use of healthy animals to study human disorders has also been questioned. Use of animal models that mimic the clinical symptoms have been recommended by the authors in the editorial. Many of the points argued here have been considered in the current study.

Drugs (especially drugs of abuse) can stimulate the brain reward system stronger than and more reliably than any natural stimulus, consolidating responses to drug-associated stimuli (Hyman and Malenka, 2001). Several studies have exploited this effect of drugs on the brain and developed place preference apparatus. The place preference apparatus has been used to study the response of laboratory animals (mainly rats and mice) to addictive drugs. Animals are conditioned to a drug in a particular environment and to vehicle or placebo in another. When in a drug-free state it has been shown that animals will prefer an environment previously associated with rewarding effects of a drug to the

one where they experienced neutral or aversive effects (Carlezon, 2003). The phenomenon is known as conditioned place preference (CPP).The strength of the CPP paradigm rests in this ability of drugs to stimulate the reward system of the brain especially within a time period short enough to allow laboratory manipulations.

The spontaneously hypertensive rats (SHR) are a widely used animal model for the study of ADHD because their behaviour mimics the major symptoms of ADHD. The SHR has been bred from the nomotensive Wistar Kyoto rat (WKY) for its hypertensive trait (Sagvolden, 2000; Hendley, 2000). However, the SHR do not only develop hypertension spontaneously but also display poorly sustained attention in behavioural tasks, are impulsive and overactive: behavioural characteristics commonly exhibited by children with ADHD (Sagvolden, 2000; Sagvolden and Seargent, 1998).

Experimenters have used the open-field test to measure locomotor activity of rats (Ferguson and Cada, 2003; Sagvolden *et al.*, 1993). SHR have been shown to be more active in open-field tests relative to WKY though not necessarily more active than other strains (Sagvolden *et al.*, 1993). Sagvolden *et al.*, (1992) demonstrated the impulsive behaviour (inability to withhold inappropriate response) behaviour of SHR in fixed interval schedule of reinforcement when SHR continued lever pressing during the extinction phase of the test. A reward was rendered to the rats if they pressed the lever when the house lights were on and no reward when the lights were off. SHR reportedly pressed the lever more than SD, WKY, Wistar and hooded rats, demonstrating both their impulsivity and problem with sustained attention (Sagvolden *et al.*, 1992). Similar

behaviour has been reported for boys with ADHD when compared to normal boys (Sagvolden *et al.*, 1998). The behaviour of SHR may be comparable (to some extent) to the behaviour of children with ADHD.

Many researchers have used the SHR model to try to explain the behaviour of ADHD children. Some of their findings include the following:

A microcomputer-assisted high-resolution image analysis showed in SHR a higher density of DA D-1 receptors and a lower density of D-3 autoreceptors in anterior forebrain of SHR compared to WKY (Sadile, 2000).

Immunocytochemistry revealed a reduced number of elements positive for Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in anterior portions of the shell of SHR when compared to WKY (Papa *et al.*, 1996); Sadile, 1996; Papa *et al.*, 1998).

Increased NE and decreased DA turnover has been reported in the frontal cortex of SHR than WKY (De Villiers *et al.*, 1995)

Sagvolden *et al.*, (1992) showed that SHR were more sensitive to immediate reinforcement and less sensitive to delayed reinforcement when compared to the behaviour of WKY in fixed-interval schedules of reinforcement of bar-presses by water.

Reports suggesting a deficiency in DA transmission in caudate-putamen, NAC and PFC of SHR have been provided by several studies (Russell, 2000; Russell *et al.*, 2000; Russell *et al.*, 1995; Russell *et al.*, 1998; Russell, 2002; Russell and Wiggins 2000). However, other researchers using other rat models for ADHD (Naples-High Excitabilty rats) have reported a hyperfunctioning mesocortical DA system (Sadile *et al.*, 2003).

Leo *et al.*, (2003) demonstrated that tyrosine hydroxylase and DAT gene were significantly and transiently reduced in SHR compared to WKY. Their results are consistent with down-regulation of DA neurotransmission occurring during postnatal development and that ADHD has a genetic component. The data in their experiment support previous studies that demonstrated altered DA metabolism and a hypodopaminergic nigrostriatal system in ADHD.

Previous studies carried by Russell *et al.*, laboratory using SHR and WKY as controls have shown K^+ and /or electrically induced release of DA from the prefrontal cortex, NAC and caudate-putamen slices of SHR to be lower than those of the control WKY rats (Russell *et al.*, 1995). Impaired autoreceptor mediated feedback inhibition of norepinephrine release in PFC of SHR has been reported relative to the control (Russell 2002). A two-fold greater glutamate stimulated release of norepinephrine from prefrontal cortex slices of SHR was observed relative to the controls (Russell and Wiggins, 2000). Lehohla *et al.*, (2001) found that basal uptake of $^{45}Ca^{2+}$ into barrel cortex of the SHR was lower than that of the controls, suggesting a disturbance in calcium metabolism in the somatosensory cortex of the SHR. More recent work in Dr Russell's lab demonstrated no

significant difference between glutamate-stimulated release of DA from NAC core of SHR and WKY but a significantly greater DA release from NAC core of SHR than NAC shell of SHR (Russell, 2003).

These studies collectively and individually provide insight into the neural disturbances of SHR and possible extrapolation to neural disturbances of ADHD. It is clear from these findings that no single factor can be singled out as the cause of ADHD-like symptoms in SHR. The current study will reinforce the existing body of knowledge about the behaviour of SHR and possibly add new insights into understanding the neurochemistry of this animal model of ADHD. Animal models of disorders offer valuable information in understanding clinical symptoms of diseases and form a basis for clinical investigations.

The aim of the study was (i) to investigate the behaviour of SHR when allowed free access to a running wheel using Wistar Kyoto rats (WKY) as controls, (ii) to establish whether methylphenidate (MP) administered orally can reduce SHR hyperactivity, (iii) to carry out preliminary experiments to repeat previously reported results on glutamate and potassium stimulated release of dopamine (DA) from nucleus accumbens (NAC) core and shell of SHR and WKY, (iv) to investigate the effect of pre-pubertal exposure to orally administered MP on norepinephrine (NE) release from prefrontal cortex (PFC) slices of five-week-old SHR and WKY and (v) to establish whether pre-adolescent exposure to MP (orally) increases susceptibility of the rats to cocaine-induced place preference in adulthood (Postnatal day 60).

CHAPTER 2:MATERIALS AND METHODS

ANIMALS

Eighty-four male SHR and seventy-two male WKY were used in this study. Sixty-one other rats (mixture of males and females, SHR and WKY) were used to characterise and test place preference apparatus prior to using it for experimantation. The rats were obtained from the University of Cape Town animal unit. The University of Cape Town animal ethics committee approved the use of these rats in this study. All the rats used in the study had free access to drinking water and commercial pellets. They were housed in a temperature-controlled room with temperatures in the range 22°C to 24°C. Except where otherwise indicated the experimental rats were housed under an 1100H to 2300H dark/light cycle (lights off from 1100H to 2300H).

2.0 STATISTICAL ANALYSIS

Data was analysed using STATISTICA 6.1 for 2004 supplied by the University of Cape Town. ANOVA was used to test the effects. Significant effects were further analysed using Post-hoc Tukey's HSD test. Results are reported as mean ± S.E.M.

2.1 COMPARISON OF VOLUNTARY RUNNING DISTANCES OF FOUR- TO SIX-WEEK OLD SHR AND WKY.

Five male SHR and six male WKY aged four to six weeks at the beginning of the experiment were used to compare voluntary running distances of SHR and WKY. The objective of the experiment was to establish whether SHR (a model for ADHD) would run more than the control WKY as they (SHR) are said to be hyperactive (Sagvolden, 2000). The rats weighed between 46g and160g at the beginning of the running experiment. The rats were housed in cages with attached free running wheels separated from the cages by trap doors. The rats had free access to the running wheels. The running wheels were freely moving permitting movement in both directions. A counter activated by a crank mechanism recorded the number of revolutions. The circumference of the wheel was 1m i.e. 1revolution was equal to 1m. The number of revolutions in a 24-hour period were converted to meters and served as a measure of the distance the rats covered per day. Readings from the counter were taken at the same time daily between 1630H and 1730H for five consecutive days. This experiment was conducted under regular light/dark cycle (lights on from 0600H and off at 1800H).

Repeated measures ANOVA was used to analyse the daily running distances of SHR and WKY. Significant effects were further analysed using Tukey's HSD test (section 3.1). Results are reported as mean ± S.E.M

2.2 EFFECT OF METHYLPHENIDATE ON VOLUNTARY RUNNING OF SHR.

To test the effect of methylphenidate on running of SHR, sixteen male SHR and four male WKY were used. On the day before MP treatment began, the animals were weaned at postnatal day 20 (P20), weighed, put into individual cages and given 0.01ml condensed milk. The rats received condensed milk twice a day to get them used to licking the condensed milk off the Petri dishes before they were introduced to the MP treatment. The 5mg MP hydrochloride tablet (Triomed (Pty) Ltd, South Africa, trade name "ritaphen 5") was dissolved in water and made up to either 5ml or 10ml to make solutions of 1mg/ml or 0.5mg/ml concentrations respectively. Making solutions of different concentrations was to enable drawing out reasonable volumes of the drug as the rats' mass increased i.e. the less concentrated solution (0.5mg/ml MP) was used when the rats were still small to avoid drawing out very small volumes of the drug and the 1mg/ml MP was used for bigger rats to avoid using large volumes. Different volumes of the 1mg/ml MP solution (0.5ml/kg, 1ml/kg and 2ml/kg) were used for MP doses of 0.5, 1.0 and 2.0mg/kg respectively while adjusted volumes the 0.5mg/ml solution (1ml/kg, 2ml/kg and 4ml/kg) were used for the same MP doses.

On experimental days (P21 to P35) the rats were given either 0.01ml condensed milk (vehicle) or MP doses 0.5, 1.0 and 2.0mg/kg in condensed milk twice a day between 1000H and 1100H followed by a second dose six hours later between 1600H and 1700H. The appropriate volume to give the 0.5, 1.0 and 2.0mg/kg was withdrawn into a 0.5ml

syringe and injected into the condensed milk in a Petri dish for the rats to voluntarily take. All the rats successfully licked their Petri dishes clean within five minutes. The four WKY only received condensed milk and not on MP treatment. Although vehicle-treated SHR served as controls for MP-treated SHR it was still of interest to see how vehicle-treated SHR would compare to vehicle-treated WKY. The rats had to be given MP during the activity cycle of their day (during the dark) so the first dose was given as close to the lights off as possible.

Every second or third day the rats were weighed so that the correct dosage per body mass (0.5, 1.0 and 2.0mg/kg) could be administered. The remaining MP solution was kept in the laboratory refrigerator. No solution was kept for more than one month although we were aware that MP is very stable under normal laboratory conditions (Connors *et al.*, 1986).

After the two-week pre-running MP treatment, the rats were transferred to the cages with running wheels. The setup was similar to the one described in the previous section (section 2.1) with the exception that the running wheels had rubber stoppers that permitted movement in only one direction. The presumption was that if the wheel ran in one direction, it would give a more accurate measure of the rat "deliberately" wanting to run as opposed to the wheel turning whatever direction the rat moved. The rats continued to receive MP treatment (or vehicle) while in the running as they did before being put into the running wheel cages for a further five days. Rats weighed between 51g and 114g at the beginning of the running experiment. Rats were in the running wheel cages for five

days with daily revolution counter readings recorded at the same time daily as described in section 2.1. Repeated measures ANOVA was used to compare the running data of the rats. Significant effects were further analysed using Tukey's HSD test (section 3.2). Results are reported as mean ± S.E.M.

2.3. GLUTAMATE-STIMULATED RELEASE OF [^{3}H]DOPAMINE FROM NUCLEUS ACCUMBENS CORE AND SHELL OF FOUR- TO SIX-WEEK-OLD SHR AND WKY

Five male SHR and five male WKY aged four to six weeks and weighing between 66g and 149g were used to determine 1mM glutamate-stimulated fractional release of [^{3}H]DA from NAC core and shell.

2.3.1 CHEMICALS AND REAGENTS

The reagents used in this study were chemically pure and of analytical grade. They were supplied by MERCK (Germany) except for: [3H]DA (PerkinElmer Life Sciences, Inc., Boston MA USA), liquid scintillator (Zinsser analytic. UK), glutamate (Sigma Chemical Co. St Louis MO USA).

Before the day of the experiment, 1litre stock solutions of the following chemicals were prepared and kept in the laboratory refrigerator: NaCl (1.18M), KCl (47mM), $MgCl_2.6H_2O$ (12mM), $NaH_2PO_4.H_2O$ (10 mM), $CaCl_2.2H_2O$ (13mM), D-Glucose (110 mM) and $NaHCO_3$ (250 mM). Solutions were discarded if not used within one month. In addition 14.7mg glutamic acid, 14mg EDTA and 10 mg ascorbic acid were pre-weighed and kept in airtight Epperndorf vials.

2.3.2 EXPERIMENTAL PROCEDURE

On the day of the superfusion experiment, at least two hours before the time of sacrifice, 1SHR and 1WKY were removed from the animal house to the laboratory. This gave the rats time to get used to their new environment and to overcome the stress of being moved before they were sacrificed.

Krebs buffer was made by mixing of 100ml of the stock solutions (section 2.3.1 above) of NaCl, KCl, NaH_2PO_4, $MgCl_2$, $NaHCO_3$ and D-Glucose. This was gassed with 95%0_2/5%CO_2 for ten minutes. EDTA (14mg) was added. The solution was gassed for a further 10 minutes before addition of 100ml of 13mM $CaCl_2$. Three hundred millilitres of water were added to make the final volume 1litre. The final concentration of the Krebs buffer was 118mM NaCl, 4.7mM KCl, 1.0mM NaH_2PO_4, 1.2mM $MgCl_2$, 25mM $NaHCO_3$, 11mM D-Glucose and 1.3 mM $CaCl_2$.

The waterbath was switched on and thermostat adjusted to keep the temperature of the waterbath at 37°C. The waterbath had a rubber tube connected to it, which circulated water through to the closed Perspex bath of *in vitro* superfusion system (University of Cape Town). A 250ml beaker was filled with Krebs buffer and suspended in the waterbath. The Krebs buffer was gassed with the 95% 0_2/5%CO_2 throughout the duration of the experiment to maintain a pH of about 7.4. Some Krebs buffer was put in the freezer to be used to cool rat brains after sacrifice.

Incubation buffer was made by dissolving 10mg ascorbic acid in 10ml Krebs buffer. This was continuously aerated and kept cool by placing it on a bed of ice.

A 10mM glutamate solution was prepared by dissolving 14.7mg glutamate in 1ml distilled water containing 2 drops of 1M NaOH. The solution made up to 100ml with Krebs buffer to give a final concentration of 1mM glutamate. The solution was poured into a beaker and suspended in the waterbath and kept aerated.

Two rows of large vials (25ml) were placed under the fraction collector (University of Cape Town) followed by seven rows of smaller (5ml) vials and aligned with needles of the superfusion chambers. These vials were used to collect the elute from the superfusion chambers.

Superfusion chambers were fitted with small cotton wool plugs, and filled with Krebs buffer.

Rats were sacrificed by cervical dislocation and/or decapitation between 1300H and 1400H one at a time. The rat brain was quickly removed from the skull and put into the ice cold buffer for 15minutes. This was to slow down enzyme activity and make the brain firm for cutting. The brain was placed on a piece of filter paper and put on the stage of a McIlwain tissue chopper (Mickel Laboratory Co. UK) set to cut 900μm thick slices and manually sliced. The nucleus accumbens was dissected out from the third to fifth slices corresponding to approximately 11.2 – 9.48 mm anterior to the interaural line (Paxinos

and Watson 1986). The NAC was identifiable ventral to the caudate putamen (see Figure1 below). The outer shell was dissected out and separated from the core. Each was individually cross chopped into 300μm X 300μm slices automatically using the tissue chopper. The chopped slices were put in a vial containing 1ml incubation buffer and incubated for ten minutes in the waterbath. The slices were aerated by placing the gas tube against the side of the vial to prevent loss of slices and the vial was occasionally swirled to mix with the gas.

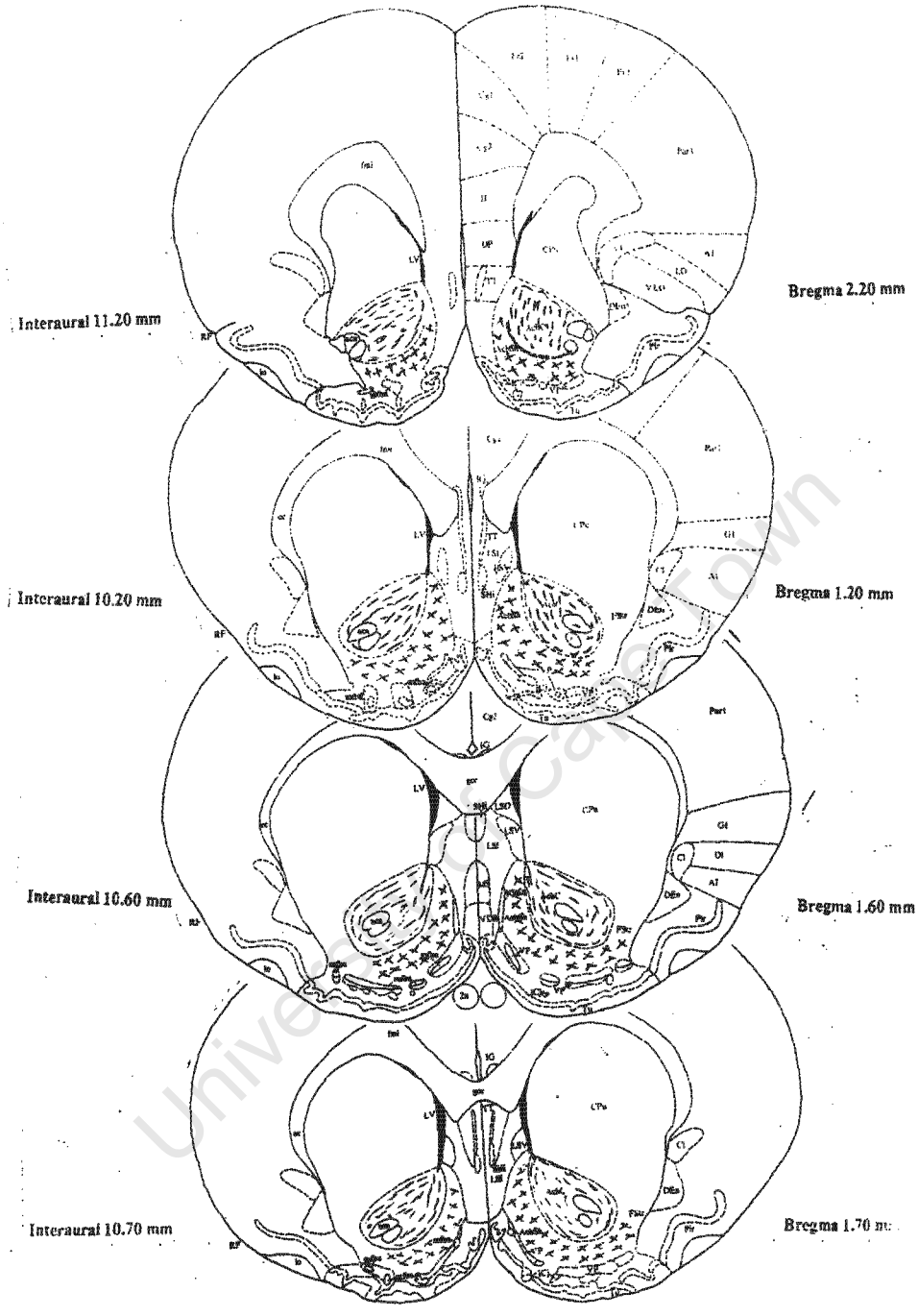


Figure1. Depiction of the coronal brains sections from which the nucleus accumbens core and shell were dissected. Used with permission from Russell *et al.*, (1996):

core shell

After ten minutes, with fluorescent lights off, 2.5µl [^{3}H]DA was pipetted into each of the vials containing incubation buffer and brain slices. The tissue was incubated for fifteen minutes with the radioactively labelled neurotransmitter to allow it to be taken up into axon terminals and label the stored DA (Russell *et al* 1996).

The incubation buffer (supernatant) was removed from the slices and discarded into the appropriate radioactive waste bin. One millilitre fresh incubation buffer was added to the slices to rinse them before being loaded into the *in vitro* superfusion chambers. The *in vitro* superfusion chamber consisted of 16 chambers (1ml plastic syringes) embedded in a perspex waterbath through which water at 37°C circulated. The chambers were sealed with stoppers (syringe plungers) pierced with stainless steel needles. When loaded, tissue slices rested on cotton wool plugs which were fitted into the chambers. Buffer flowed through the chambers via the stainless steel needles connected by plastic tubing to a multichannel peristaltic pump and buffer reservoirs suspended in a waterbath at 37°C on one side and fraction collector on the other. (Adopted from Russell *et al.*, 1996). See Appendix D1 for photograph of superfusion apparatus.

Using a 1ml Gilson pipette, slices were loaded into the superfusion columns. Each brain area of each rat was loaded into two columns.

The pre-warmed Krebs buffer was pumped from the beaker to the superfusion chambers at the rate of 0.25ml/min using a Watson Marlow peristaltic pump. The buffer flowed over the slices to wash off the excess radioactivity for eighty minutes into the large vials

in the fraction collector. The fraction collector was then engaged to collect five-minute fractions into the 5ml vials. To establish a pre-stimulation baseline, three five-minute fractions were collected. The inlet tubes of the peristaltic pump were transferred from the Krebs to the glutamate solution and slices stimulated for one minute. The inlet tubes were returned to the Krebs buffer after one minute. Post stimulation, one more five minute fraction was collected to establish a post stimulation baseline then the inlet tubes were removed from the Krebs buffer. The slices (pellet) were collected into a vial and 1ml of 0.1M NaOH added to dissolve the tissue and release the radioactivity from the slices. The slices were left in the NaOH overnight before 3.4ml liquid scintillator was added.

The radioactivity in the five-minute fractions of the chamber elute was determined using a pre-calibrated Packard 1900 CA Tri-Carb liquid scintillation analyser. The fractional release of [^{3}H]DA was calculated as the amount of radioactivity in the collected elute divided by the sum of the radioactivity in that sample and subsequent fractions including the radioactivity in the slices (Russell *et al.*, 1996). Glutamate evoked release of transmitter was calculated as the difference between the radioactivity during the 5-minute stimulation period and the pre-stimulation baseline (Russell *et al.*,1996).

Repeated measures ANOVA was used to analyse the effects. Significant effects were further analysed with post-hoc Tukey's HSD test (section 3.3). Results are reported as mean ± SEM.

2.4 POTASSIUM-STIMULATED RELEASE OF [^{3}H]DOPAMINE FROM NUCLEUS ACCUMBENS CORE AND SHELL OF FOUR- TO SIX-WEEK OLD SHR AND WKY.

Five male SHR and five male WKY aged four to six weeks and weighing between 60 and 149g were used to determine potassium stimulated release of [3H]DA from NAC core and shell.

2.4.1 CHEMICALS AND REAGENTS.

The chemicals and reagents used in this experiment were the same as those used in the glutamate stimulated release of [^{3}H]DA in the preceding section (section 2.3.1) with the exception that KCl was used in place of glutamate.

2.4.2 EXPERIMENTAL PROCEDURE.

The same experimental procedure as described in section 2.3.2 was followed with the exception that 25mM KCl solution was used to evoke release of neurotransmitter instead of glutamate.

To prepare 50ml of 25mM KCl solution for stimulation, 5ml of stock solutions of NaH_2PO_4, $MgCl_2$, $NaHCO_3$, D-glucose, 250mM KCl (not 47mM) and 4.14 ml NaCl were

mixed and gassed with 95%O_2/5%CO_2 for ten minutes. EDTA 0.7mg was added and the solution gassed for a further ten minutes. Five millilitres of $CaCl_2$ were added and the solution made up to 50ml with distilled water. The Krebs buffer for stimulation should be isomolar. The solution was suspended in the waterbath and kept aerated.

Same statistical analysis as for section 2.3.2 was used (section 3.4).

Results are reported as mean ± SEM.

2.5 GLUTAMATE-STIMULATED RELEASE OF [^{3}H]NOREPINEPHRINE FROM PREFRONTAL CORTEX SLICES OF FOUR- TO SIX-WEEK OLD SHR AND WKY.

Six male SHR and six male WKY aged four to six weeks and weighing between 60 and 161 were used to determine 100μM glutamate stimulated fractional release of [^{3}H]NE from PFC slices.

2.5.1 CHEMICALS AND REAGENTS.

The reagents used in this experiment were similar to the reagents used in section 2.3.1 with the exception that [^{3}H]NE (Amersham Pharmacia Biotech. UK) was used instead of [^{3}H]DA.

2.5.2 EXPERIMENTAL PROCEDURE.

The same experimental procedure as in section 2.3.2 was followed with the following exceptions:

PFC was obtained from the first two slices cut.

The slices were incubated with 2.68μl [^{3}H]NE (2.68μCi)

100μM glutamate was used for stimulation instead of 1mM glutamate. A lower concentration of glutamate was used for PFC because it produced sufficient stimulation. For dopamine release 1mM glutamate was the lowest concentration that evoked sufficient

stimulation as previously established in the laboratory. The rest of the procedure was unchanged.

A 1mM glutamate solution was prepared by dissolving 14.7mg glutamate in 10ml distilled water containing 2 drops of 1M NaOH. One millilitre of this solution was added to 99ml Krebs buffer to give a final concentration of 100μM glutamate. The solution was poured into a beaker and suspended in the waterbath and kept aerated.

One-way ANOVA was used to compare the results (section 3.5). Results are reported as mean ± SEM.

2.6 EFFECT OF TWO-WEEK TREATMENT WITH METHYLPHENIDATE ON [^{3}H] NOREPINEPHRINE RELEASE FROM PREFONTAL CORTEX SLICES OF FIVE-WEEK-OLD SHR AND WKY.

Twenty-day old male SHR and male WKY were obtained from University of Cape Town animal unit. The animals were treated with 2.0mg/kg MP or vehicle from P21 for 14 days as described in section 2.2. At P35 (five weeks of age) the animals weighed between 80 to 100g. At P21 they weighed between 20 and 40g. The animals were not given any MP on the day of experiment but were all given condensed milk (vehicle).

2.6.1 CHEMICALS AND REAGENTS.

The chemicals and reagents used for this experiment were the same as those used in section 2.5.1.

2.6.2 EXPERIMENTAL PROCEDURE.

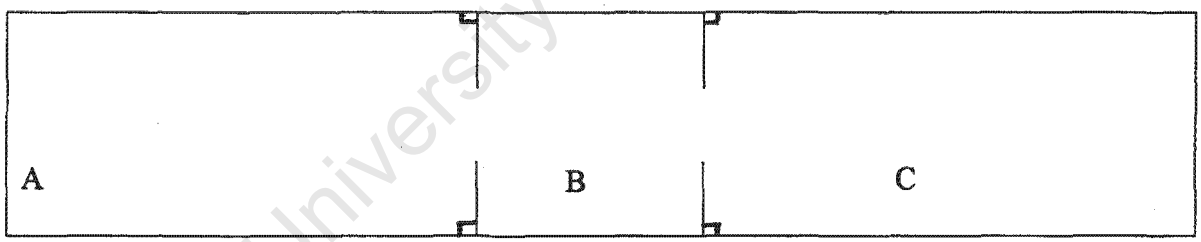
The experimental procedure used in section 2.5.2 was followed. However, four rats were sacrificed per experiment: 1SHR and 1WKY treated with 2mg/kg MP, 1SHR and 1WKY vehicle-treated. The PFC slices from each rat were loaded into two columns of the superfusion chamber.

Factorial ANOVA was used to analyse the results (section 3.6). Significant effects were further analysed with post-hoc Tukey's HSD test. Results are reported as mean ± SEM.

2.7 ACCLIMATISING PLACE PREFERENCE CAGES (PRELIMINARY TESTING).

2.7.1 MATERIALS.

Place preference apparatus consisted of two identical cuboidal polythene containers measuring 58cm X 33cm X 45cm. Each container was divided into three compartments, two larger compartments separated by a middle smaller one. The middle compartment had two trap doors that led to each of the two larger side compartments. The two larger compartments measured 24cm X 33cm X 45cm. The middle compartment measured 10cm X 33cm X 45cm. Adjustable lights were fixed to the lids of each compartment. See sketch below. Diagram not to scale



Key:

A Black larger chamber with sieve floor and brighter lights.

B Grey small middle chamber with smooth Perspex floor and brightest lights.

C White larger chamber with metal grid floor and comparatively dim lights.

□ Infrared detector

Following a similar protocol to Carlezon, (2003), the compartments differed in terms of wall colour, floor texture and light intensity. One of the larger compartments was painted black and the other white. The middle compartment was painted grey. The white compartment had dim light (because the walls were more reflective); the black (less reflective) had slightly brighter light than the white and the lights were brightest in the grey middle compartment (to discourage rats from spending too much time in it). The white compartment was fitted with removable metal grid floor, the black with metal sieve (tighter mesh) floor while the grey had smooth Perspex floor.

Infrared beams were used to detect movement across the compartments. Beams were positioned close to the entrance of each compartment hence when the rat crossed the beam from the neutral middle chamber to the side chambers, it triggered the counter to record the time spent there. Crossing over from the side chambers to the middle stopped the counter. The time that a rat spent in a given chamber was recorded as the cumulative sum of all the times that it spent in the particular compartment in the thirty-minute (1800s) screening sessions. The middle chamber acted as a bridge between the black and the white compartment and therefore the time rats spent in it was not recorded because it was not associated with any drug treatment.

2.7.2 EXPERIMENTAL PROCEDURE.

To set up the cages 48 rats were used. The rats were a mixture of both males and females and SHR and WKY but had not been in the place preference apparatus before. Without

metal floors, i.e. when both floors were smooth, the rats showed a preference for the black compartment even though its lights were slightly brighter than the white one. The sieve floor was put in the white compartment and the grid in the black. There was a large preference for the black chamber. When the floors were swopped (without tampering with the lights) the preference shifted to the white chamber. The less preferred sieve floor was put in the initially preferred black compartment. Further adjustments to curb bias were done by increasing or decreasing light intensity in the compartments.

After characterising the apparatus, thirteen rats were used to test the two cages for balance. Rats were individually placed in the middle chamber with both trap doors open and they were allowed to freely explore the entire cage for thirty minutes. The cumulative time that each rat spent in each of the two large chambers was recorded and used to establish whether one chamber was preferred over the other.

Repeated measures ANOVA was used to test the effects (section 3.7). Results are reported as mean ± SEM.

No further adjustment to the cages was done. All experiments were done under these settings.

It was observed during these screening sessions that WKY spent comparatively more (though not significant) time in one compartment than the other but their preference was

not limited to a particular compartment, i.e. some spent more time in the black while others spent more time in the white.

2.8 EFFECT OF TWO-WEEK TREATMENT WITH METHYLPHENIDATE ON COCAINE-INDUCED CONDTIONED PLACE PREFERENCE OF SIXTY-DAY-OLD SHR AND WKY.

Twelve male SHR and twelve male WKY were used to determine the response of sixty-day-old rats to a challenge of 10mg/kg cocaine.

2.8.1 MATERIALS.

The place preference apparatus described in section 2.7.1 were used for this experiment.

2.8.2 EXPERIMENTAL PROCEDURE.

SHR and WKY were obtained from University of Cape Town animal unit when they were twenty days old. They were either MP treated or treated with vehicle (condensed milk) as described in section 2.2. The animals received no further treatment after P35. The rats were left in the 1100H to 2300H dark cycle until P45. This was to allow them to overcome the stress of being discontinued from MP (or condensed milk) before they were moved to a different light/dark cycle. After P45 the rats were moved to the regular 12-hour light/dark cycle (lights on from 0600H to 1800H). The animals were left in this light/dark cycle until P60 when the place preference experiments resumed.

The place preference experiment had to be done during the light phase of the animals' light/dark cycle. According to Carlezon, (2003) when lights are off in the experiment room during the light phase of the animals' activity cycle, exploratory behaviour is maximised. Entry to the experimental room during experimental sessions was minimised. Lights in the room were always switched off as soon as the animal was put into the place preference cage.

The conditioning place preference experiment was a four-day protocol. On day one (P60), a naive experimental animal was weighed, brought to the experimental room and placed in the middle compartment of the place preference apparatus with both trap doors open and allowed to freely explore the apparatus for thirty minutes (1800s). The time that it spent in each compartment was recorded. The animal was returned to the animal room after the thirty-minute session. This was the preconditioning screening session. The preconditioning screening sessions were conducted between 0900H and 1300H.

To minimise animal to animal cues and reduce odours, the entire apparatus was cleaned with ethanol after each animal had been in the apparatus. The lid was left open to allow the alcohol to evaporate before putting the next animal in the apparatus.

A solution of 20mg/ml cocaine hydrochloride was made and kept in the laboratory refrigerator (for no longer than five days). According to the mass of the animals, the appropriate volume would be drawn on day two to give the rats 20mg/kg. The rats were

given an equivalent volume when they were saline injected. The rats weighed between 200g and 260g.

On day two, the animal was given an intraperitoneal (IP) saline injection and confined to the compartment that it showed 'preference' for in the preconditioning screening session for one hour. The animal was returned to the animal room immediately after the saline conditioning was over. All animals always received saline injection in their preferred compartment.

Four and half hours later, the animal was brought to the experiment room, given an IP injection of 10mg/kg cocaine hydrochloride and confined to the 'less preferred' compartment for one hour. The animal was returned to the rat room as soon as the hour was over. This marked the end of the first conditioning session. All animals received cocaine injection in the less preferred compartment, drug side (DS) minus saline side (SS) is always negative (see Table 3.8a and Table 3.8b).

On the third day, the procedure for day two was repeated. It was ensured that each rat had treatment (saline or cocaine) at exactly the same time as the previous day. This marked the end of the second conditioning session.

On the fourth day of the experiment, the animal was brought to the experimental room and placed in the middle compartment of the place preference apparatus (in a drug free state) and allowed to freely explore the entire apparatus as was done on the first day. The

time that the rat spent in each compartment was recorded. This was the post-conditioning screening time.

Repeated measures ANOVA was used to test the effects (section 3.8.1). Significant effects were analysed with post-hoc Tukey's HSD test.

The same protocol was repeated for a different set of animals challenged with 20mg/kg cocaine hydrochloride. Repeated measures ANOVA was used to test the effects (section 3.8.2)

CHAPTER 3: RESULTS

3.1 COMPARISON OF VOLUNTARY RUNNING DISTANCES OF FOUR- TO SIX-WEEK-OLD SHR AND WKY

Five male SHR and six male WKY aged four to six weeks were used to compare voluntary running distance of SHR and WKY (section 2.1). SHR were expected to run more than WKY as they are reportedly hyperactive. Repeated measures ANOVA (Appendix 3.1) revealed a significant strain effect ($F_{(1,9)}$ = 21.18, p = 0.0013), a significant day effect ($F_{(4,36)}$ = 9.12, p = 0.000034) and a significant strain*day effect ($F_{(4,36)}$ = 6.244, p = 0.00064). Total mean daily distance run by SHR was significantly greater than distance run by WKY. Day 1 running distance for SHR was significantly less than other days ($p < 0.05$). SHR ran significantly greater distances per day than WKY on all days tested (Table 3.1, figure 3.1).

Table 3.1. Daily voluntary running distances of SHR and WKY.

Days (24 Hours)	Mean daily distance run (m)	
	SHR (n = 5)	WKY (n = 6)
1	4344 ± 1061*	380 ± 81
2	7700 ± 1664*	1487 ± 782
3	7994 ± 1791*	816 ± 185
4	9765 ± 2131*	828 ± 162
5	8576 ± 1878*	891 ± 140

*Significantly greater than WKY.

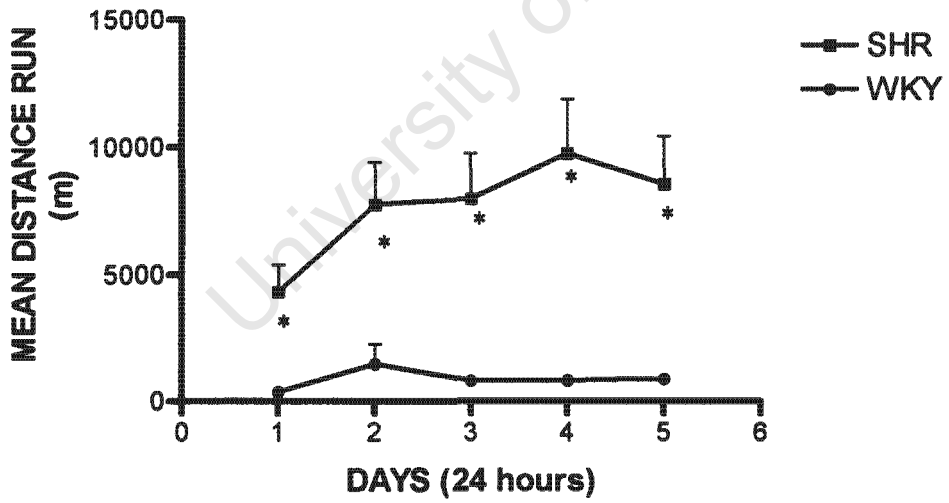


Figure 3.1. Mean daily running distances of SHR and WKY.

*Significantly greater than WKY.

3.2 EFFECT OF METHYLPHENIDATE ON VOLUNTARY RUNNING OF FIVE-WEEK-OLD SHR.

Sixteen male SHR and four male WKY aged five weeks at the beginning of the running experiment were used to test the effect of oral doses of methylphenidate (0.5, 1.0, and 2.0mg/kg) on the voluntary running of SHR (section 2.2). Four SHR were assigned to each of the treatment doses, four SHR were used to compare running of SHR to vehicle-treated WKY. MP is the most frequently prescribed drug for ADHD which is known to reduce hyperactivity in patients. The aim of the study was to test whether MP can reduce voluntary running of SHR. It was expected that MP would reduce the running of SHR.

Repeated measures ANOVA (Appendix 3.2) showed that MP significantly reduced the running of SHR. Untreated SHR ran significantly more than the control WKY ($p < 0.001$) and treated SHR. MP doses of 0.5, 1.0, 2.0mg/kg significantly reduced the running of SHR. The 2.0mg/kg MP-treated SHR ran significantly less than 0.5mg/kg and 1.0mg/kg MP-treated SHR. No significant difference was observed between the 2.0mg/kg MP-treated SHR and vehicle-treated WKY (Table 3.2, Figure 3.2).

Table 3.2. Daily voluntary running distances of vehicle-treated SHR, WKY and MP-treated SHR.

Days (24Hours)	**Distance run in 24 hours (m)**				
	Vehicle SHR (n=4)	**Vehicle WKY (n=4)**	**0.5mg/kg MP-SHR (n=4)**	**1.0mg/kg MP-SHR (n=4)**	**2.0mg/kg MP-SHR (n=4)**
1	3505 ± 415*	1089 ± 420	1528 ± 99	2339 ± 311	803 ± 211
2	3024 ± 614*	1062 ± 408	1186 ± 345	2040 ± 349	948 ± 175
3	3481 ± 796	1031 ± 370	1565 ± 764	1981 ± 507	1138 ± 120
4	3842 ± 238*	1230 ± 114	1918 ± 97	2344 ± 225	1669 ± 723
5	3785 ± 304**	1340 ± 222	1841 ± 317	2065 ± 354	1524 ± 403

*Significantly greater than 0.5mg, 2.0mg MP-treated SHR and vehicle-treated WKY.

**Significantly greater than all MP-treated SHR and vehicle-treated WKY.

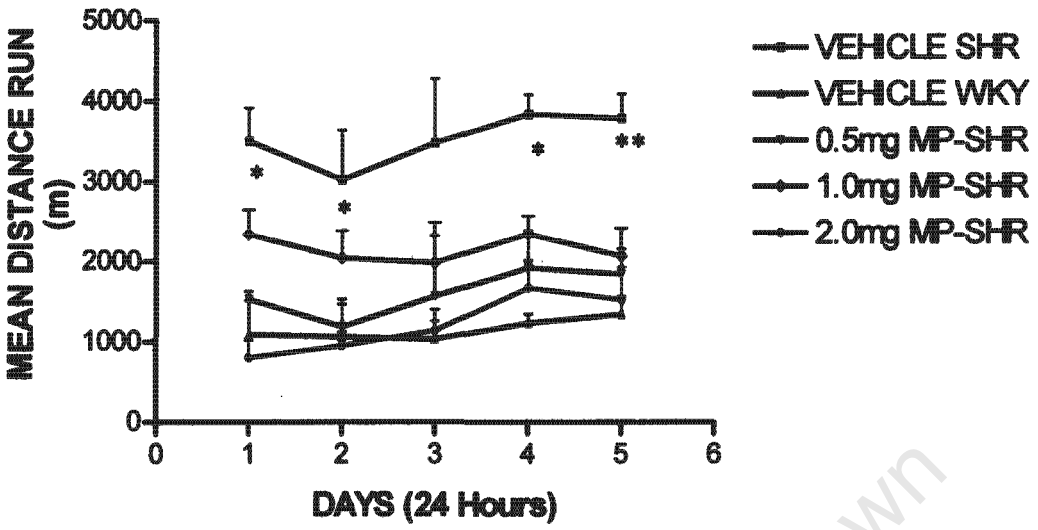


Figure 3.2. Running distances of vehicle-treated SHR, WKY and Methylphenidate-treated SHR.

* Significantly different from 0.5mg MP-SHR, 2.0mg MP-SHR and vehicle-treated WKY.

**Significantly different from all MP-treated SHR and vehicle-treated WKY.

3.3 GLUTAMATE-STIMULATED RELEASE OF [^{3}H]DOPAMINE FROM NUCLEUS ACCUMBENS CORE AND SHELL OF FOUR- TO SIX-WEEK-OLD SHR AND WKY.

Five male SHR and five male WKY were used to determine 1mM glutamate-stimulated fractional release of [^{3}H]DA from NAC core and shell of four- to six-week-old SHR and WKY (Section 2.3). A repeated measures ANOVA (Appendix 3.3) revealed a significant effect of brain area ($F_{(1,8)} = 40.8$, $p = 0.00021$) but no strain effect ($F_{(1,8)} = 0.7034$, $p = 0.429$). There was no significant area*strain effect ($F_{(1,8)} = 0.127$, $p = 0.730$). A concentration of 1mM glutamate evoked a significantly greater fractional release of [^{3}H] DA from NAC core than from NAC shell of both SHR ($p < 0.01$) and WKY ($p < 0.05$). The NAC core fractional release was twice that of the shell for both strains. The NAC core fractional release of SHR was not significantly different from the NAC core fractional release of WKY, neither was the SHR NAC shell significantly different from WKY NAC shell (Table 3.3, Figure 3.3).

Table 3.3. 1mM Glutamate-stimulated fractional release of [^{3}H] DA from NAC core and shell.

Area	Fractional release above pre-stimulation baseline	
	SHR (n=5)	**WKY (n=5)**
Core	0.0030 ± 0.00041*	0.0025 ± 0.00035**
Shell	0.0015 ± 0.00039	0.0012 ± 0.00033

*Significantly greater than SHR shell ($p < 0.01$)

**Significantly greater than WKY shell ($p = 0.01$)

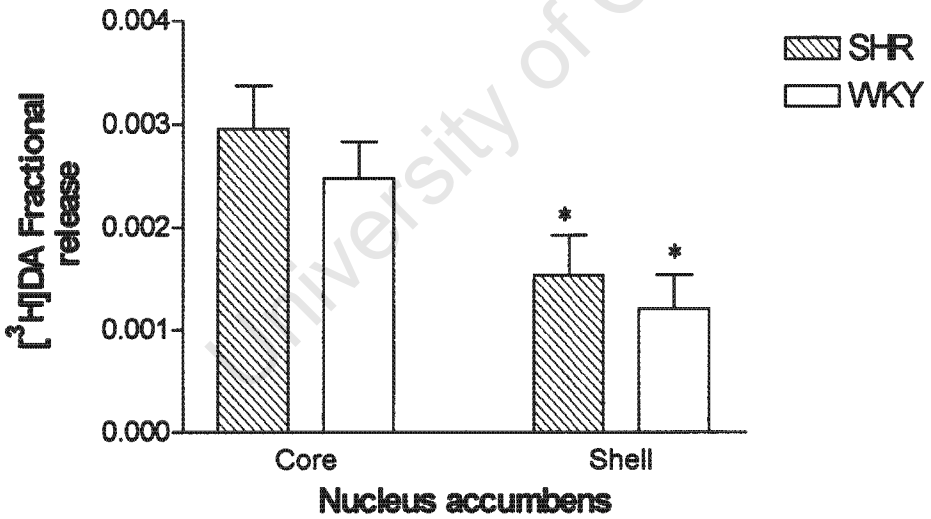


Figure 3.3. Glutamate-stimulated release of DA from NAC core and shell.

*Significantly less than corresponding core value.

3.4 POTASSIUM-STIMULATED RELEASE OF [^{3}H] DOPAMINE FROM NUCLEUS ACCUMBENS CORE AND SHELL OF FOUR- TO SIX-WEEK-OLD SHR AND WKY.

Five male SHR and five male WKY aged four to six weeks were used to determine 25mM K^+-stimulated fractional release of [^{3}H]DA from the NAC core and shell (section 2.4). Similar to glutamate-stimulated fractional release, a repeated measures ANOVA (Appendix 3.4) showed a significant effect of brain area ($F_{(1,8)} = 56.7$, $p = 0.000067$) but no strain effect ($F_{(1,8)} = 0.249$, $p = 0.879$). There was no significant area*strain effect ($F_{(1,8)} = 0.940$, $p = 0.767$). Depolarisation by 25mM K^+ produced a significantly greater fractional release of [^{3}H]DA from the core than from the shell of both SHR ($p < 0.005$) and WKY ($p < 0.005$). Neither the core of SHR nor shell was significantly different from the corresponding area in WKY (Table 3.4, Figure 3.4).

Table 3.4. 25 mM K^+-stimulated fractional release of [^{3}H] DA from NAC core and shell of SHR and WKY.

Area	**Fractional release above pre-stimulation baseline.**	
	SHR (n=5)	**WKY (n=5)**
Core	0.075 ± 0.0059*	0.075 ± 0.0055**
Shell	0.052 ± 0.0049	0.050 ± 0.0054

*Significantly greater than SHR shell ($p = 0.004$).

**Significantly greater than WKY shell ($p = 0.003$)

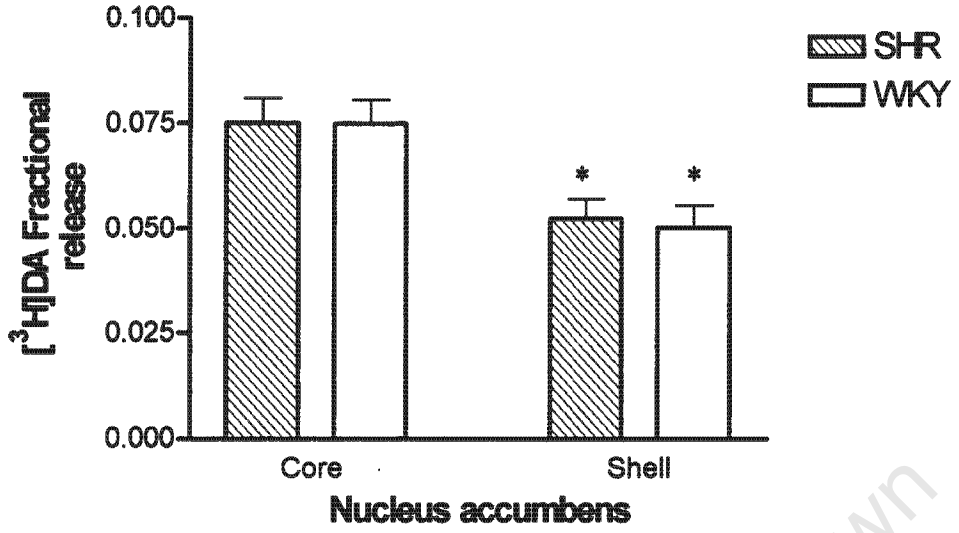


Figure 3.4. K^+-stimulated release of [^{3}H]DA from NAC core and shell.

*Significantly less than corresponding core value.

Since no significant difference was established between SHR and WKY, DA release was not investigated further.

3.5 GLUTAMATE-STIMULATED RELEASE OF [^{3}H]NOREPINPHRINE FROM PREFRONTAL CORTEX SLICES OF FOUR- TO SIX-WEEK-OLD SHR AND WKY.

Six male SHR and six male WKY aged four to six weeks were used to determine 100μM glutamate-stimulated fractional release of [^{3}H]NE from prefrontal cortex slices (section 2.5). One-way ANOVA (Appendix 3.5) of the results showed a significant strain effect ($F_{(1,10)} = 6.18$, $p = 0.032$). The SHR had a significantly greater glutamate-stimulated fractional release of [^{3}H]NE from their prefrontal cortex slices than WKY ($p < 0.05$). The glutamate-stimulated fractional release of [^{3}H]NE from SHR prefrontal cortex slices was twice that from WKY (Figure 3.5).

Table 3.5. 100μM Glutamate-stimulated fractional release of [^{3}H]NE from PFC slices of untreated SHR and WKY.

Fractional release above pre-stimulation baseline	
SHR (n=5)	**WKY (n=5)**
0.0022 ± 0.0005*	0.0009 ± 0.0003

*Significantly greater than WKY NE release.

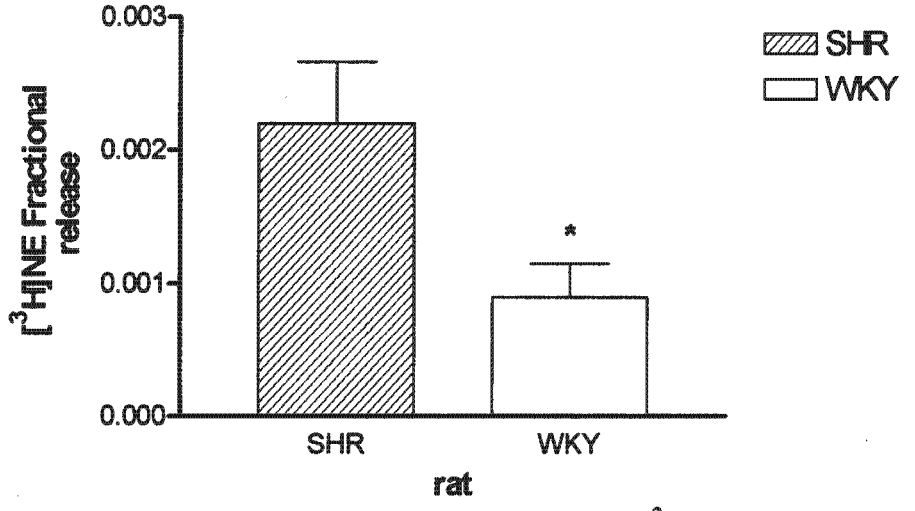


Figure 3.5. Glutamate-stimulated release of [^{3}H]NE from PFC slices of untreated SHR and WKY.

*Significantly less than SHR.

3.6. EFFECT OF TWO-WEEK TREATMENT WITH METHYLPHENIDATE ON [^{3}H] NOREPINEPHRINE RELEASE FROM PREFRONTAL CORTEX SLICES OF FIVE-WEEK-OLD SHR AND WKY.

Sixteen male SHR and sixteen male WKY were used to determine the effect of two-week treatment with MP on 100μM glutamate-stimulated fractional release of [^{3}H]NE from PFC slices of five-week old SHR and WKY (section 2.6). Factorial ANOVA (Appendix 3.6) of the results revealed a significant strain*treatment effect ($F_{(1,28)}$ = 13.154, p = 0.005). There was no strain or treatment effect, ($F_{(1,28)}$ = 0.36, p = 0.554 and $F_{(1,28)}$ = 2.95, p = 0.097 respectively). MP-treated WKY released significantly more [^{3}H] NE than vehicle-treated WKY ($p < 0.005$) whereas MP- treated SHR fractional release was not significantly different from vehicle-treated SHR. There was no significant difference between MP- treated SHR and MP-treated WKY [^{3}H]NE fractional release. Vehicle-treated SHR [^{3}H] NE fractional release was significantly more than vehicle-treated WKY ($p < 0.05$). (Table 3.6, Figure 3.6)

Table 3.6. 100μM Glutamate-stimulated fractional release of [^{3}H] NE from PFC slices of five-week old MP- treated and vehicle-treated SHR and WKY.

Fractional release above pre-stimulation baseline			
MP WKY (n=8)	**VEHICLE WKY (n=8)**	**MP SHR (n=8)**	**VEHICLE SHR (n=8)**
0.0016 ± 0.00028 *	0.00063 ± 0.00005	0.0011 ± 0.00011	0.0014 ± 0.00021

* Significantly greater than vehicle WKY.

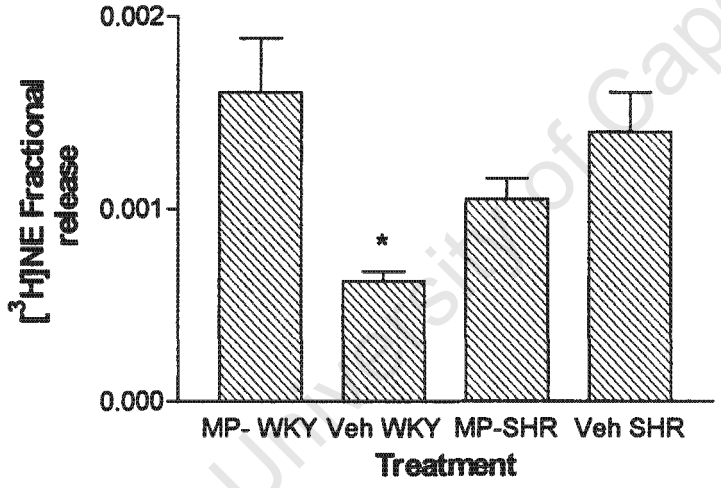


Figure 3.6. Fractional release of [^{3}H]NE from PFC slices of MP-treated and vehicle-treated WKY and SHR.

*Significantly less than MP-treated WKY and SHR and vehicle-treated SHR.

3.7. ACCLIMATISING PLACE PREFERENCE CAGES (PRELIMINARY TESTING)

Thirteen rats (males and females) were used to test the two cages that were to be used for conditioned place preference. This was to determine whether there was any significant prior preference for either of the two larger compartments of the cages (black versus white compartment), (section 2.7). Repeated measures ANOVA (Appendix 3.7) of the data revealed no significant cage effect, ($F_{(1,11)} = 0.064$, $p = 0.80$), no colour effect, ($F_{(1,11)} = 0.592$, $p = 0.46$) and no colour*cage effect ($F_{(1,11)} = 0.799$, $p = 0.39$). There was no significant difference between the times spent in either of the two larger compartments of the two cages prior to conditioning. (Table 3.7, figure 3.7).

Table 3.7. Time spent by rats in black and white compartments of conditioning place preference cages.

CAGE	Time spent in compartments (seconds)	
	BLACK	WHITE
1 (n=6)	788 ± 125	765 ± 117
2 (n=13)	639 ± 117	905 ± 141

No significant time difference between black and white compartments.

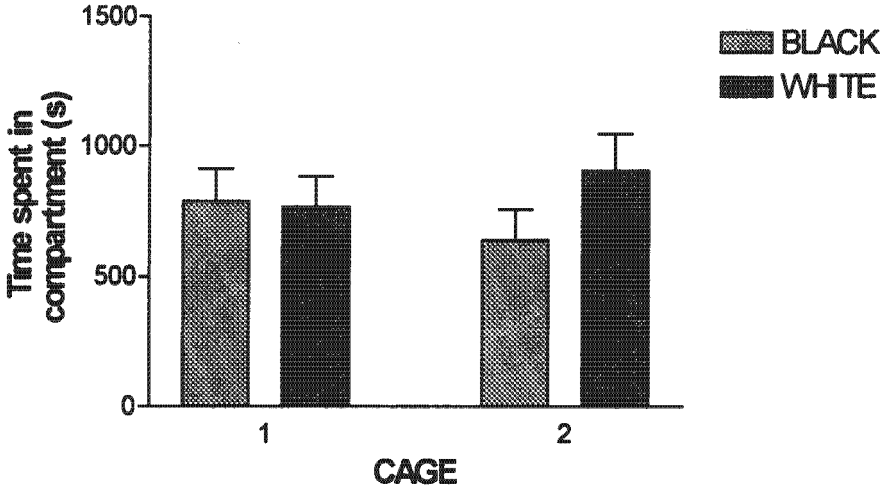


Figure 3.7. Time spent by rats in black and white compartments of conditioning place preference cages.

3.8 EFFECT OF TWO-WEEK TREATMENT WITH METHYLPHENIDATE ON COCAINE-INDUCED CONDITIONED PLACE PREFERENCE OF SIXTY-DAY-OLD SHR AND WKY.

3.8.1 RESPONSE TO 10mg/kg COCAINE CHALLENGE.

Twelve male SHR and twelve male WKY were used to determine the response of SHR and WKY to 10mg/kg cocaine (drug) challenge at P60 after 14 days treatment with 2mg/kg MP from P21 to P35 (section 2.8). A repeated measures ANOVA (Appendix 3.8.1) of the time differences between the drug-paired and saline-paired compartments before and after conditioning with cocaine, revealed no strain effect ($F_{(1,20)} = 0.145$, $p = 0.71$), no treatment effect ($F_{(1,20)} = 3.05$, $p = 0.96$), and no strain*treatment effect ($F_{(1,20)} = 0.54$, $p = 0.47$). There was a significant cocaine, and cocaine*strain effect ($F_{(1,20)} = 36.36$, $p = 0.000007$) and ($F_{(1,20)} = 10.48$, $p = 0.0041$) respectively. No significant cocaine*treatment or cocaine*strain*treatment was observed ($F_{(1,20)} = 1.30$, $p = 0.27$, $F_{(1,20)} = 1.10$, $p = 0.31$ respectively). Post conditioning with 10mg/kg cocaine, there was a significant increase in the time difference between the drug-paired and saline-paired compartments for MP-treated WKY ($p < 0.0005$) and vehicle-treated WKY ($p < 0.05$). No significant difference between the pre-conditioning and post-conditioning time difference was observed for both vehicle- treated and MP-treated SHR (Table 3.8a, Figure 3.8a)

Table 3.8a. Time difference between drug-paired and saline-paired compartments in conditioned place preference before and after conditioning with 10mg/kg cocaine.

Rat and Treatment	PRE-CONDITIONING TIME (s)			POST-CONDITIONING TIME (s)		
	Drug Side (DS)	Saline Side (SS)	DS - SS	Drug Side (DS)	Saline Side (SS)	DS - SS
MP-SHR	652	802	-150	818	450	368
MP-SHR	694	710	-16	860	377	483
MP-SHR	623	769	-146	345	718	-373
MP-SHR	687	708	-21	1016	380	636
MP-SHR	619	815	-196	595	855	-260
MP-SHR	576	724	-148	753	532	221
MP-WKY	585	926	-341	1158	372	786
MP-WKY	490	1024	-534	1046	500	546
MP-WKY	750	784	-34	1498	155	1343
MP-WKY	463	1168	-705	1150	321	829
MP-WKY	428	1130	-702	434	1095	-661
MP-WKY	860	866	-6	1800	0	1800
VEH-SHR	520	981	-461	584	650	-66
VEH-SHR	529	816	-287	626	797	-171
VEH-SHR	723	899	-176	830	530	300
VEH-SHR	617	840	-223	508	714	-206
VEH-SHR	675	710	-35	774	650	124
VEH-SHR	510	825	-315	766	601	165
VEH-WKY	614	897	-283	557	1018	-461
VEH-WKY	609	938	-329	1180	349	831
VEH-WKY	266	1211	-945	568	924	-356
VEH-WKY	286	1360	-1074	1050	362	688
VEH-WKY	505	900	-395	602	603	-1
VEH-WKY	657	760	-103	876	405	471

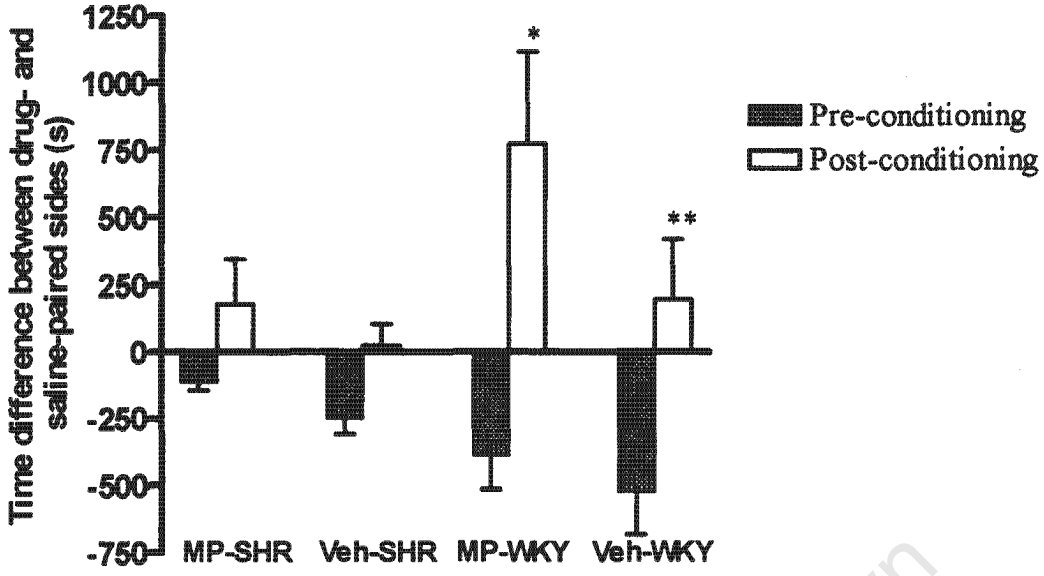


Figure 3.8a. Comparison of time differences between drug-paired and saline paired compartments in a 10mg/kg cocaine-induced place preference.

*Significantly different from MP-treated WKY pre-conditioning time difference.

**Significantly different from vehicle-treated WKY pre-conditioning time difference.

3.8.2 RESPONSE TO 20mg/kg COCAINE CHALLENGE.

Twelve male SHR and twelve male WKY were used to determine the effect of 20mg/kg cocaine challenge at P60, following treatment with 2mg/kg MP from P21 to P35 (Section 2.8). A repeated measures ANOVA (Appendix 3.8.2) of the time differences between the drug-paired and saline-paired compartments before and after conditioning with cocaine, revealed no strain effect ($F_{(1,20)} = 1.57$, $p = 0.23$), no treatment effect ($F_{(1,20)} = 0.83$, $p = 0.37$), and no strain*treatment effect ($F_{(1,20)} = 1.06$, $p = 0.32$). There was a significant cocaine effect ($F_{(1,20)} = 73.78$, $p = 0.0$), cocaine*strain effect ($F_{(1,20)} = 14.67$, $p = 0.0010$) and cocaine*treatment effect ($F_{(1,20)} = 10.02$, $p = 0.0048$). No cocaine*strain*treatment effect was observed ($F_{(1,20)} = 0.057$, $p = 0.81$). Post conditioning with 20mg/kg cocaine, vehicle-treated SHR ($p < 0.05$), vehicle-treated WKY ($p < 0.0005$) and MP-treated WKY ($p < 0.005$), showed a significant increase in the time spent in the drug-paired compartment as evidenced by the increased time difference between the drug-paired and saline-paired compartments. No significant change in the pre-conditioning and post-conditioning time difference was observed for MP-treated SHR (Table 3.8a, Figure 3.8a).

Table 3.8b. Time difference between drug-paired and saline-paired compartments in conditioned place preference before and after conditioning with 20mg/kg cocaine.

Rat and Treatment	PRE-CONDITIONING TIME (s)			POST-CONDITIONING TIME (s)		
	Drug Side (DS)	Saline Side (SS)	DS - SS	Drug Side (DS)	Saline Side (SS)	DS - SS
MP-SHR	680	688	-8	607	630	-23
MP-SHR	602	927	-325	438	899	-461
MP-SHR	524	1090	-566	649	900	-251
MP-SHR	437	1148	-711	368	1090	-722
MP-SHR	381	1130	-749	560	670	-110
MP-SHR	645	788	-143	639	648	-9
MP-WKY	640	986	-346	1080	407	673
MP-WKY	358	1270	-912	465	1080	-615
MP-WKY	483	1100	-617	560	912	-352
MP-WKY	281	1300	-1019	1432	236	1196
MP-WKY	559	953	-394	1222	318	904
MP-WKY	507	892	-385	1332	318	1014
VEH-SHR	317	1348	-1031	736	402	334
VEH-SHR	634	921	-287	720	455	265
VEH-SHR	409	1102	-693	1130	318	812
VEH-SHR	277	1245	-968	1214	307	907
VEH-SHR	670	803	-133	600	789	-189
VEH-SHR	543	890	-347	644	648	-4
VEH-WKY	186	1580	-1394	1010	639	371
VEH-WKY	392	1110	-718	1190	305	885
VEH-WKY	323	1333	-1010	1326	293	1033
VEH-WKY	316	1264	-948	1280	249	1031
VEH-WKY	341	1261	-920	1133	438	695
VEH-WKY	360	1128	-768	1095	371	724

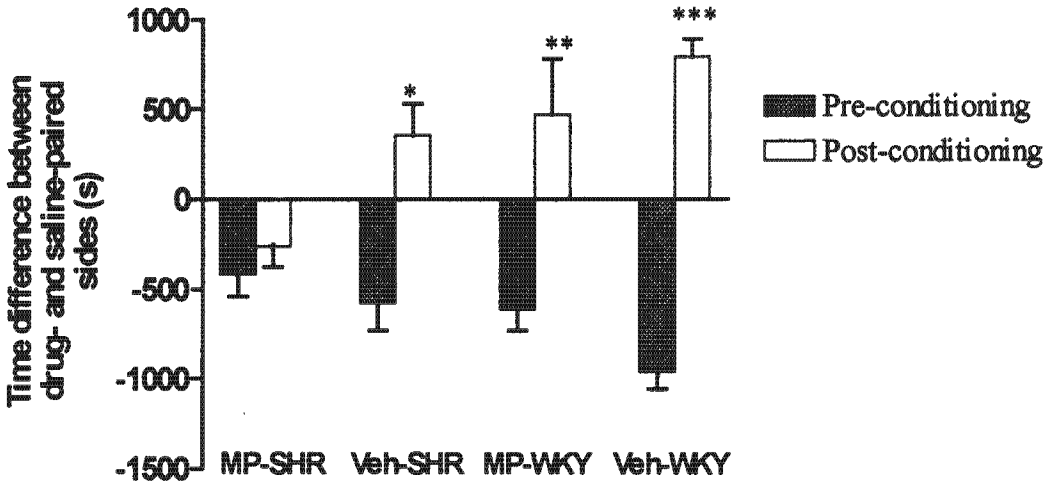


Figure 3.8b. Comprarison of time difference between drug-paired and saline-paired compartments in a conditioned place preference induced by 20mg/kg cocaine.

*Significantly different from vehicle-treated SHR pre-conditioning time difference.

**Significantly different from MP-treated WKY pre-conditioning time difference.

*** Significantly different from vehicle-treated WKY pre-conditioning time difference.

CHAPTER 4: DISCUSSION

The preliminary running data show that SHR run significantly greater distances per day than WKY. Day 1 running distance for SHR was significantly different from other days. With the exception of Day 1 distance for SHR daily running distance was not significantly different within strain.

SHR are reported to be hyperactive under a variety of behavioural paradigms (Sagvolden 2000). The results of this preliminary running experiment show that SHR ran significantly greater distances per day than WKY which may serve as an indicator of their hyperactivity. However, Sherwin, (1998) cautions that wheel running may not be an accurate measure of animal activity because it is not analogous with any naturally occurring behaviour. It is still feasible here to propose that SHR were hyperactive relative to the controls because given the chance to display the same "unnatural" behaviour, they ran more than WKY.

When comparing SHR to other rat strains, Sagvolden *et al.*, (1993) found that SHR were more active than WKY in 7.5-minute free-exploration open-field test though less active than Wistar and Sprague-Dawley (SD) rats. In 7.5-minute forced exploration open-field test, the SHR was still more active than WKY but less active than Wistar and SD rats. Although our experiment did not compare SHR to Wistar and SD rats, it agrees with the finding that SHR are more active than WKY. Ferguson and Cada, (2003) also reported

more SHR activity (than WKY and SD) in Residential Figure-Eight Maze activity both short-term (12 minutes) and long-term (14 days).

Contrary to our findings, Ferguson and Cada, (2003) did not find SHR more active than WKY in the running wheel though they were more active than SD. Their results indicate that in the first 10 minutes into the running wheel WKY were more active than SHR. They reported no significant difference between the times the SHR and WKY spent in the running wheel during the 1-hour baseline sessions they performed prior to injecting rats with drugs. No significant differences between SHR and WKY were reported for long-term running wheel activity either. Making this data more difficult to compare with ours is the fact that differences that were observed in 10-12 minute sessions all levelled out to equal activity during 1-hour baseline sessions. One wonders why baseline sessions could not be same duration as short term sessions or vice versa. Since our data does not include short term activities, it is difficult to account for the differences between their finding and ours. The authors themselves wrote "the finding that the SHR in this study were not hyperactive relative to the WKY is difficult to explain".

Tarr *et al.*, (2004) found that male Long Evans (LE) rats progressively increased their running distance during the week. The same finding is reported by Ferguson and Cada, (2003) using SHR, WKY and SD rats. Although Day 1 running distance for SHR was significantly less than the succeeding days' running, our experiment did not show significant progressive increase in the running distance covered by the rats (both SHR and WKY). Tarr *et al.*, (2004) reported greater distance on running day 7 than the first

day. Ferguson and Cada, (2003) reported the progressive increase over 14 days. Our experiment only reports 5 days of running and this may be too short a time to observe the increase. Ferguson and Cada, (2003) reported that SHR displayed initial hypoactivity in the running wheels and increased their activity within 24 hours of unlimited access. This may explain why the first day's running distance is significantly less than succeeding days' running in our experiment. The rats may have been initially less active but may have increased their activity during the course of the day to levels which were significantly more than the WKY's running. (We have no empirical evidence or reason to suggest that the SHR may have run less than WKY at the beginning of the running experiment). Sagvolden *et al.* (1993) also reported initial hypoactivity in SHR relative to other rat strains they used. It may be this initial hypoactivity that made Day 1 running less than the succeeding days running.

Sherwin, (1998) described wheel running as "self-reinforcing and perceived by animals as 'important'." If the reinforcement is derived from increased extracellular dopamine in the NAC (Wilson and Marsden, 1995) then SHR may be expected to run more than WKY to achieve the same level of satisfaction as WKY. Tarr *et al.*, (2004) found, using an *in vitro* superfusion technique, that LE rats that ran more than others had decreased release of DA from striatum. SHR have decreased release of DA from striatum compared to WKY (Russell, 2003) and this may account for their need to run more.

When SHR had been pre-treated with MP prior to being placed in the running wheels, it was observed that the MP-treated SHR generally ran less than vehicle-treated SHR and

did not run significantly more than vehicle-treated WKY. With the exception of Day 3 running, vehicle-treated SHR ran more than vehicle-treated WKY. No significant difference in running was observed on Day 3 between all groups though vehicle-treated SHR demonstrated an insignificant tendency ($p = 0.055$) to be more active than vehicle-treated WKY.

The results of this running experiment agree with the previously reported data for untreated rats. Vehicle-treated SHR were more active in the running wheels than WKY. This was an expected outcome as SHR had demonstrated significant hyperactivity in the preliminary running experiment.

Methylphenidate treatment had a significant effect on SHR. From the first day the rats were placed in the running wheels there was a significant difference between the vehicle-treated SHR and 0.5mg and 2.0mg/kg MP-treated SHR though vehicle-treated SHR was not significantly different from 1.0mg/kg MP-treated SHR. On test days (except Day3), 0.5mg/kg and 2.0mg/kg MP-treated SHR were significantly different from vehicle-treated SHR. On Day 5 all MP-treated SHR ran significantly less than vehicle-treated SHR.

Methylphenidate is the most widely prescribed drug for treatment of ADHD known to improve both behavioural and cognitive aspects of ADHD patients (Volkow *et al.*, 2002; Barkely *et al.*, 2003; Sproson, *et al.*, 2001; Solanto, 1998). The ability of MP to reduce

the running of SHR may be taken as a positive indication of reduced hyperactivity in these rats.

Contrary to our findings, Wultz *et al.*, (1990) reported no "paradoxical" inhibition of SHR following MP. Several factors may account for the differences between these studies. The rats in the current study had been MP-treated for two weeks prior to being placed in the running wheels, rats started MP treatment at P21 and the obvious difference between a free-exploration open-field activity and a running wheel. Other difference as mentioned in chapter one relate to age of experimental animals and dose of MP used.

The results of the glutamate-stimulated release of DA from the NAC core and shell experiment show a significantly greater release of dopamine from the nucleus accumbens core than the shell. They show no strain effect.

The significant difference in the dopamine release between the NAC core and shell falls into the existing body of evidence that these two regions of the NAC are functionally different (Jongen-relo *et al.*, 2002; Zahm, 1999; Zahm and Brog, 1992; Heimer et al., 1991; Meridith *et al.*, 1993). A study by Russell, (2003) showed 1mM glutamate-stimulated release of DA from NAC core of four to six-week-old SHR to be significantly higher than the SHR shell. The DA release from SHR core was not significantly different from the WKY core release. This is in agreement with the current results. In agreement with the present results, Hedou *et al*, (1999) found a higher concentration of baseline DA in the NAC core of male Wistar rats than the shell, using dual-probe microdialysis prior

to injecting the rats with cocaine. King *et al.*, (1997), Heidbreder and Feldon, (1998) Heidbreder *et al.*, (1999) also reported higher NAC core basal concentrations of DA than the shell. Although these studies did not measure glutamate-stimulated release, they suggest that there is a difference in DA concentrations in the two sub regions of the NAC.

The current study does not show any difference between the SHR, a widely used model of ADHD, and the control WKY. The study by Russell (2003) did not find the WKY NAC core to be significantly different from the shell. There was also an insignificant tendency for the SHR shell to be lower than the WKY shell, which the current study did not reproduce. Although the two experiments used the same strains and ages of rats, more animals should have been used to establish the differences found (n=4, Russell and n=5 for the current study). Another experiment done in the same laboratory, also found no significant difference in glutamate-stimulated DA release between NAC shell and core of male adult Long Evans rats (Tarr *et al.*, 2004). The Long Evans rats in this particular study had been used for voluntary running experiment for seven days before being used for the glutamate-stimulated release of DA. The effect of age and exercise in this study may account for the lack difference. Wu *et al.*, (1999) also found no significant difference between basal DA output in NAC core and shell using dual-needle microdialysis before introduction of psychological stress. The animals were tested 24 hours after insertion of the needles. The stress of the surgical procedure may have elevated the shell DA levels as it has been established that the shell is more susceptible to stress (Barrot *et al.*, 2000) than the core. Levels of DA have been reportedly elevated in the shell in response to stress, while remaining unaltered in the core (Wu *et al.*, 1999;

Murphy *et al.*, 2000; King *et al.*, 1997). Their experiment (Wu *et al.*, 1999) did confirm that DA levels remained almost unaltered in the core after introduction of psychological stress but increased in the shell. Twenty-four hours may not have been sufficient for the shell to recover from the stress of the surgery.

Interestingly, Carboni *et al.*, (2003) found a higher extracellular DA concentration in the NAC shell of six-week-old SHR than aged-matched WKY using in vivo microdialysis. Although this may not account for the difference of this finding to others, it is worth noting that the SHR are more susceptible to stress than the WKY (Chieuh and McCarty, 1992; Carboni *et al.*, 2003) as the authors themselves acknowledged. In the light of this, the animals should have been given more than 24 hours to recover from the stress of the surgery. The increased DA in the shell of SHR may still be partly from the effect of the probe implantation.

In the absence of a common denominator to the experiments above, the current results may be interpreted in the light of the existing structural difference between the NAC core and shell. The NAC core has been reported to have a higher density of dopamine neurons than the shell (Cilax *et al.*, 1995; Meredith *et al.*, 1993) and Meredith *et al.*, (1993) reported that NAC core cells surface area available for synaptic contact is 50% greater than the surface area for shell. These differences would favour more DA release from the NAC core than shell. Berridge *et al.*, (1997) found more dopamine β-hydroxylase fibres in the shell than the core. Beta hydroxylase converts DA to NE. The presence of more β-hydroxylase fibres in the shell than the core may mean that more DA is converted to NE

in the shell than the core, result in less DA in the shell relative to the core. These findings may help explain the higher concentration of DA in the core when compared to the shell. The current study agrees with other published data that the NAC core has a higher concentration of DA than the shell and that there is no significant difference between glutamate-stimulated DA release from NAC core and shell of SHR and WKY at this age.

A study by Russell showed that K^+-induced release of DA from the NAC of twelve- to fourteen-week-old SHR was significantly lower than that of their age-matched WKY (Russell, 2000). Since the mesolimbic DA neurons in the ventral tegmental area of the midbrain that project to the NAC play an important role in reward and reinforcement of proper behaviour (Kandel *et al.*, 2000; Arnsten, 1998), this study suggested that the impairment in DA transmission of SHR could account for their behavioural disturbances. The rats used in the study by Russell were old and at this age the SHR would have already developed hypertension and brain damage. It is thus difficult to compare the results of these two studies.

The significance of a higher K^+-evoked release of DA in the NAC core than the shell may be accounted for by the differences between NAC core and shell mentioned for the glutamate-stimulated release above.

Results of 100μM glutamate stimulated fractional release of [^{3}H]NE from PFC slices of four- to six-week old SHR and WKY revealed a significant strain effect. SHR had a greater glutamate stimulated release of [^{3}H]NE than WKY. This finding had been

demonstrated earlier by Russell and Wiggins, (2000). It was necessary to repeat this finding so that results generated in the laboratory could be compared and to establish ability to reproduce earlier findings.

As previously demonstrated by Russell and Wiggins, glutamate stimulated release of NE from PFC slices of SHR was twice that of WKY. PFC is needed to facilitate planning and organised behaviour (Arnsten, 1998). PFC uses working memory to guide behaviour so that the organism may be freed from dependence on environmental cues and allows inhibition of inappropriate behaviour and distractive stimuli (Arnsten, 1998). Both DA and NE are necessary neurotransmitters in the regulation of PFC function (Arnsten, 1998). Excess or too little of both of these catecholamines may impair PFC function (Arnsten, 1998). When animals are aroused and have high NE release in the PFC they respond well to distracters and poorly to targets. The results of this experiment have shown SHR to have greater NE release from their PFC when compared to WKY. This may help explain why SHR demonstrate poor attentional processes when compared to WKY (Sagvolen *et al.*, 1993; Sagvolden, 2000). They may be more alert to environmental cues and distracters as a result of the high NE release. It is important to note that no part of the brain works in isolation so that though NE release from PFC may be cited here as a probable reason for inattention in SHR other brain circuits are involved in modulating PFC function and the attentional process.

Results from five-week-old SHR and WKY pre-treated with 2.0mg/kg MP for two weeks before sacrifice at P35 showed increased glutamate-stimulated release of [^{3}H]NE from

PFC slices of MP-treated WKY relative to vehicle-treated WKY. No significant difference was observed between vehicle-treated SHR and MP-treated SHR.

Even though 2.0mg/kg MP reduced running of SHR in experiments reported earlier, this did not appear to have altered glutamate-stimulated [^{3}H]NE release from PFC. However, the same dose significantly increased glutamate-stimulated release of [^{3}H]NE from PFC slices of WKY. Since no behavioural or any other tests were done to MP-treated WKY, the significance of the increased [^{3}H]NE may not be accurately interpreted. It may have been more informative to have treated some WKY prior to placing them in cages with attached running wheels in the running experiments reported earlier to investigate whether the increased release of [^{3}H]NE may be correlated to increased or decreased running. As the data stands we have no empirical evidence to account for the increased [^{3}H]NE release.

Prior to using the conditioned place preference apparatus for the experiment, the two cages were tested for balance. The results of the test show no significant cage effect. This suggests that the two cages are similar. There was no colour effect and no colour*cage effect. These results show that there is no prior preference for any of the two larger compartments. The times spent in the two compartments (black versus white) were not significantly different from each other for both cages. It was important to establish this since this experiment had not been done in our laboratory before. Equally important was the need to ascertain that any preference for a particular compartment after conditioning may be attributed to the treatment associated with that compartment. Since this

preliminary test showed no significant baseline preference for any compartment, the apparatus may be assumed to be unbiased.

When rats were challenged with 10mg/kg cocaine at P60 following a pre-adolescent treatment with either MP or vehicle (from P21-P35), the time differences between the cocaine-paired and saline-paired compartments before and after conditioning showed no strain effect.

The results also showed no treatment effect on the difference between time spent in the cocaine-paired and saline-paired compartments. There was no significant interaction between strain and treatment i.e. treatment did not affect SHR and WKY differently.

There were significant cocaine, and cocaine*strain effects. Post conditioning with 10mg/kg cocaine, there was a significant shift in the time that both MP-treated and vehicle-treated WKY spent in the drug-paired and the saline-paired compartments (figure 3.8a). There was a significant increase in the time spent in the drug-paired compartment.

The results also show that cocaine affected the two strains differently as evidenced by the significant cocaine*strain effect. The effect of the cocaine was only on WKY. Cocaine had no significant effect on either MP-treated or vehicle-treated SHR.

The absence of a significant cocaine*treatment or cocaine*strain*treatment interaction suggests that prior exposure to MP had no significant influence on the acquisition of

cocaine-induced conditioned place preference in SHR and WKY. The shift in place preference was a result of cocaine and can not be attributed to prior exposure to MP.

The existing body of knowledge stands divided on whether treatment (of both human and animal subjects) with MP predisposes users to increased vulnerability to drugs of abuse. Using conditioned place preference, Andersen *et al.*, (2002) and Carlezon *et al.*, (2003) reported that early exposure (pre-adolescent) to MP (2mg/kg) reduced the rewarding effects of cocaine and increased aversion to the drug in adulthood. Similarly Beiderman *et al.*, (1999) and Wilens *et al.*, (2003) also reported that administration of stimulants (therapeutic doses) such as MP to children reduces their risk of drug abuse. Conversely, Brandon *et al.*, (2001) reported enhanced self-administration of low dose cocaine (75µg/kg/infusion) in adult rats following adolescent exposure to low (2mg/kg) dose MP. Similar findings were reported by Meririnne *et al.*, (2001). They found that conditioning with 1.25 to 20mg/kg MP increased preference for the drug-paired compartment and that a dose of MP (0.31mg/kg) that did not induce place preference earlier increased preference for the drug-paired compartment following a 7-day sensitisation treatment with MP at doses of 0.62 to 20mg/kg. They concluded that rewarding properties of MP are sensitised by prior exposure to the drug. The Brandon (2001) and Meririnne (2001) findings support each other in that prior exposure to MP produces sensitisation to low typically non-rewarding doses of a drug.

The results of our current study show a difference in response to 10mg/kg cocaine challenge at adulthood between SHR and WKY. A dose of cocaine that produced

conditioned place preference in both MP-treated and vehicle-treated WKY did not produce a similar effect in the SHR. The response to 10mg/kg cocaine in this study does not indicate that prior exposure to MP predisposes rats to cocaine preference neither does it show that MP treatment increases aversion to cocaine. This may serve to highlight the need to recognise that different rat strains may respond differently to cocaine (and possibly other psychostimulants). Consistent with this finding, Yang *et al.*, (2003) demonstrated strain differences in behavioural response to chronic administration of increasing doses of methylphenidate to eight-week old Sprague-Dawley rats (SD), WKY and SHR. They showed that repeated administration of 2.5mg/kg (IP) MP evoked behavioural sensitization in SD and WKY while having no significant effect on SHR. In our study the SHR responded differently to WKY in acquisition of cocaine-induced place preference. It is a noteworthy point to realise that differences in rat strains may account for differences in findings between experimenters investigating similar matters.

In our experiment we used MP-treated rats pre-adolescently (P21 to P35) similar to Andersen *et al.*, (2002) (P20 to P35) and started behavioural testing at P60 as they did but our results did not reproduce aversion to cocaine that they reported. Although the experimental procedures differed in such aspects as the time of weaning (P20 versus P25), mode of MP administration (oral versus IP) or even the time interval between successive doses (6 hours versus 4 hours), differences between strains of rats used may posit an explanation for the difference between results of these studies. Robinson and Berridge, (2000) highlighted that there is individual variation in susceptibility to

sensitisation even in animals, with some showing robust sensitisation while others may not sensitise at all. He attributed the variation to such factors as genetics, hormones (including sex) and experience. This may offer an explanation for the difference between our results and others who used the same dose of MP and cocaine.

Psychomotor sensitisation as a result of exposure to stimulants has been correlated to brain neuroadaptations associated with increased liability to abuse, (White and Kalivas 1998).

Repeating the protocol for conditioned place preference with 20mg/kg cocaine produced no significant strain effect, treatment effect or strain*treatment interaction. There was a significant cocaine effect, cocaine*strain effect and cocaine*treatment effect. No significant cocaine*strain*treatment effect.

As with 10mg/kg cocaine-induced place preference, both MP-treated and vehicle-treated WKY showed a significant increase in the time they spent in the drug-paired compartment after conditioning with cocaine. There was a significant increase in the time spent in the drug-paired compartment. This cocaine-induced conditioned place preference by WKY may demonstrate that this dose of cocaine is rewarding but does not support the view that prior exposure to MP makes the rats more susceptible to cocaine-induced conditioned place preference since both MP-treated and vehicle-treated WKY showed a significant preference for the cocaine-paired compartment. Both doses of cocaine failed

to support any suggestion that MP pre-treated rats were more prone to cocaine-induced conditioned place preference than those which were not.

On a very interesting note, 20mg/kg cocaine induced a conditioned place preference in vehicle-treated SHR but failed to produce a similar effect in the MP-treated SHR. The difference in response to cocaine between MP-treated SHR and MP-treated WKY signify that cocaine had a different effect on the two strains and may not signify effect of MP pre-treatment. Results of SHR pretreated with MP suggest that MP prevented cocaine induced place preference in SHR.

As stated previously, assessing the behaviour of WKY post conditioning with both 10- and 20mg/kg cocaine, we have no empirical evidence to suggest that pre-treatment is either beneficial or detrimental in terms of whether it sensitises or protects against sensitisation to cocaine. The results of the 20mg/kg cocaine-induced conditioned place preference in SHR are consistent with the results of Andersen *et al.*, (2002) and Carlezon, (2003) in the experiments where they report decreased responsiveness to cocaine in SD rats exposed to MP pre-adolescently. In our experiment, a dose of (10mg/kg) cocaine produced no significant conditioned place preference in either MP-treated or vehicle-treated SHR whereas 20mg/kg cocaine produced a significant increase in the time that vehicle-treated SHR spent in the drug-paired compartment. This may suggest that SHR need relatively larger doses of cocaine to experience similar reward that WKY acquire with a smaller dose. As Robinson and Berridge, (2000) pointed out, a given dose of a drug may produce sensitisation in one animal but fail to produce a similar effect in

another animal. This may help explain why the earlier dose did not produce conditioned place preference in vehicle-treated SHR when it did in WKY. These same authors also reported that sensitisation is dose-dependent. Larger doses produce more robust sensitisation. This explanation fits the behaviour of the vehicle-treated SHR since they responded to a high dose of cocaine when a lower dose failed to produce a similar effect.

Using Swiss Webster mice, Itzhak and Martin, (2002) were able to demonstrate that 20mg/kg MP reinstated preference for a compartment that was previously cocaine-paired. They used cocaine to induce conditioned place preference and followed this with eight days saline injections in both the cocaine-paired and saline-paired compartments to produce an extinction phase. After the extinction phase, the mice were challenged with 20mg/kg MP which reproduced preference for the previously cocaine-paired compartment. Cross-sensitisation to MP may suggest that MP has the same rewarding effects as cocaine at this dose.

The reinforcing effects of cocaine are attributed to its ability to inhibit dopamine reuptake by the dopamine transporter (DAT), thereby increasing the concentration of extracellular dopamine (Sproson, *et al.*, 2001; Kandel *et al.*, 2000; Keating *et al.*, 2001; Spanagel and Wiss, 1999; Solanto, 1998). The amount of DA released is said to depend on vesicular stores and the sensitivity of presynaptic autoreceptors while the DAT is the major inhibitor of reuptake. Russell, (2000) and Russell *et al.*, (1995) found decreased potassium- and glutamate-stimulated release of DA from NAC of adult SHR when compared to age-matched WKY. DA neurons in the NAC (and other parts) are important

in expressing reinforcement and reward (Arnsten, 1998; Hyman and Malenka, 2001; Solanto, 1998). This may in part shed some light into why SHR (vehicle-treated) did not respond as WKY in the 10mg/kg cocaine conditioning. It might be feasible to assume that since their dopamine levels are low, they may require a much higher dose of cocaine to sufficiently block the DAT to raise extracellular dopamine to rewarding levels. Levels of DA are comparatively higher in WKY and extracellular DA may reach rewarding levels with a dose lower than that required for SHR. Volkow *et al.*, (2002) reported similar finding using PET in healthy human subjects. They reported that for a given dose of MP-induced DAT blockade, individual differences in response to MP were due to DA release. For the same amount of blockade, MP induced smaller changes in DA in individuals with low cell activity than those with high cell activity (Volkow *et al.*, 2002). If this explanation may be extrapolated to our finding, it may help explain why SHR (with low DA release) did not respond as WKY (with high DA release) when challenged with the same dose of cocaine, a DAT blocker.

This explanation however, fails to account for the behaviour of MP-treated SHR even after conditioning with a higher dose of cocaine. MP treatment during the preadolescent period may have induced changes in SHR that are consistent with decreased response to the rewarding effects of cocaine. The behaviour of MP-treated SHR in the conditioned place preference experiment may highlight an important consideration to be taken in the use of MP. As a model for ADHD, SHR may help to point out (with a lot of limitations) that MP may not predispose subjects with ADHD symptoms to drug abuse but its safety in 'healthy' individuals may be more elusive. It is however, not practical to extrapolate

data from rats to human given the complexities that differentiate the two. Animal models may only be used to highlight a phenomenon to be further investigated in humans. Wilens *et al.*, (2003) and Barkley *et al.*, (2003) reported that use of stimulants to treat ADHD does not predispose children to substance abuse later in life but rather both studies reported reduced risk of substance abuse disorders. The results from MP-treated SHR may be comparable to these findings.

It is feasible to conclude from the results of this experiment that there is strain difference in response to cocaine-induced place preference. Pre-treatment with MP does not predispose rats to increased vulnerability to cocaine-induced conditioned place preference.

CHAPTER 5:SUMMARY

The current study looked at the effects of methylphenidate on a rat model for attention-deficit hyperactivity disorder. Results from the running experiments indicate that SHR ran more than WKY. These results demonstrated that SHR displayed more locomotor activity when compared to WKY. This finding supports other reports that classify SHR as behavioural models of hyperactivity, a behavioural output comparable to ADHD hyperactivity.

Methylphenidate reduced running of SHR. A dose of 2mg/kg given orally twice a day was the most effective dose in reducing the running of SHR. This finding supports reported data on the ability of MP to reduce hyperactivity in ADHD and ADHD-like symptoms.

Glutamate and potassium stimulated release of [^{3}H]DA from NAC core was significantly greater than from shell for both SHR and WKY. There was no significant difference however between corresponding NAC sub-territories of SHR and WKY.

Glutamate stimulated release of [^{3}H]NE from PFC slices of SHR was significantly greater than [^{3}H]NE release from WKY. A two-fold greater release was observed for SHR than WKY. This previously reported result in Dr Russell laboratory emphasizes the disturbances of PFC NE sytem in a rat model for ADHD and offers a possible explanation for their ADHD-like symptoms.

MP treatment did not significantly alter [^{3}H]NE release from PFC slices of SHR but significantly increased release from MP-treated WKY relative to vehicle treated WKY. Raised levels of [^{3}H]NE in MP-treated WKY may argue in favour of the much disputed "paradoxical" effect of treating "normal subjects" with psychostimulants. Our results however did not investigate any behavioural patterns in MP-treated WKY. Only SHR running was recorded in the running experiments reported in this study. It would have been more informative if the some behavioural output had been investigated to see if this reported increase in glutamate-stimulated [^{3}H]NE in WKY correlated with changes in locomotor (or other) activity.

Both vehicle-treated and MP-treated WKY showed cocaine-induced conditioned place preference when challenged with 10mg and 20mg/kg cocaine at adulthood. Only vehicle treated SHR showed preference for the cocaine-paired compartment when challenged with 20mg/kg cocaine but not 10mg/kg cocaine. The results from this experiment suggest that SHR may be less likely to be affected by reinforcing and rewarding properties of addictive drugs such as cocaine because vehicle-treated SHR did not show cocaine-induced CPP to 10mg/kg cocaine. These results also do not show any evidence to support that chronic MP-treatment predisposes users to abuse of psychostimulants. If these data could be extrapolated to clinical settings, then one would be justified in suggesting that MP may render its users less liable to drug abuse.

REFERENCES.

Anderson, S.L., Arvanitogiannis, A., Pliakas, A.M., LeBlanc, C., Carlezon, W.A. Jr. (2002) Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat. Neurosci.* 5:13-14

Arnsten, A.F.T. (1998) Catecholamine modulation of prefrontal cortical cognitive function. *Trends Cognit. Sci.* 11: 436-447

Barkley, R.A., Fischer, M., Smallish, L., Fletcher, K. (2003) Does the treatment of attention-deficit/hyperactivity disorder with stimulants contribute to drug use/abuse? A 13-year prospective study. *Pediatrics.* 111: 97-109

Barrot, M., Marinelli, M., Abrous, D.N., Rouge-Pont, F., Le Moal, M., Piazza, P.V. (2000) The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *Eur. J. Neurosci.* 12: 973-979

Beirderman, J. Wilens T, Mick E., Spencer T., Faraone, S.V. (1999) Pharmacotherapy of attention-deficit/hyperactivity disorder reduces risk for substance use disorder. *Pediatrics.* 104: e20

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

Bolaños, 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. *Biol. Psychiatry.* 54: 1317-1329

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: 651-661

Brandon, C.L., Marinelli, M., White, J.F. (2003) Adolescent exposure to methylphenidate alters the activity of rat midbrain dopamine neurons. *Biol. Psychiatry.* 54:1338-1344

Budygin, E.A., John, C.E., Mateo, Y., Jones, S.R. (2002) Lack of cocaine on dopamine clearance in the core and shell of the nucleus accumbens of dopamine transporter knock-out mice. *J. Neurosci.* 22: R222

Carboni, E., Silvagni, A., Valentini, V., Di Chiara, G. (2003) Effects of amphetamine, cocaine and depolarization by high potassium on extra dopamine in the nucleus accumbens shell of SHR rats. An in vivo microdyalisis study. *Neurosci. and Biobehav. Revs.* 27: 653-659

Carlezon, W.A. (2003) Place conditioning to study drug reward and aversion. *Methods mol .med.* 84: 243-249

Carlezon, W.A., Mague, S.D., Andersen, S.L. (2003) Enduring behavioral effects of early exposure to methylphenidate in rats. *Biol. Psychiatry*. 54: 1338-1344

Castellanos, F.X., Tannock, R. (2002) Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. *Nat. Neurosci. Revs.* 3: 617-628

Cervo, L., Samanin, R. (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Res.* 673: 242-250

Chieuh, C.C., McCarty, R. (1992) Sympatho-adrenal hypereactivity to foot-shock stress but not to cold exposure in spontnaneously hypertensive rats. *Physiol. Behav*. 52: 49-57

Cilax, B.J., Heilman, C., Demchyshyn, L.L., Pristupa, Z.B., Ince, E., Hersch, S.M., Nisnik, H.B., Levey, A.I. (1995) The Dopamine transporter: immunocytochemical characterization and localization in brain. *J. Neurosci*. 15: 1714-1723

Connors, K.A., Gordon, L.A., Valentino, J.S. (1986). Chemical stability of pharmaceuticals: A handbook for pharmacists. 2nd Edition. John Wiley and Sons. New York.

De Villiers, A.S., Russell, V.A., Sagvolden, T., Searson, A., Jaffer, A., Taljaard, J.J. (1995) Alpha 2-adrenoceptor mediated inhibition of [^{3}H]dopamine release from nucleus accumbens slices and monoamine levels in a rat model for attention-deficit hyperactivity disorder. *Neurochem. Res.* 20: 427-433

Ferguson, S.A., Cada, A.M. (2003) A longitudinal study of short- and long-term activity levels in male and female Spontaneously Hypertensive, Wistar-Kyoto, and Sprague-Dawley rats. *Behav.Neurosci.*117: 271-282

Gaytan, O., al-Rahim, S., Swann, A., Dafny, N. (1997) Sensitization to locomotor effects of methylphenidate in the rat. *Life Sci.* 61: 101-107

Gerasimov, M.R., Franceschi, M., Volkow, N.D., Gifford, A., Gatley, J.S., Marsteller, D., Molina, E.P., Dewey, L.S. (2000) Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. *J. Pharmacol. Exp.Ther.* 295: 51-57

Haber, S.N., Fudge, J.L., Mcfarland, R.N. (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J. Neurosci.* 20: 2369-2382

Hedou, G., Feldon, J., Heidbreder, C.A. (1999) Effects of cocaine on dopamine in subregions of the rat prefrontal cortex and their effects to subterritories of the nucleus accumbens. *Eur. J. Pharmacol.* 372: 143-155

Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., Wohltmann, C. (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*. 41: 89-125

Hendley, E.D. (2000) WKHA rats with genetic hyperactivity and hyperreactivity to stress: a review. *Neurosci. and Biobehav. Revs.* 24: 41-44

Homberg, J.R., Wardeh, G., Raasø, H.S., Schoffelmeer, A.N.M., de Vries, T. J. (2003) Neuroadaptive changes in mesocorticolimbic dopamine and acetylcholine neurons following cocaine or saline self-administration are dependent on pre-existing individual differences. *Neuroscience*.121: 829-836

Hyman, E.S., Malenka, C.R. (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat. Neurosci. Revs.* 2: 695-702

Ilgin, N., Senol, S., Gucuyener, K., Gokcora, N., Sener, S. (2001) Is increased D2 receptor availability associated with response to stimulant medication in ADHD? *Dev. Med. Child Neurol*. 43: 755-760

Itzhak, Y., Martin, J.L. (2002) Cocaine-induced conditioned place preference in mice: induction, extinction and reinstatement by related psychostimulants. *Neuropsychopharmacolgy*. 26: 130-134

Izenwasser, S., Coy, A.E., Ladenheim, B., Loeloff, R.J., Cadet, J.L., French. D. (1999) Chronic methylphenidate alters locomotor activity and dopamine transporters differently from cocaine. *Eur. J. Pharmacol.* 373: 187-193

Jacobs, G.H., Fenwick, J.A., Williams, G.A. (2001) Cone-based vision of rats for ultraviolet and visible light. *J. Exp. Biol.* 204: 2439-2446

Jongen-relo, A.L., Kaufmann, S., Feldon, J. (2002) A differential involvement of the shell and core subterritories of the nucleus accumbens of rats in attentional processes. *Neuroscience*. 111: 95-109

Kandel, E.R., Schwartz, J.H., Jessell, T.M. (2000) Principles of neural science, 4th Edition. International Edition: McGraw Hill.

Keating, G.M., McClellan, K., Jarvis, B. (2001) Methylphenidate (OROS formulation). *CNS Drugs*. 15: 495-500

Kehoe, W.A. (2001) Treatment of attention deficit hyperactivity disorder in children. *Ann Pharmacother*. 35: 1130-1134

King, D., Zigmond, M.J., Finlay, J.M. (1997) Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increase in extracellular dopamine in the nucleus accumbens core and shell. *Neuroscience*. 77: 141-153

Kramer, A.F., Cepeda, N.J., Cepeda, M.L. (2001) Methylphenidate effects on task-switching performance in attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry.* 40: 1277-1284

Krause, K-H., Dresel, S.H., Krause, J., la Fougere, C., Ackenheil, M. (2003) The dopamine transporter and neuroimaging in attention deficit hyperactivity disorder. *Neurosci. and Biobehav. Revs.* 27: 605-613

Kuczenski, R., Segal, D.S. (2001) Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. *J. Pharmacol. Exp.Ther.* 296: 876-883

Lacroix, D., Ferron, A. (1988) Electrophysiological effects of methylphenidate on the coeruleo-cortical noradrenergic system in the rat. *Eur. J. Pharmacol.* 149: 277-285

Lehohla, M., Russell, V., Kellaway, L. (2001) NMDA-stimulated Ca^{2+} uptake into barrel cortex slices of spontaneously hypertensive rats. *Metab Brain Dis.* 16: 135-143

Leo, D., Sorrentino, E., Volpicelli, F., Eyman, M., Greco, D., Viggiano, D., di Porzio, U., Perrone-Capano, C. (2003) Altered midbrain dopaminergic neurotransmission during development in an animal model of ADHD. *Neurosci. and Biobehav. Revs.*27: 661-669

Machetti, A., Magar, R., Lau, H., Murphy, E.I., Jensen, P.S., Conners, C.K., Findling, R., Wineburg, E., Carotenuto, I., Einarson, T.R., Iskedjian, M. (2001) Phamacotherapies for attention-deficit/hyperactivity disorder: expected cost analysis. *Clin Ther.* 23: 1904-1921

Madras, B.K., Miller, M.G., Fischman, A.J. (2002) The dopamine transporter: relevance to attention deficit hyperactivity disorder (ADHD). *Behav. Brain Res.* 130: 57-63

Marinelli, M., White, J.F. (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *J. Neurosci.* 20: 8867-8885

Martini, F.H., (1998) Fundamentals of anatomy and physiology, 4th edition, Prentice Hall Int, Inc.: UK.

Mathieu, J.F., Ferron, A., Dewar, K.M. (1989) Acute and chronic effects of methylphenidate on cortical adrenoceptors in the rat. *Eur. J. Pharmacol.* 162: 173-178

Meredith, G.E, Pennartz, C.M.A., Groenewegen, H.J. (1993) The cellular framework for chemical signaling in the nucleus accumbens. *Prog. Brain Res.* 99

Meririnne, E., Kankaanpää, A., Seppälä, T. (2001) Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and effects of D1- and D2-receptor antagonists. *J. Pharmacol. Exp.Ther.* 298: 539-550

Mogenson, G.J., Jones, D.L., Yim, C.Y. (1980) From motivation to action; functional interface between the limbic system and the motor system. *Prog. Neurobiol.*14: 69-97

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. *J. Child Adolesc. Psychopharmacol.* 11: 15-24

Moll, G.H., Heinrich, H., Trott, G.E., Wirth, S., Rothenberger, A. (2000) Deficient intracortical inhibition in drug-naïve children with attention-deficit hyperactivity disorder is enhanced by methylphenidate. *Neurosci. Lett.* 284: 121-125

Murphy, C.A., Pezze, M., Feldon, J., Heidbreder, C. (2000) Differential involvement of dopamine in the shell and core of the nucleus accumbens in the expression of latent inhibition to an aversively conditioned stimulus. *Neuroscience*. 99: 469-477

Papa, M., Diewald, L., Carey, M.P., Espisito, F.J., Gironi Carnevale, U.A., Sadile, A.G. (2002) A rosto-caudal dissociation in the dorsal and ventral striatum of the juvenile SHR suggests an anterior hypo- and a posterior hyperfunctioning mesocorticolimbic system. *Behav. Brain Res.* 130: 171-179

Papa, M., Sagvolden, T., Seargent, J.A., Sadile, A.G. (1996) Reduced CaMKII-positive neurones in the accumbens shell of an animal model of attention-deficit hyperactivity disorder. *Neuroreport*. 7: 3017-3020

Papa, M., Sergeant, J.A., Sadile, A.G. (1998) Reduced transduction mechanisms in the anterior accumbal interface of an animal model of attention-deficit hyperactivity disorder. *Behav. Brain Res.* 94: 187-195

Paxinos, G., Watson, C. (1986) The rat brain in stereotaxic coordinates. Academic Press. New York

Paxinos,G., Watson, C. (1986) The rat brain in stereotaxic co-ordinates. 2nd Edition. New York.

Penner, M.R., Mcfayden, M.P., Carrey, N., Brown, R.E. (2001) Effects of chronic and acute methylphedinate hydrochloride (Ritalin) administration on locomotor activity, ultrasonic vocalizations, and neuromotor development in 3- to 11-day-old CD-1 mouse pups. *Dev. Psychobiol.* 39: 216-228

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

Robinson, T.E., Berridge, K.C. (2000) Animal models in craving research: The psychology and neurobiology of addiction: an incentive – sensitisation view. *Addiction.* 95: S91- S117

Russell, V. (2003) Dopamine hypofunction possibly results from a deficit in glutamate-stimulated release of dopamine in the nucleus accumbens shell of a rat model for attention deficit hyperactivity disorder – the spontaneously hypertensive rat. *Neourosci. and Biobehav. Revs.* 27: 671-682

Russell, V., Allie, S., Wiggins, T. (2000) Increased noradrenaline activity in prefrontal cortex slices of an animal model for attention-deficit hyperactivity disorder – the spontaneously hypertensive rat. *Behav. Brain Res.* 117: 69-74

Russell, V., de Villiers, A., Sagvolden, T., Lamm, M., Taljaard, J.F.J. (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. *Brain Res.* 676: 343-351

Russell, V.A. (2000) The nucleus accumbens motor limbic interface of the spontaneously hypertensive rat as studied in vitro by the superfusion slice technique. *Neurosci. and Biobehav. Revs.*24: 133-136

Russell, V.A. (2001) Increased AMPA receptor function in slices containing the prefrontal cortex of spontaneously hypertensive rats. *Metab Brain Dis.* 16: 145-151

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

Russell, V.A. (2003) In vitro glutamate-stimulated release of dopamine from nucleus accumbens core and shell of spontaneously hypertensive rats. *Metab Brain Dis.* 18: 161-168

Russell, V.A., Lamm, C.L.M., de Villiers, A.S., Reyneke, L., Taljaard, J.F.J. (1996) [^{3}H]Dopamine and [^{14}C]acetylcholine release from rat prefrontal cortex, nucleus accumbens and caudate-putamen slices. *Neurosci. protocols.* 60: 1-18

Russell, V.A., Wiggins, T.M. (2000) Increased glutamate-stimulated norepinephrine release from prefontal cortex slices of spontaneously hypertensive rats. *Metab Brain Dis.* 15: 297-304

Sadile, A.G. (2000) Multiple evidence of a segmental defect in the anterior forebrain of an animal model of hyperactivity and attention deficit. *Neurosci. and Biobehav. Revs.* 24: 161-169

Sagvolden, T. (2000) Behavioural validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (ADHD). *Neurosci. and Biobehav. Revs.* 24: 31-39

Sagvolden, T., Aesa, H., Zeiner, P., Berger, D. (1998) Altered reinforcement mechanisms in attention-deficit/hyperactivity disorder. *Behav. Brain Res.* 94: 61-71

Sagvolden, T., Mertger, 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. *Behav. Neural Biol.* 58: 103-112

Sagvolden, T., Seargent, J.A. (1998) Attention-deficit/hyperactivity disorder-from brain dysfunction to behaviour. *Behav. Brain Res.* 94: 1-10

Sagvolden. T., Pettersen, M.B., Larsen, M.C. (1993). Spontaneously hypertensive rats (SHR) as a putative model of childhood hyperkinesis: SHR behavior compared to other rat strains. *Physiol. Behav.* 54: 1047-1055

Schultz, W., Apicella, P., Ljungberg, T., Romo, R., Scarnati, E. (1993) Reward-related activity in the monkey striatum and substantia nigra. *Prog. Brain Res.* 99: 227-235

Sesack, S.R., Hawrylak, V.A., Matus, C., Guido, M.A., Levey, A.I. (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J. Neurosci.* 18: 2697-2708

Sherwin, C.M. (1998) Voluntary wheel running: a review and novel interpretation. *Anim. Behav.* 56: 11-27

Solanto, M.V. (1998) Neuropsychophamacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and intergration. *Behav. Brain Res.* 94: 127-152

Solanto, M.V. (2002) Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behav. Brain Res.* 130: 65-71

Sonuga-Barke, E.J.S., Taylor, E., Sembi, S., Smith, J. (1992) Hyperactivity and delay aversion-I. The effects of delay on choice. *J. Child Psychol. Psychiat.* 33: 387-398

Spanagel, R., Weiss, F. (1999) The dopamine hypothesis of reward: past and current status. *TINS.* 22: 521-527

Sproson, E.J., Chantrey, J., Hollis, C., Marsden, C.A., Fonel, K.C. (2001) Effect of repeated methylphenidate administration on presynaptic dopamine and behaviour in young adults rats. *J. Psychopharmacol.* 15: 67-75

Tarr, B.A, Kellaway L.A., Gibson, A., Russell, V.A. (2004) voluntary running distance is negatively correlated with striatal dopamine release in untrained rats. *Behav. Brain Res.* (Article in press).

Taylor, E. (1998) Clinical foundations of hyperactivity research. *Behav. Brain Res.* 94: 11-24

Viggiano, D., Vallone, D., Ruocco, L.A., Sadile, A.G. (2003) Behavioural, pharmacological, morpho-functional molecular studies reveal a hyperfunctioning mesocortical dopamine system in an animal model of attention deficit and hyperactivity disorder. *Neurosci. and Biobehav. Revs.* 27: 683-689

Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Franceschi, D., Maynard, L., Ding, Y.S., Gatley, S.J., Gifford, A., Zhu, W., Swanson, J.M. (2002) Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeuitic implications. *Synapse*: 43: 181-187

Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gerasimov, M., Maynard, L., Ding, Y.S., Gatley, S.J., Gifford, A., Franceschi, D. (2001) Therapeutic doses of methylphenidate significantly increase extracellular dopamine in human brain. *J. Neurosci.* 21: RC121

Volkow,N.D., Insel, T.R. (2003) What are the long term effects of methylphenidate treatment? *Biol. Psychiatry.* 54: 1307-1309

White, F.J., Kalvis, P.W. (1998). Neuroadaptations involved in amphetamine and cocaine addiction. *Drug Alcohol Depend.* 51: 141-153

Wilens, T.E., Faraone, S.V., Biederman, J., Gunawardene, S. (2003) Does stimulant therapy of attention-deficit/hyperactivity disorder beget later substance abuse? A meta-analytic review of the literature. *Pediatrics.* 111: 179-185

Wilson, W.M., Marsden, C.A. (1995) Extracellular dopamine in the nucleus accumbens of the rat during treadmill running. *Acta Physiol Scand.* 155: 465-466

Wu, Y.L., Yoshida, M., Emoto, H., Tanaka, M. (1999) Psychological stress selectively increases extracellular dopamine in the 'shell' but not in the 'core' of the rat nucleus accumbens: a novel dual-needle probe simultaneous microdialysis study. *Neurosci. Lett.* 275: 69-72

Wultz, B., Sagvolden, T., Moser, E.I., Moser, M.B. (1990) The spontaneously hypertensive rat as an animal model of attention-deficit hyperactivity disorder: effects of methylphenidate on exploratory behavior. *Behav. Neural Biol.* 53: 88-102

Yang, P.B., Amini, B., Swann, C., Dafny, N. (2003) Strain difference in the behavioral responses of male rats to chronically administered methylphenidate. *Brain Res.* 971: 139-152

Yeomans, J.S., Mathur, A., Tampakeras, M. (1993) Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. *Behav. Neurosci.* 107: 1077- 1087

Zahm, D.S. (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. *Ann N Y Acad Sci*. 877: 113-128

Zahm, D.S., Brog, J.S. (1992) On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience*. 50: 751-767

Zigmond, M.J., Bloom, F.E., Landis, S.C., Roberts, J.L., Squire, L.R. (1999) Fundamentals of Neuroscience. Academic Press.

APPENDICES

APPENDIX 3.1.

STATISTICAL ANALYSIS OF VOLUNTARY RUNNING DISTANCES OF FOUR- TO SIX-WEEK-OLD SHR AND WKY.

Daily running distances of SHR and WKY (m).

	1 STRAIN	2 DAY 1	3 DAY 2	4 DAY 3	5 DAY 4	6 DAY 5
1	SHR	316	2818	3405	4452	3926
2	SHR	5106	8665	8408	10588	8573
3	SHR	5492	1268	12173	14216	10462
4	SHR	6164	9016	11553	14374	14484
5	SHR	4244	5571	4422	5193	5336
6	WKY	565	1132	1620	1489	955
7	WKY	516	838	884	733	751
8	WKY	284	535	448	780	598
9	WKY	568	599	837	988	1087
10	WKY	85	253	329	288	521
11	WKY	265	749	779	991	1438

Repeated measures ANOVA of NAC core and shell fractional release of [^{3}H] dopamine of SHR and WKY.

All effects summary.

Repeated Measures Analysis of Variance (GLUTAMATE NAC CORE VS SHELL) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0.000084	1	0.000084	71.96794	0.000029
STRAIN	0.000001	1	0.000001	0.70341	0.425987
Error	0.000009	8	0.000001		
AREA	0.000009	1	0.000009	40.82854	0.000211
AREA*STRAIN	0.000000	1	0.000000	0.12798	0.729786
Error	0.000002	8	0.000000		

Post-hoc, Tukey HSD test.

Tukey HSD test; variable DV_1 (GLUTAMATE NAC CORE VS SHELL) Approximate Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = .00000, df = 10.955						
Cell No.	STRAIN	AREA	{1} .00296	{2} .00154	{3} .00248	{4} .00121
1	SHR	CORE		0.006261	0.892924	0.029502
2	SHR	SHELL	0.006261		0.326837	0.960950
3	WKY	CORE	0.892924	0.326837		0.011844
4	WKY	SHELL	0.029502	0.960950	0.011844	

Post-hoc, Newman-Keuls test.

Newman-Keuls test; variable DV_1 (GLUTAMATE NAC CORE VS SHELL) Approximate Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = .00000, df = 10.955						
Cell No.	STRAIN	AREA	{1} .00296	{2} .00154	{3} .00248	{4} .00121
1	SHR	CORE		0.003685	0.501737	0.029502
2	SHR	SHELL	0.003685		0.100713	0.642640
3	WKY	CORE	0.501737	0.100713		0.006983
4	WKY	SHELL	0.029502	0.642640	0.006983	

APPENDIX 3.4

STATISTICAL ANALYSIS OF POTASSIUM-STIMULATED RELEASE OF [^{3}H] DOPAMINE FROM NAC CORE AND SHELL OF FOUR TO SIX WEEK OLD SHR AND WKY.

	1 STRAIN	2 CORE	3 SHELL
1	SHR	0.0811	0.0509
2	SHR	0.0856	0.0584
3	SHR	0.053	0.0402
4	SHR	0.0734	0.04429
5	SHR	0.0823	0.0675
6	WKY	0.0656	0.0558
7	WKY	0.067	0.0398
8	WKY	0.0674	0.0477
9	WKY	0.0803	0.0393
10	WKY	0.0944	0.0683

Repeated measures ANOVA of NAC core and shell fractional release [3H] dopamine of SHR and WKY.

All effects summary.

Effect	Repeated Measures Analysis of Variance (Potassium NAC Shell vs Core) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.079669	1	0.079669	322.3033	0.000000
STRAIN	0.000006	1	0.000006	0.0249	0.878581
Error	0.001977	8	0.000247		
AREA	0.002830	1	0.002830	56.6844	0.000067
AREA*STRAIN	0.000005	1	0.000005	0.0940	0.766939
Error	0.000399	8	0.000050		

Post hoc. Tukey HSD test.

Cell No.	Tukey HSD test; variable DV_1 (Potassium NAC Shell vs Core) Approximate Probabilities for Post Hoc Tests Error: Between; Within: Pooled MS = .00015, df = 11.105 STRAIN	AREA	{1} .07508	{2} .05226	{3} .07494	{4} .05018
1	SHR	CORE		0.004197	0.999999	0.034377
2	SHR	SHELL	0.004197		0.055578	0.996552
3	WKY	CORE	0.999999	0.055578		0.002585
4	WKY	SHELL	0.034377	0.996552	0.002585	

Post-hoc. Newman-Keuls test.

Cell No.	Newman-Keuls test; variable DV_1 (Potassium NAC Shell vs Core) Approximate Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = .00015, df = 11.105 STRAIN	AREA	{1} .07508	{2} .05226	{3} .07494	{4} .05018
1	SHR	CORE		0.002480	0.989232	0.034377
2	SHR	SHELL	0.002480		0.013428	0.839817
3	WKY	CORE	0.989232	0.013428		0.001544
4	WKY	SHELL	0.034377	0.839817	0.001544	

APPENDIX 3.5

STATISTICAL ANALYSIS OF GLUTAMATE-STIMULATED RELEASE OF [^{3}H] NOREPINEPHRINE FROM PREFRONTAL CORTEX SLICES OF FOUR- TO SIX-WEEK-OLD SHR AND WKY.

	1 Strain	2 NE release
1	SHR	0.0011
2	SHR	0.0014
3	SHR	0.0023
4	SHR	0.0037
5	SHR	0.0013
6	SHR	0.0034
7	WKY	0.0002
8	WKY	0.0006
9	WKY	0.0019
10	WKY	0.0006
11	WKY	0.0013
12	WKY	0.0008

One-way ANOVA of [^{3}H] NE fractional release from PFC slices of four- to six-week-old SHR and WKY.

	Univariate Tests of Significance for NE release (UNTREATED SHR WKY PFC NE RELEASE Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0.000029	1	0.000029	35.15854	0.000145
Strain	0.000005	1	0.000005	6.18293	0.032180
Error	0.000008	10	0.000001		

Post Hoc. Tukey HSD test.

Cell No.	Strain	{1} .00220	{2} .00090
1	SHR		0.032329
2	WKY	0.032329	

Tukey HSD test, variable NE release (UNTREATED SHR WKY PFC NE RELEASE)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = .00000, df = 10.000

T-test, independent, by groups. Four- to six-week-old SHR and WKY. Fractional [^{3}H] NE release from the PFC slices.

Variable	Mean SHR	Mean WKY	t-value	df	p	Valid N SHR	Valid N WKY	Std.Dev. SHR	Std.Dev. WKY	F-ratio Variances	p Variances
NE release	0.002200	0.000900	2.486549	10	0.032180	6	6	0.001128	0.000307	3.456522	0.199648

T-tests: Grouping: Strain (UNTREATED SHR WKY PFC NE RELEASE)
Group 1: SHR
Group 2: WKY

APPENDIX 3.6

STATISTICAL ANALYSIS OF FIVE-WEEK-OLD (35 DAYS) SHR AND WKY [^{3}H]NE RELEASE FROM PFC SLICES.

The rats were either treated with 2mg/kg MP or vehicle (condensed) milk twice a day from day 21

	1 strain	2 treatment	3 NE release
	WKY	2mg MP	0.001
	WKY	2mg MP	0.002
	WKY	2mg MP	0.002
	WKY	2mg MP	0.00054
	WKY	2mg MP	0.000
	WKY	2mg MP	0.00
	WKY	2mg MP	0.001
	WKY	2mg MP	0.002
	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	WKY	Vehicle	0.000
1	SHR	2mg MP	0.001
1	SHR	2mg MP	0.001
1	SHR	2mg MP	0.000
2	SHR	2mg MP	0.00
2	SHR	2mg MP	0.001
2	SHR	2mg MP	0.001
2	SHR	2mg MP	0.000
2	SHR	2mg MP	0.000
2	SHR	Vehicle	0.001
2	SHR	Vehicle	0.001
2	SHR	Vehicle	0.002
2	SHR	Vehicle	0.000
2	SHR	Vehicle	0.001
3	SHR	Vehicle	0.000
3	SHR	Vehicle	0.00
3	SHR	Vehicle	0.001

Factorial ANOVA of [^{3}H]NE fractional release from PFC slices of five week old SHR and WKY.

Effect	Univariate Tests of Significance for NE release (35 DAY RATS Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	0.000044	1	0.000044	162.7619	0.000000
strain	0.000000	1	0.000000	0.3575	0.554684
treatment	0.000001	1	0.000001	2.9545	0.096677
strain*treatment	0.000004	1	0.000004	13.1540	0.001132
Error	0.000008	28	0.000000		

Post Hoc. Tukey HSD test.

Cell No.	Tukey HSD test; variable NE release (35 DAY RATS) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00000, df = 28.000					
	strain	treatment	{1} .00161	{2} .00063	{3} .00105	{4} .00140
1	WKY	2mg MP		0.004075	0.164952	0.857251
2	WKY	Vehicle	0.004075		0.374557	0.028033
3	SHR	2mg MP	0.164952	0.374557		0.540648
4	SHR	Vehicle	0.857251	0.028033	0.540648	

Post hoc Newman-Keuls test

Cell No.	Newman-Keuls test; variable NE release (35 DAY RATS) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00000, df = 28.000					
	strain	treatment	{1} .00161	{2} .00063	{3} .00105	{4} .00140
1	WKY	2mg MP		0.004075	0.099642	0.434817
2	WKY	Vehicle	0.004075		0.112709	0.015588
3	SHR	2mg MP	0.099642	0.112709		0.188234
4	SHR	Vehicle	0.434817	0.015588	0.188234	

APPENDIX 3.7

STATISTICAL ANALYSIS OF TIME SPENT IN CONDITIONED PLACE PREFERENCE (CPP) CAGES (PRELIMINARY TESTING).

Time (s) spent in black and white compartments of cages 1 and 2.

	1 cage	2 black	3 white
1	1	663	975
2	1	458	1124
3	1	749	601
4	1	1409	382
5	1	695	746
6	1	511	1105
7	1	1032	427
8	2	885	758
9	2	307	1341
10	2	563	730
11	2	348	1300
12	2	1020	467
13	2	712	833

Repeated measures ANOVA of the time spent in black and white compartments CPP cages 1 and 2).

	Repeated Measures Analysis of Variance (CCP balancing cages) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	15837802	1	15837802	1146.609	0.000000
cage	890	1	890	0.064	0.804272
Error	151940	11	13813		
COLOUR	123564	1	123564	0.592	0.457884
COLOUR*cage	166896	1	166896	0.799	0.390392
Error	2296298	11	208754		

Post Hoc. Tukey HSD test.

Cage*colour effect.

Cell No.	cage	COLOUR	{1} 788.14	{2} 765.71	{3} 639.17	{4} 938.17
	Tukey HSD test: variable DV_1 (CCP balancing cages) Approximate Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = 1113E2, df = 12.449					
1	1	black		0.999727	0.162538	0.849434
2	1	white	0.999727		0.902149	0.092251
3	2	black	0.162538	0.902149		0.677788
4	2	white	0.849434	0.092251	0.677788	

Cage effect.

Cell No.	cage	{1} 776.93	{2} 788.67
	Tukey HSD test: variable DV_1 (CCP balancing cages) Approximate Probabilities for Post Hoc Tests Error: Between MS = 13813., df = 11.000		
1	1		0.804413
2	2	0.804413	

Colour effect.

Cell No.	COLOUR	{1} 719.38	{2} 845.31
	Tukey HSD test: variable DV_1 (CCP balancing cages) Approximate Probabilities for Post Hoc Tests Error: Within MS = 2088E2, df = 11.000		
1	black		0.497047
2	white	0.497047	

APPENDIX 3.8.1

STATISTICAL ANALYSIS OF MP-TREATED SHR AND WKY RESPONSE TO 10 mg/kg COCAINE CHALLENGE AT P60.

Time difference between drug-paired and saline paired compartments before and after conditioning with 10mg/kg cocaine.

	1 Strain	2 Treatment	3 Pre	4 Post
1	SHR	MP	-150	368
2	SHR	MP	-16	483
3	SHR	MP	-146	-373
4	SHR	MP	-21	636
5	SHR	MP	-196	-260
6	SHR	MP	-148	221
7	WKY	MP	-341	786
8	WKY	MP	-534	546
9	WKY	MP	34	1343
10	WKY	MP	-705	829
11	WKY	MP	-702	-661
12	WKY	MP	-6	1800
13	SHR	vehicle	461	-66
14	SHR	vehicle	-287	171
15	SHR	vehicle	-176	300
16	SHR	vehicle	-223	-206
17	SHR	vehicle	-35	124
18	SHR	vehicle	-315	165
19	WKY	vehicle	-283	-461
20	WKY	vehicle	-329	831
21	WKY	vehicle	945	356
22	WKY	vehicle	-1074	688
23	WKY	vehicle	-395	-1
24	WKY	vehicle	-103	471

(The rats had been either MP-treated or vehicle-treated (condensed milk) for two weeks from P21 to P35 and left without any further treatment until P60).

Repeated measures ANOVA of time difference between drug-paired and saline-paired compartments before and after conditioning with 10mg/kg cocaine

	Repeated Measures Analysis of Variance (SadiCocaine) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7228	1	7228	0.02914	0.866162
Strain	36135	1	36135	0.14571	0.706693
Treatment	756765	1	756765	3.05158	0.095999
Strain*Treatment	133247	1	133247	0.53730	0.472057
Error	4959827	20	247991		
COCAINE	4478019	1	4478019	36.36466	0.000007
COCAINE*Strain	1290680	1	1290680	10.48123	0.004127
COCAINE*Treatment	160199	1	160199	1.30092	0.267519
COCAINE*Strain*Treatment	136001	1	136001	1.10442	0.305831
Error	2462841	20	123142		

Post-hoc, Tukey HSD test.

	Tukey HSD test: variable DV_1 (SadiCocaine) Approximate Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = 1956E2, df = 35.934									
Cell No	Strain	Treatment	COCAINE	{1} -249.5	{2} 24.333	{3} -112.8	{4} 179.17	{5} -521.5	{6} 195.33	{7} -387.0	{8} 773.83
1	SHR	vehicle	Pre		0.867950	0.999673	0.672545	0.977128	0.631431	0.999659	0.00487
2	SHR	vehicle	Post	0.867950		0.999265	0.999263	0.378658	0.998588	0.715366	0.21039
3	SHR	MP	Pre	0.999673	0.999265		0.827856	0.837198	0.914457	0.976116	0.02111
4	SHR	MP	Post	0.672545	0.999263	0.827856		0.122440	1.000000	0.333787	0.46564
5	WKY	vehicle	Pre	0.977128	0.378658	0.837198	0.122440		0.035533	0.999705	0.00031
6	WKY	vehicle	Post	0.631431	0.998588	0.914457	1.000000	0.035533		0.300392	0.49859
7	WKY	MP	Pre	0.999659	0.715366	0.976116	0.333787	0.999705	0.300392		0.00041
8	WKY	MP	Post	0.004877	0.210390	0.021114	0.465641	0.000314	0.498595	0.000041	

APPENDIX 3.8.2

STATISTICAL ANALYSIS OF MP-TREATED SHR AND WKY RESPONSE TO 20 mg/kg COCAINE CHALLENGE AT P60.

Time difference between drug-paired and saline-paired compartments before and after conditioning with 20mg/kg cocaine.

	1 strain	2 treatment	3 pre	4 post
1	SHR	MP	-8	-23
2	SHR	MP	-325	-461
3	SHR	MP	-566	-251
4	SHR	MP	-711	-722
5	SHR	MP	-749	-110
6	SHR	MP	-143	-9
7	WKY	MP	-346	673
8	WKY	MP	-912	-615
9	WKY	MP	-617	-352
10	WKY	MP	-1019	1196
11	WKY	MP	-394	904
12	WKY	MP	-385	1014
13	SHR	vehicle	-1031	334
14	SHR	vehicle	-287	265
15	SHR	vehicle	-693	812
16	SHR	vehicle	-968	907
17	SHR	vehicle	-133	-189
18	SHR	vehicle	-347	-4
19	WKY	vehicle	-1394	371
20	WKY	vehicle	-718	885
21	WKY	vehicle	-1010	1033
22	WKY	vehicle	-948	1031
23	WKY	vehicle	-920	695
24	WKY	vehicle	-768	724

(The rats had been either MP-treated or vehicle- (condensed milk) treated for two weeks from P21 to P 35 and left without any further treatment until P60).

Repeated measures ANOVA of time difference between drug-paired and saline-paired compartments before and after conditioning with 20mg/kg cocaine.

All effects summary.

Effect	Repeated Measures Analysis of Variance (20mg Cocaine preference, aversion exp Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1105347	1	1105347	6.63049	0.018078
strain	261075	1	261075	1.56607	0.225218
treatment	138460	1	138460	0.83056	0.372965
strain*treatment	176419	1	176419	1.05826	0.315894
Error	3334132	20	166707		
COCAINE	11505208	1	11505208	73.78230	0.000000
COCAINE*strain	2288133	1	2288133	14.67368	0.001045
COCAINE*treatment	156313	1	156313		
COCAINE*strain*treatment	8911	1	8911	0.05714	0.813501
Error	3118691	20	155935		

Post-hoc, Tukey HSD test.

Cell No.	Tukey HSD test; variable DV_1 (20mg Cocaine preference, aversion exp) Approximate Probabilities for Post Hoc Tests Error: Between, Within, Pooled MS = 1613E2, df = 39.955 strain	treatment	COCAINE	{1} -417.0	{2} -262.7	{3} -576.5	{4} 354.17	{5} -512.2	{6} 470.00	{7} -959.7	{8} 789.83
1	SHR	MP	pre		0.996803	0.996812	0.036457	0.989246	0.009761	0.339477	0.000275
2	SHR	MP	post	0.996803		0.872310	0.206937		0.084531	0.078568	0.004864
3	SHR	vehicle	pre		0.872310		0.011160		0.001427	0.730751	0.000145
4	SHR	vehicle	post	0.036457	0.206937				0.999593	0.000161	0.597770
5	WKY	MP	pre	0.989246		1.000000	0.003743		0.002666		0.000140
6	WKY	MP	post	0.009761	0.084531					0.000137	0.865779
7	WKY	vehicle	pre	0.339477	0.078568	0.730751	0.000161	0.811842	0.000137		0.000165
8	WKY	vehicle	post	0.000275	0.004864	0.000145	0.597770	0.000140	0.865779	0.000165	

APPENDIX D1

PHOTOGRAPH OF SUPERFUSION CHAMBER