A Study of an Animal Model of Attention Deficit/Hyperactivity Disorder using *in vivo* Chronoamperometry and Behavioural Responses to Methylphenidate and Guanfacine

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Declaration

I, Jennifer Hsin-Wen Hsieh, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signature:

Date:

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Prof Vivienne Ann Russell

EVERYTHING

For guidance, patience, grief, misunderstandings, understandings, concessions, disapprovals, encouragement, approvals, more patience, being my surrogate mother for three years, and even more guidance:

Prof Vivienne Ann Russell

Abstract

BACKGROUND: Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood disorder that is behaviorally characterized by developmentally inappropriate levels of inattention, impulsivity and hyperactivity. Among several hypotheses of ADHD etiology the prevailing hypothesis implicates a hypodopaminergic system due largely to the successes of the use of psychostimulants such as methylphenidate, that increase extracellular levels of dopamine (DA) and/or norepinephrine (NE) for the treatment of symptoms. In this study, the spontaneously hypertensive rat (SHR), the most widely accepted model for ADHD, is compared with the Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rat strains as controls. *METHODS:* The SHR and WKY rat strains were first genetically screened for purity using SNPs from three chromosomal sites. *In vivo* chronoamperometry, a technique that allows for the measurement of micromolar DA concentrations at high spatial (30 μm) and temporal (5 Hz) resolutions was set up for the first time in Africa by this laboratory. This technique was used to measure a) the level of dopamine release by potassium stimulation and b) the rate of clearance of exogenously applied dopamine. Recordings were taken from within the anaesthetized rat's striatum (AP 1 mm; ML 2.5 mm from bregma (Paxinos and Watson, 2005)) every 500 μm from DV co-ordinates 3.5 to DV 7.5 mm relative to dura, between P 32 and P 37. Behaviour in the open field apparatus and voluntary running were measured in response to methylphenidate or guanfacine treatment, since an alternative noradrenergic hypothesis suggests that a hypernoradrenergic system may be the cause of ADHD symptoms, and α_{2A}-adrenoreceptor agonists such as guanfacine have been shown to be effective in treating ADHD. *RESULTS:* Initial genetic screening suggested a possible but unconfirmed crossing of SHR and SD strains. The *in vivo* chronoamperometry study revealed that SHR release more dopamine than SD and WKY strains, in response to potassium stimulation. SHR cleared exogenously applied dopamine at rates that were more similar to SD and significantly faster than that of WKY. Methylphenidate and guanfacine treatment had no effect on voluntary running or open field behaviour in any strain. *CONCLUSION:* SHR were significantly more active than WKY in terms of voluntary behaviour in cages with attached running wheels and in the open field. The SHR showed decreased DAT activity in the rising phase of the DA peaks while the WKY showed decreased DAT activity in both the rising and falling phase of the DA peaks. Additional genetic screening is required to confirm whether an SHR and SD crossing of strains occured, however the present results suggest that new animal stocks should be purchased.

Abbreviations

3'UTR 3'-untranslated region.

5-HTT serotonin transporter.

ADHD Attention-Deficit/Hyperactivity Disorder.

ASD Autism Spectrum Disorder.

ASPD Antisocial Personality Disorder.

BD Bipolar Disorder.

BDNF brain derived neurotrophic factor.

CC corpus callosum.

CD Conduct Disorder.

CFV cresyl fast violet.

CM condensed milk vehicle.

CN caudate nucleus.

COMT catechol-O-methyltransferase.

CVLM caudal ventrolateral medulla.

DA dopamine.

DAT dopamine transporter.

DBH dopamine β-hydroxylase.

DCD Developmental Co-ordination Disorder.

DRD4 dopamine receptor type 4.

DRD5 dopamine receptor type 5.

DSP-4 N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride.

DTI diffusion tensor imaging.

EtBr ethidium bromide.

FAST Fast Analytical Sensing Technology.

fMRI functional Magnetic Resonance Imaging.

HCN Hyperpolarization-activated Cyclic Nucleotide-gated.

KO knock-out.

L-DOPA L-dioxyphenylalanine.

LC locus coeruleus.

LOD Limit of Detection.

MAO-A monoamine oxidase A.

MPH methylphenidate.

NAc nucleus accumbens.

NE norepinephrine.

NET norepinephrine transporter.

NO nitric oxide.

NTF neurotrophic factor.

OCD Obsessive Compulsive Disorder.

ODD Oppositional Defiance Disorder.

PBS phosphate buffered saline.

PCB polychlorinated biphenyl.

PCR polymerase chain reaction.

PET positron emission tomography.

PFC pre-frontal cortex.

SD Sprague-Dawley.

SHR spontaneously hypertensive rat.

SNAP-25 synaptosome-associated protein of 25 kDa.

SPECT single photon emission computed tomography.

ST striatum.

TE Tris EDTA.

TH tyrosine hydroxylase.

UCT University of Cape Town.

VMAT2 vesicular monoamine transporter 2.

VNTR variable number of tandem repeats.

WKY Wistar-Kyoto.

ZFN zinc-finger nuclease.

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1 Introduction

1.1 Attention-Deficit/Hyperactivity Disorder

Attention-Deficit/Hyperactivity Disorder (ADHD) is a childhood disorder that is behaviorally characterized by developmentally inappropriate levels of inattention, impulsivity and/or hyperactivity (American Psychiatric Association, 2000). Three subtypes of ADHD have been defined, including the predominantly inattentive (ADHD-I), the predominantly hyperactive (ADHD-H) and a combined sub type (ADHD-C), each diagnosed by various specific behaviours that are evident in at least two or more settings (American Psychiatric Association, 2000).

Children with ADHD have been shown to be at increased risk of school failure, academic underachievement, juvenile delinquency, substance abuse, incarceration, eventually receiving lower paying occupations in adulthood, divorce, and of living in poverty (Fischer et al., 1993; Barkley et al., 2004, 2006; Loo et al., 2009). ADHD is also associated with other psychiatric disorders in about two thirds of cases (Gillberg et al., 2004). In a study of Swedish school-aged children, approximately 50% and 25% of children with ADHD also met the criteria for Oppositional Defiance Disorder (ODD) and Conduct Disorder (CD), respectively (Cormier, 2008; Kadesjo and Gillberg, 2001). Other documented co-morbidities include Bipolar Disorder (BD) (Bernardi et al., 2010), Antisocial Personality Disorder (ASPD) (Black et al., 2010), Developmental Co-ordination Disorder (DCD) (Bart et al., 2010), Autism Spectrum Disorder (ASD), Obsessive Compulsive Disorder (OCD), depression and anxiety disorder and tic disorders (Cormier, 2008; Spencer et al., 1999a,b). The impulsive characteristic of ADHD leads to a certain disregard of consequences and carelessness, together with the variety of co-morbidities, they frequently result in harming themselves or others (Blum et al., 2008).

The symptoms of ADHD are usually discerned before the age of seven (Tan and Appleton, 2005), and occurs in between 3% to 7% of school-aged children (Faraone et al., 2003). Although symptoms of this disorder have been known to dissipate as the patient age increases, 70% of children diagnosed with ADHD, or between 1% to 6% of the adult population, continue to exhibit symptoms in adult life (Biederman, 1998; Kessler et al., 2005; Cormier, 2008; Kolar, 2008). A recent German study on the psychological status of soldiers with ADHD showed that cumulative adaptability problems were significantly correlated with agression, partnership problems and dissocial symptoms (Zimmermann et al., 2010). This study concluded that although ADHD does not automatically exclude

soldiers from military duty, 44.4% with ADHD were not able to continue their service (Zimmermann et al., 2010).

In the United States, it was estimated that two-and-a-half billion dollars per year are spent on the diagnosis and treatment of ADHD (Chan et al., 2002). This disorder is extremely costly to the afflicted individuals, their families, and society (Birnbaum et al., 2005), and when children go undiagnosed and untreated, further potential costs are immense (Blum et al., 2008). In South Africa. the contraction and spread of HIV/AIDS is of particular concern, since ADHD is also associated with increased sexual risk taking behaviour in adolescents (Brown et al., 2010).

1.2 Treatments

The first report of successful drug treatment for 'hyperactivity' occurred in 1937, with the use of *dl*-amphetamine (Bradley, 1937; Heal et al., 2008; Cormier, 2008). Currently, stimulant treatments include methylphenidate (Ritalin, Concerta), dextroamphetamine (Dexedrine) and a *dl*-amphetamine extended release, mixed salts formulation (Adderal XR); non-stimulant medications include atomoxetine, bupropion, tricyclic antidepressants, clonidine, guanfacine and modafinil (Strange, 2008; Kolar, 2008). Some side effects of stimulant treatment include decreased appetite, headache, insomnia, nausea, dry-mouth, irritability, anxiety, nervousness, increased blood pressure and weight loss; while non-stimulant treatments have side effects such as dry mouth, drowsiness and constipation (Kolar, 2008; Medori et al., 2008). In rare cases stimulants have caused seizures, psychosis, hypertension, severe anorexia and hepatotoxicity (Kolar, 2008; Swanson et al., 2001). Generally, the stimulant treatments have been shown to be more effective than the non-stimulants, however some patients are unable to tolerate stimulant side effects, while patients with substance abuse co-morbidity may require the non-stimulant treatments to prevent drug-primed relapse (Strange, 2008).

Methylphenidate has been used safely for about 50 years (Taylor et al., 2004) and has been shown to be effective in improving working and visual memory (Wright and White, 2003; Rhodes et al., 2004), reading skills (Keulers et al., 2007), non-verbal and visual-spatial learning (O'Toole et al., 1997; Bedard et al., 2004), academic performance (Yang et al., 2004; McGough et al., 2006; Dommett et al., 2008) and in reducing hyperactivity (Arnold et al., 1978; Posey et al., 2007). As a result, it is still one of the most commonly prescribed drugs for the treatment of ADHD (Faraone et al., 2001), with decreasing age of prescription onset (Kollins et al., 2006). Indeed, these cognitive enhancing effects of methylphenidate are also experienced when used by healthy individuals (Elliott et al., 1997), and illicit use by students to aid academic performance is common (McCabe et al., 2006; Wilens et al., 2008). However, methylephenidate is not consistently effective (Ad-

vokat, 2010). Some studies report that 68% of the ADHD treated group were considered responders (Spencer et al., 2005), other studies have reported only 21% responders (Greenhill et al., 2006) and a recent study reported that 15% of ADHD subjects dropped out of the study due to lack of efficacy (Bejerot et al., 2010). Methylphenidate was shown to be ineffective in elderly healthy volunteers (Turner et al., 2003), and although young healthy individuals showed improvements in various cognitive aspects (Elliott et al., 1997), there is high variability in response to methylphenidate that could in part be due to differences in dopamine tone between individuals (Volkow et al., 2002a). A rodent study found that dopamine D4 receptor knock-out (KO) mice showed increased glucose metabolism in the pre-frontal cortex (PFC), while homologous D4 positive and heterozygot mice had decreased glucose metabolism in the PFC, and thus proposed a genetic explanation for the differential response to methylphenidate (Michaelides et al., 2010). PET scans of methylphenidate's effects on DAT blockade and DA release showed that a 60 mg dose of methylphenidate blocked 60±11% of DAT, and also caused a 16±8% increase in extracellular DA levels in healthy individuals, however the DAT blockage and DA release were not correlated (Volkow et al., 2002b). Thus Volkow *et. al* suggest that the individual difference in response to methylphenidate could be due to individual differences in DA release (Volkow et al., 2002b; Forssberg et al., 2006; Swanson and Volkow, 2009).

The mechanisms underlying methylphenidate's therapeutic actions are heavily researched but as yet unclear. It is a known dopaminergic and noradrenergic reuptake blocker, with higher binding affinities for the norepinephrine transporter (NET) than dopamine transporter (DAT) (Markowitz et al., 2006). Human positron emission tomography (PET) studies with use of [^{11}C]-raclopride binding displacement measures have shown that oral administration of therapeutic doses of methylphenidate increased levels of dopamine in the striatum of both normal subjects (Volkow et al., 2001), and those with ADHD (Rosa-Neto et al., 2005), although the level of increase was lower in adults with ADHD than controls (Volkow et al., 2007b). This increase in striatal dopamine levels was also observed in a similar study performed in rodents and primates (Schiffer et al., 2006). Norepinephrine levels in the brain were also increased with methylphenidate treatment (Kuczenski and Segal, 1997, 2001). A whole-cell patch clamp study showed that methylphenidate increased intrinsic excitability of prefrontal cortical neurons that was mediated via the α_2-adrenoreceptor (Andrews and Lavin, 2006), and support a behavioural study that showed improvement in the performance of a delayed alternation task in rats through actions of α_2-adrenoreceptor and dopamine D1 receptors in the PFC (Arnsten and Dudley, 2005). Since methylphenidate is generally effective in treating ADHD symptoms, this evidence strongly implicates the involvement of both the dopaminergic and the noradrenergic systems in ADHD aetiology.

Although generally effective, the side effects of stimulants are not tolerable in some

patients, especially those with tic disorders or at risk of substance abuse or relapse (Scahill et al., 2001; Brennan and Arnsten, 2008), and alternative medications have received much research attention (Glazer, 2010). It was initially shown that clonidine, a general noradrenergic agonist, produced beneficial effects in the treatment of ADHD symptoms with fewer side effects than methylphenidate (Hunt et al., 1985, 1986; Hunt, 1987). Subsequently, guanfacine was also shown to be effective in treating ADHD symptoms in children (Horrigan and Barnhill, 1995) and since it is less hypotensive, less sedating and has a longer half-life than clonidine, guanfacine became prefered over clonidine for the treatment of ADHD symptoms (Horrigan and Barnhill, 1995; Jakala et al., 1999a; May and Kratochvil, 2010).

Guanfacine is a selective α_{2A}-adrenoreceptor agonist (Scholtysik, 1986; Uhlen and Wikberg, 1991a,b) that has been FDA approved for the treatment of hypertension since 1986 (Nakamichi et al., 1988; Horrigan and Barnhill, 1995). Guanfacine (0.6 mg/kg) in a rat model of ADHD showed improvements in sustained attention and reduced hyperactivity and impulsiveness (Sagvolden, 2006). Low or high doses of guanfacine improved the performance of aged monkeys in the delayed-response task (Rama et al., 1996), while a high dose of guanfacine (0.1 mg/kg) improved reversal learning performance in aged rhesus monkeys (Steere and Arnsten, 1997). In humans, guanfacine was shown to improve planning and working memory performance (Jakala et al., 1999a) as well as paired associates learning (Jakala et al., 1999b) in healthy volunteers. A double-blind placebo controlled study found that guanfacine and dextroamphetamine displayed similar efficacy levels in adults with ADHD (Taylor and Russo, 2001), and that guanfacine was effective and safe to use for 24 months without intolerable adverse effects (Faraone and Glatt, 2009; Biederman et al., 2008a,b; Posey and McDougle, 2007). Thus, guanfacine has been recently approved by the FDA (September, 2009) for the treatment of ADHD (May and Kratochvil, 2010).

The selectivity of guanfacine for the α_{2A}-adrenoreceptor (Jarrott et al., 1982; Scholtysik, 1986) was highlighted by a rodent study where it improved spatial working memory of wild type and α_{2C}-adrenoreceptor KO mice, but not of α_{2A}-adrenoreceptor KO mice (Franowicz et al., 2002). Stimulation of α_{2A}-adrenoreceptors was found to inhibit cAMP signalling in the post-synaptic spines of PFC neurons (Wang et al., 2007). Increased cAMP levels activate Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels (Shin et al., 2004) and decrease membrane resistance, while inhibition of cAMP closes HCN channels and increases membrane resistance (Wang et al., 2007). An increase in membrane resistance in the dendritic spines of PFC neurons would strengthen signal input by preventing the dissipation of regional potential difference, and since HCN channels were co-localized with α_{2A}-adrenoreceptors in the dendritic spines of PFC neurons (Wang et al., 2007), Arnsten *et al.* hypothesize that guanfacine improves working memory function through

the inhibition of cAMP and HCN channels, that is mediated by the stimulation of α_{2A}-adrenoreceptors (Wang et al., 2007; Brennan and Arnsten, 2008), and strongly supports the involvement of the noradrenergic system in the aetiology of ADHD.

Since both methylphenidate and guanfacine have shown degrees of efficacy in treating ADHD symptoms, including that of hyperactivity (Taylor et al., 2004; Minzenberg and Carter, 2008; Biederman et al., 2008b; Sagvolden, 2006) and do so via different hypothesized neural mechanisms, we chose to focus the study on the effects of methylphenidate and guanfacine on voluntary running in an animal model of ADHD, the SHR.

1.3 Genetic Associations

Familial and adoption studies have found that ADHD is highly heritable (Faraone and Doyle, 2001; Biederman, 2005; Albrecht et al., 2010). Although attempts to determine the causal genes for this disorder are extensive, findings from genetic studies to date have been inconsistent (Banaschewski et al., 2010). The only concensus is that ADHD is a heterogenous disorder with a complex genetic architecture, and that the overall phenotype is likely due to multiple interacting genes, each with small effects (Smalley, 1997; Bobb et al., 2005b; Faraone et al., 2005; Curatolo et al., 2008; Durston et al., 2009).

Given the mechanisms of the drugs that are successful in the treatment of ADHD, many candidate gene studies are based on the hypothesis of the involvement of the dopaminergic, noradrenergic and serotonergic systems.

Genes in the dopaminergic system associated with ADHD, include the 9 or 10 repeat alleles of the 3'-untranslated region (3'UTR) variable number of tandem repeats (VNTR) of the DAT1 (SLC6A3) (Dresler et al., 2010; Bellgrove et al., 2008), dopamine β-hydroxylase (DBH), dopamine receptor type 4 (DRD4) 7-repeat allele (Gornick et al., 2007) and the dopamine receptor type 5 (DRD5) (Bobb et al., 2005b; Faraone et al., 2005; Barkley et al., 2006; Lasky-Su et al., 2007). The DRD5 marker has in fact been linked more specifically to ADHD-I and ADHD-C (Lowe et al., 2004). Studies have also reported associations with adrenergic genes (Comings et al., 2000), particularly the NET1 (Bobb et al., 2005a; Kim et al., 2008). Recent genome-wide association scans have additionally implicated the α_{1A}- and β_2-adrenoreceptors (Lasky-Su et al., 2008), while a polymorphism (C-1291G) in the promoter region of the α_{2A}-adrenoreceptor gene has been linked specifically to the ADHD-I subtype (da Silva et al., 2008; Kiive et al., 2010). A population based study with samples of twins associated monoamine oxidase A (MAO-A) and serotonin transporter (5-HTT) genes and platelet MAO-B activity with disruptive behavior scores in adolescents (Malmberg et al., 2008), implicating serotonergic involvement.

Genes involved in general neural development and signalling have also been implicated, for example a microsatellite in the synaptosome-associated protein of 25 kDa (SNAP-25)

(Mill et al., 2005b; Zhang et al., 2010) was found to have weak associations in a linkage study. An association study of 10 genes which encode neurotrophic factors (NTFs) and their receptors in both adult and child ADHD produced data which suggested that variations in NTFs may be involved in the genetic susceptibility to ADHD (Ribases et al., 2008). The study also suggested NTF-3 and neurotrophic receptor kinase 2 (NTRK2) to be involved in the molecular basis of the age dependent changes in ADHD symptoms throughout the life span of the patient, and further support the contribution of the cytokine neurotrophic factor receptor (CNTFR) locus as a predisposition factor for the disorder (Ribases et al., 2008). NTF-3 is involved in the differentiation and survival of dopaminergic and noradrenergic neurons and has been further implicated in a preliminary case-control family-based Korean study (Cho et al., 2010). Among others, the cadherin-13 (CDH13), brain derived neurotrophic factor (BDNF) and catechol-O-methyltransferase (COMT) were also found to be significantly associated candidate genes in more recent genome wide association studies (Lasky-Su et al., 2008), and thus new avenues of research for the genetic causes for ADHD have been suggested (Banaschewski et al., 2010).

In addition to genetic factors, environmental factors such as maternal smoking (Milberger et al., 1996), lower maternal folate status (Schlotz et al., 2010), early exposure to polychlorinated biphenyls (PCBs) (Kuehn, 2010; Sagiv et al., 2010) and higher serum levels of manganese (Farias et al., 2010) have been associated with the development of ADHD. Indeed, there is certainly evidence that gene $\times$ environment interactions have a highly relevant role in the development of ADHD. A study of the interaction between maternal smoking and the 10 repeat allele of the 40 bp VNTR in the DAT1 gene showed that children that were homozygous for the 10 repeat allele had increased symptoms of hyperactivity, impulsivity and oppositional behaviour only if they were exposed to pre-natal smoking (Kahn et al., 2003). A study of the interaction between the C-1291G polymorphism in the α_{2A}-adrenoreceptor gene and family maltreatment on 429 children showed that while the boys with the CC genotype and low maltreatment scores showed less overactivity than G allele carries, CC genotype boys with higher maltreatment scores were more overactive, and more inattentive (Kiive et al., 2010).

ADHD is diagnosed on the basis of behaviour. Whether a subject participating in a scientific study is grouped as 'ADHD' or 'control' is determined by behaviour. A hypothetical subject with a predisposing homozygous 10 repeat allele in the DAT1 VNTR may not have had pre-natal exposure to maternal smoking and thus be grouped as 'control', while another hypothetical subject with the same genotype could be grouped as 'ADHD' if he was pre-natally exposed to maternal smoking. Results from such a study would find no linkage of ADHD with this gene. In fact, genetic linkage and association studies differ in whether they control for the exposure to certain environmental factors (e.g. maternal smoking), gender, co-morbidity, method of recuitment and age (Banaschewski

et al., 2010), each of which could alter the subjects' behaviour and significantly influence the results. Further study of gene × environment effects on the aetiology of ADHD has the potential to explain the discrepancy between studies regarding the causal genes of ADHD (Banaschewski et al., 2010). However, gene × environment interaction research is a highly complex field, and current statistical approaches are not equipped to handle this complexity (Banaschewski et al., 2010). Once these challenges have been overcome, gene × environment studies, along with more controlled genetic studies would provide more valuable insights into the aetiology of ADHD, which may provide strategies for individualized prevention and therapy (Wermter et al., 2010).

1.4 Structural Alterations

Volumetric anatomical studies of brain structures have identified the PFC (Castellanos et al., 1996; Makris et al., 2007), caudate nucleus (CN) (Tremols et al., 2008), striatum (ST) (Castellanos et al., 1994, 1996; Tremols et al., 2008; Buitelaar et al., 1996), substantia nigra (Romanos et al., 2010), corpus callosum (CC) (Luders et al., 2009), hippocampus (Plessen et al., 2006), anterior cingulate (Makris et al., 2010), cerebellar vermis (Castellanos et al., 2001) and pulvinar nucleus in the thalamus (Ivanov et al., 2010) as regions of defect in ADHD.

Cortical networks that support attention and executive function were found to be thinner in adults with ADHD (Makris et al., 2007). In particular, there was bilateral cortical thinning in the orbital frontal cortex, lateral superior and middle frontal gyri. Right cortical thinning occurred in the angular gyrus, supramarginal gyrus and anterior cingulate cortex. Left cortical thinning occurred in the posterior cingulate cortex (Makris et al., 2007).

Inter-hemispheric connections are required to sustain attention and motor control (Weber et al., 2005; Tassinari et al., 1994). An MRI comparison of the CC between ADHD and control subjects confirmed the CC to be significantly thinner in ADHD subjects in the anterior, but particularly the posterior callosal sections (Luders et al., 2009). The decreased callosal thickness could be caused by decreased myelination of the axons, or decreased number of fibers that connect the parietal and prefrontal cortices. Impairment or lack of inter-hemispheric connections may contribute to the symptoms of ADHD. These callosal abnormalities may be due to maturational delays, as many symptoms of ADHD dissipate as the subject age increases, however, it has been found that 70% of those diagnosed with childhood ADHD continue to have adult ADHD (Biederman, 1998), which suggests that these abnormalities in the CC could be permanent (Luders et al., 2009).

Defects in visuospatial memory and temporal processing in individuals with ADHD (Bedard et al., 2004; Barkley, 1997) have implicated the limbic system. An MRI study

of hippocampus and amygdala morphology found that the head of the hippocampus was bilaterally larger in the ADHD group compared to the control group (Plessen et al., 2006). This study suggested that the enlargement of the hippocampus may be a compensatory response to the disturbance in time perception and delay aversion in ADHD subjects (Plessen et al., 2006).

Another MRI study found ADHD patients to have decreased right caudate body volume, as well as an inverse asymmetry of the caudate body and head as compared to the controls. ADHD patients had a larger right caudate head volume than the left, and a larger left caudate body volume than the right (Tremols et al., 2008). This newer study suggested that a previous (less advanced) MRI study had found a larger right caudate volume (compared to left caudate) in ADHD subjects (Hynd et al., 1993) because detailed distinctions between caudate body and caudate head had not been made, since the technique to do so accurately was not available at that time (Tremols et al., 2008). Regardless of direction of striatal symmetry, abnormalities of the ST have been found in additional cases (Castellanos et al., 1994, 1996; Mataro et al., 1997) and thus is a structure that is clearly involved in the pathophysiology of ADHD.

The most recent high resolution structural MRI study used a technique that created a 'cytoarchitechnotic' map of the thalamic surface based on cellular structure (Ivanov et al., 2010). A comparison between control and ADHD subjects, in the degree to which the ADHD surface needed to be stretched or shrunk, showed that ADHD subjects not on medication had bilaterally smaller pulvinar nuclei, while those on medication were more similar to normal subjects. Additionally, severity of hyperactivity was associated with a decreased volume in the lateral nuclei of the thalamus, while severity of inattention was associated with an increase in size of nuclei in the right medial-posterior thalamus (Ivanov et al., 2010), and suggests that further developments in structural imaging, including functional Magnetic Resonance Imaging (fMRI) and diffusion tensor imaging (DTI) studies may well provide diagnostic signals for ADHD (Shaw, 2010).

The aetiology of ADHD is likely to be both genetic and developmental. Genetic aberrations could be the sole cause of structural abnormalities, and poorly regulated development of neuronal networks as a result of genetic dysfunctions could lead to functional problems when specific activities (such as sustained attention) are required, which exhibits as a behavioural symptom. However, neural compensations are known to solve functional problems, afforded by the high plasticity of the brain. Indeed, there are degrees of severity of ADHD symptoms, and individuals with ADHD are not completely unable to function, or devoid of all faculties of attention (Taylor, 1998). Thus, the structural defects found in the imaging studies could be that of the original genetic defect or the result of the subsequent (although perhaps inadequate) neural compensations. Structural studies are important for determining target areas to refine further studies, and could prove to be

useful for diagnosis (Ivanov et al., 2010; Shaw, 2010), however the abnormalities could be a cause or a compensation of the disorder.

The present project elected to study the ST since it was found to be an area of defect in humans (Tremols et al., 2008) and has been the focus of many neurochemical studies in our SHR model of ADHD (Russell et al., 1995; Heal et al., 2008). The ST also fundamentally controls activity/hyperactivity (Tucker and Williamson, 1984), and additionally, it is an area with extensive dopaminergic activity that would allow for the most selective measurements of dopamine concentrations with use of carbon fibre *in vivo* chronoamperometry (elaboration in Introduction section 1.7).

1.5 Animal Models

ADHD is a behaviourally diagnosed disorder, based on the presence of reported symptoms from a detailed history of the patient, observations from the family and subjective reports from school or other observers (Thapar and noz Solomando, 2008), thus accurate diagnosis is difficult and highly variable between practitioners (Fulton et al., 2009). This has caused problems not only with identifying appropriate treatment measures for patients, but also hampers scientific study of the disorder in humans, since comparative groups are determined based on these variable behavioural diagnoses. The huge genetic variety of human populations and practical impossibility to control for environmental confounding factors further challenge the ability of human studies to distill specific information. Additionally, and most importantly, current methods used to directly study specific details of neurochemical function, basic cellular abnormalities, and their correlation to observed behaviours can be highly invasive and unethical to perform in humans.

Animal models are advantageous in ADHD research because it is possible to breed genetically homogenous animals, control the experimental environment easily and specifically, they have simpler nervous systems, more easily quantifiable behaviours and a greater variety of interventions may be applied (Sagvolden et al., 2005). This allows for the detailed research of various behaviours and the neurocorrelates thereof, in order to determine the aetiology of ADHD (and other disorders).

A good animal model of ADHD must satisfy the requirements for face, construct and predictive validities. Face validity requires that animal behaviour mimics the fundamental characteristics of the people with the disorder, thus the animal must present symptomatic behaviours such as hyperactivity, impulsivity and inattention. Construct validity requires that the animal model conforms to the theoretical rationale of the disorder, and predictive validity requires that the animal model be able to predict aspects of behaviour, genetics and neurobiology in humans that were previously unknown (Sagvolden et al., 2005; Willner, 1984).

In order to confirm face validity of the animal models, their behaviour requires to be comparable with that of ADHD. However, 'impulsivity', 'inattention' and 'hyperactivity' are difficult to determine accurately even with psychometric tests that are specifically designed to measure these aspects of behaviour in children (Johansen et al., 2002). Thus, these behaviours, in both humans and rats, need to be defined in ways that allow empirical measurement and quanfication, in order to draw meaningful comparisons between the studies.

The study of animal operant behaviour in response to a discriminating stimulus (e.g. light cue) rather than respondant behaviour, in response to a direct stimulus (e.g. foot-shock) was pioneered by Skinner in the 1930s (Hilgard, 1939). Skinner studied the pattern of operant responses (lever presses) as a result of reinforcers (food), and discovered 'laws' that governed the operant behaviours in different reinforcing situations. After the presentation of a single reinforcement as a result of an operant response (lever press due to spontaneous rat exploration), the pattern named 'reflex' was discovered, where lever presses inevitably continued after it resulted in the presentation of a reinforcement. The 'extinction curve' was observed when the number and rate of lever presses decreased over time if reinforcement was discontinued, and the 'resistence to extiction' was the potential number of lever presses that would occur without further reinforcement. Reinforcement of the operant behaviour at periodic intervals (every 3, 6 or 12 mins) resulted in a characteristically uniform rate of responding. Reinforcers presented in fixed ratios, where reinforcement was presented every 10th, 20th or 192nd lever press, resulted in a typical acceleration of responses (Skinner, 1938; Hilgard, 1939). The paradigms used to measure behavioural patterns have since been refined and further developed in order to measure additional and more complex behaviours empirically. In particular, the 'delay-of-reinforcement gradient' is a behavioural pattern found where the shorter the time between the operant response and reinforcement delivery, the higher the likelihood of the response being performed again, and it was proposed that individuals with ADHD have steeper and shorter delay gradients, which would explain the ADHD symptoms (Catania et al., 1988; Sagvolden et al., 1998; Johansen et al., 2002).

The 'inattention' observed in ADHD is specifically a deficit in *sustained* attention (Johansen et al., 2002), and appears to have a strong motivational component, since the problem is revealed only when the task is unwelcome or uninteresting (Taylor, 1998). Individuals with ADHD are able, at least temporarily, to maintain focus on tasks that are interesting to them, 'when the behaviour is maintained by potent and frequent reinforcers' (Johansen et al., 2002). Sagvolden *et. al* proposed that the impairment in sustained attention in ADHD should be more appropriately termed 'cognitive impulsiveness', and measured as the inability to *maintain* refrain from lever pressing after the extinction of reinforcements signaled by a discriminative cue (Sagvolden et al., 1992b; Johansen et al.,

2002). Impulsiveness, or 'motor impulsiveness' is characterized by bursts of responses with short inter-response times in a task with fixed interval schedule (where only 1 response will be reinforced at regular intervals), especially during the interval when additional responses will not be reinforced (Sagvolden et al., 1998). In terms of hyperactivity, children with ADHD have been shown to move twice as frequently, and cover a four fold wider area, with body movements that are more linear (side-to-side) and less complex than normal children (Taylor, 1998). Overactivity also occurs only after habituation, and not in novel environments. In humans, movements really only become 'hyper' when implicit or explicit social rules are overstepped, and depends on situational appropriateness, and hyperactivity is often described in classroom situations but not in play (Taylor, 1998). 'Social appropriateness' of activity is not measureable in a rat, and the thinner cortical structures in rodents compared to humans prevent detailed studies of top-down control of behaviour. However quantities and patterns of activity in comparison with other 'control' rat strains can be measured in environments such as the the standard open field, or other behavioural tests, in terms of total distance moved, and velocities or frequencies of movements (Johansen et al., 2002).

The heterogeneity of the ADHD symptoms has been confirmed by the wide variety of manipulations in animals that can cause the presentation of some or all of the behavioural symptoms of ADHD. While each experimental model provides valuable insight into various aspects of the disorder, animal studies of the symptoms of ADHD are extensive, and a comprehensive review of each of the models is not practical for the purpose of this dissertation. This section will thus review 3 models in detail, namely the Spontaneously Hypertensive Rat (SHR), the dopamine transporter knock-out (DAT-KO) mouse and the coloboma mutant mouse, and briefly mention the other proposed models of aspects of ADHD, since they highlight the spectrum of possible causes for the disorder.

1.5.1 The Spontaneously Hypertensive Rat (SHR)

The spontaneously hypertensive rat (SHR) was originally selectively bred for hypertension from the Wistar-Kyoto (WKY) rat strain (Okamoto and Aoki, 1963), however behavioural studies in the late 70's and early 80's began to show that the SHR, usually in comparison with the WKY or Wistar rat strains, showed higher levels of activity and lower levels of anxiety in the open field test (Knardahl and Sagvolden, 1979; Danysz et al., 1983). Much research has since been conducted on this rat strain, which has now become the most well characterised animal model for ADHD (Russell, 2007). In fact, it is also the most well characterised model for hypertension (Pravenec and Kurtz, 2010), and although most details from the hypertension studies do not appear directly relevant to the ADHD studies, it serves to bear in mind that there is much physiological data available on this rat strain that could potentially provide better context and explanations for the results

of the direct neural studies that are the focus of this subsection.
Initial open field tests found that the SHR showed hyperactivity in comparison with the WKY gradually, after habituation to the environment (Knardahl and Sagvolden, 1979; Sagvolden et al., 1993b), and that the number of lever presses in a reinforcement task was higher in SHR compared to 4 other rat strains (Sagvolden et al., 1993b), which provided promising face validity for hyperactivity. Investigations of the reinforcement processes showed that the SHR had higher response rates to immediate reinforcements than WKY, however when reinforcement delivery was delayed after the response, the number of responses from the SHR for the delayed reward decreased more *sharply* than for WKY, although the number of SHR responses were higher than that for WKY (Sagvolden et al., 1993b). The authors interpret these results as an indication of a steeper delay-of-reinforcement gradient in the SHR compared to WKY, confirmed in a later experiment that showed that SHR and WKY behaviour did not differ if reinforcements were given immediately, however the responses of the SHR decreased more than that of WKY when a delay between the response and reinforcer was introduced (Sagvolden et al., 1993a). In response to methylphnidate, both the SHR and WKY showed increased numbers of responses, however the delay-of-reinforcement gradient in both strains was decreased, and the effectiveness of the delayed reinforcer was increased. This drug effect was less pronounced in the SHR compared to WKY (Sagvolden et al., 1993b). Methylphenidate also increased both SHR and WKY activity in terms of exploratory behaviour in the open field, as well as in the operant chamber in a more recent visual stimulus position discrimination task (Wultz et al., 1990; Thanos et al., 2010). Although sub-chronic methylphenidate treatment decreased rearing activity of the SHR in the Làt-maze (Aspide et al., 2000).

In a differential low rate of reinforcement task (DRL), where the rat was required to wait 1, 2, 5, 10, 20, 30 or 60s (DRL-1s $\sim$ DRL-60s) before pressing the lever in order to receive the reinforcement, the performance of the SHR was not impaired compared to the WKY or SDs. In fact, when high response rates were required (DRL-1s), the WKY performed the most poorly in comparison to both SHR and WKY (Bull et al., 2000). This study argued that the SHR did not exhibit excessive impulsivity, but that the WKY showed a uniformly low response rate (Bull et al., 2000). Another study used the DRL schedule to evaluate the performance of SHR, WKY and Wistar rats, found that the SHR exhibited impulsivity during the acquisition phase of the experiment, however higher doses of methylphenidate (4 and 8 mg/kg) impaired the performance of all three rat strains. Impulsivity was exhibited by SHR during acquisition, but methylphenidate impaired their performance in the task requiring response inhibition. The lack of efficacy of MPH in this study suggested that the SHR may not be a reliable model for ADHD, however it is also possible that the DRL protocol may not the optimum procedure for the measurement of impulsivity (na et al., 2009).

The α_{2A}-adrenoceptor agonist guanfacine improved sustained attention and reduced overactivity and impulsiveness in SHR in a multi Random Interval/Extinction procedure where the correct lever press (signaled by a discriminating lever light over the lever) was rewarded within 180s and an incorrect lever press was followed by an extinction schedule (Sagvolden, 2006). A similar behavioural test showed that *l*-amphetamine improved poor sustained attention while *d*-amphetamine reduced overactivity and impulsiveness as well as improved sustained attention in SHR (Sagvolden and Xu, 2008).

In a spatial learning task using the Morris Water Maze, atomoxetine, a selective NET blocker, improved spatial learning during the acquisition phase of the task, reflected in decreased latency to reach the platform in treated animals. Spatial memory was also improved as shown by increased amount of time spent in the target quadrant during the probe trial in the treated animals compared to controls (Liu et al., 2008). This study also measured increased histamine release in response to atomoxetine treatment and suggested a strong histaminergic component for the improvement of spatial memory in treated SHR (Liu et al., 2008). Unfortunately, no comparative strain was used in this study.

SHR neurotransmission has been studied extensively *in vitro*, especially via superfusion experiments, in comparison with the WKY rat strain. It was found that electrically stimulated [^{3}H]DA release from the caudate putamen and PFC in SHR was lower than that of WKY, while D2-autoreceptor function measured in response to quinpirole activation showed that DA release was significantly decreased in the caudate of the SHR, but not in the PFC or NAc in comparison with the WKY (Russell et al., 1995). These responses suggested that the SHR could have region specific sensitization of D2 autoreceptors in the caudate, but not in the PFC of the SHR (Russell et al., 1995). The increased response to quinpirole was supported by higher D2 receptor mRNA expression in the SHR striatum (Vaughan et al., 1999). Application of methylphenidate or *d*-amphetamine stimulated [^{3}H]DA release in the caudate, NAc and PFC of both SHR and WKY, although the methylphenidate stimulated response was $7 \sim 17$ fold less potent than that of *d*-amphetamine, and the amount of [^{3}H]DA released from the NAc was lower in the SHR compared to WKY (Russell et al., 1998). Electrical stimulation of [^{3}H]DA release in the presence of methylphenidate resulted in similar release levels in the SHR and WKY, while the presence of *d*-amphetamine caused more DA release from SHR than WKY (Russell et al., 1998). The difference in the mechanisms of drug action between methylphenidate and amphetamine could explain the difference in DA release levels in the presence of each drug. While both methylphenidate and amphetamine block the DAT and prevent reuptake of DA, amphetamine additionally causes vesicle leakage of DA into the cytoplasm by the reversal of vesicular monoamine transporter 2 (VMAT2) and DA release into the synapse by reversal of the DAT. Thus, the similar responses between SHR and WKY in

presence of low dose methylphenidate indicated that DAT function was not different between the strains, and is unlikely the source of the dopaminergic impairment in the SHR. The ability of *d*-amphetamine to mediate increased DA release in SHR compared to WKY suggested that the SHR had higher levels of DA in the cytoplasm of neurons that was released by the reversal of DAT in the presence of *d*-amphetamine. Taken together, this study suggested that the SHR may have impaired vesicular storage of DA (Russell et al., 1998). Stimulated release of norepinephrine from PFC slices either electrically or with potassium showed no difference between the SHR and WKY, however α_{2A}-adrenoreceptor mediated inhibition of norepinephrine was less effective in the SHR (Russell et al., 2000). These results suggested that although there was no deficiency in the release of NE, the inhibitory control of NE in SHR could be deficient (Russell et al., 2000).

Recently, an *in vitro* study of dopaminergic parameters in juvenile SHR and WKY striatal synaptosomes showed similar results to the superfusion experiments in that uptake rates of DA by DAT were not significantly different between the SHR and WKY (Simchon et al., 2010). However, although basal DA release was lower in SHR, potassium, amphetamine and methylphenidate stimulated release of DA were not significantly different between strains (Simchon et al., 2010), which contrasts the superfusion studies that showed increased amphetamine but not methylphenidate stimulated release in adult SHR (Russell et al., 1998). Chronic methylphenidate treatment (21 days, i.p, 3 mg/kg) in juvenile SHR caused decreased basal DA release compared to saline treated SHR, however potassium and amphetamine stimulated release was increased in the treated group (Simchon et al., 2010). In a study of 1 month old SHR, DAT and TH expression were found to be decreased compared to WKY (Leo et al., 2003). Measurement of DAT and VMAT2 showed significantly lower densities in the SHR striatum (Simchon et al., 2010). The lower VMAT2 densities support the previously suggested impairment of vesicular storage (Russell et al., 1998).

Taken together, the *in vitro* studies have suggested a hypofunctioning dopaminergic system and a hyperfunctioning noradrenergic system in the SHR (Russell, 2002; Russell et al., 2005). The lower basal release of DA could be be due to a compensation for the lower DAT densities in SHR, and the increased amphetamine induced release of DA could be due to decreased VMAT2 densities, resulting in elevated amounts of DA in the cytoplasmic compartment of the presynaptic neurons (Simchon et al., 2010). However, DAT densities were found to be *higher* in SHR compared to WKY in adult and juvenile rats, and in addition, the D1 receptor was found to be increased in adult SHR caudate (Watanabe et al., 1997). Further studies and analysis of variations in experimental methods are required in order to draw any conclusions from the various contradicting information.

In comparison to the number of *in vitro* neurochemical studies that have been per-

formed on the SHR, *in vivo* studies have been surprisingly few, most of which have been related to hypertension (Linthorst et al., 1991; Terrazzino et al., 1994; Miyamae et al., 1995; Ferguson et al., 2003), and the results have been no less disparate.

Initially, *in vivo* microdialysis found that although basal DA levels were not different between SHR and WKY, DOPAC levels were significantly lower in SHR than WKY in the caudate of 8 week old rats (Linthorst et al., 1991). Administration of quinpirole showed that the SHR had more responsive D2 autoreceptors, indicated by a stronger inhibition of DA release, although DOPAC levels were similar between SHR and WKY after quinpirole treatment (Linthorst et al., 1991). Likewise, basal dopamine levels in both the nucleus accumbens and the ventrolateral striatum were lower in SHR than WKY rats, and levels of dopamine in the nucleus accumbens (NAc) were lower than in the the ventrolateral ST in both strains (Fujita et al., 2003). Infusion of quinpirole also showed that SHR had more responsive D2 autoreceptors, particularly in the NAc, in comparison with the WKY (Fujita et al., 2003). In terms of behaviour, this study co-administered quinpirole with the D1 agonist SK-38393, which caused stereotypic jaw movements in WKY, but not in SHR, and suggested that the postsynaptic D1 receptors are hyposensitive compared to WKY (Fujita et al., 2003). An interesting measurement of tonic DOPA levels from the caudal ventrolateral medulla (CVLM) showed that the SHR had lower tonic DOPA levels than WKY (Miyamae et al., 1995). Injection of L-DOPA into depressor sites of the CVLM decreased SHR hypertension and suggested that tonic neural activity to release DOPA in the CVLM is lost in the SHR, which results in the maintainence of hypertension in SHR (Miyamae et al., 1995).

Contradicting results however have been reported in 2 subsequent *in vivo* microdialysis studies. Measurements taken from the NAc shell of 6 week old SHR showed significantly *higher* basal DA release compared to WKY, and low dose methylphenidate and amphetamine stimulated higher levels of DA increase compared to WKY (Carboni et al., 2003). Potassium stimulated release of DA was however lower in SHR compared to WKY (Carboni et al., 2003), which could be explained by the apparent VMAT2 defect in SHR impairing DA storage (Simchon et al., 2010). A comparison of SHR to the SD rat strain showed similar patterns as those with WKY, where measurements of DA from the ST were 78% higher in SHR than SD (Heal et al., 2008). Basal NE release measured from the PFC was 26% lower in SHR than SD (Heal et al., 2008). Low dose amphetamine treatment induced significantly higher levels of NE release from the PFC in the SHR compared to SD, while only higher dose (9 mg/kg) amphetamine induced striatal DA releases that were significantly larger than SD (Heal et al., 2008). An *in vivo* microdialysis study in SHR without strain comparison showed that atomoxetine treatment (2 mg/kg, i.p) significantly increased cortical NE, DA and histamine, that was correlated with improved spatial learning (Liu et al., 2008).

The shortcomings of *in vitro* studies lie largely in that neuronal sections have been taken out of context and the study of longer loop network effects are not possible. *In vivo* microdialysis is able to take highly accurate measurements of neurotransmitter concentrations from the most relevant context in a freely-moving animal, however it has poor spatial and temporal resolution. It is possible that more modern techniques of *in vivo* microdialysis in the latter studies could provide more accurate information than the previous microdialysis studies, however, a detailed review of microdialysis method refinement does not fall within the scope of this dissertaion. At this time, no *in vivo* voltammetric studies in SHR have yet been published. The present project will thus present novel data on the measurement of DA release and uptake in SHR, at a higher spatial and temporal resolution than can be studied by microdialysis.

Neurotransmission in the striatum and PFC have been studied extensively in the SHR, and age related changes have been found. Recently, micrographs of Nissl-stained striatal serial sections measured striatal volume of SHR compared with WKY at weekly intervals between $4 \sim 10$ weeks old (Hsu et al., 2010). It was found that the striatum was significantly smaller in the SHR compared to WKY only at 5 weeks of age (equivalent to 7∼9 years in humans (Quinn, 2005), but not in the subsequent ages, and suggested that abnormalities in the striatum for ADHD occurs at the prepubertal stage (Hsu et al., 2010). The lack of size difference in the ST in subsequent ages also suggests that the developmental defect that was detectable at 5-weeks was rectified via compensatory mechanisms until it is no longer structurally detectable by the Nissl-stained method. Although neurochemical studies show clear evidence of altered function.

Dendritic morphology of neurons in medial prefrontal cortex, hippocampus and nucleus accumbens were studied at 2, 4 and 8 months of age in SHR, campared to WKY with use of the Golgi-Cox stain and Sholl analysis (Sanchez et al., 2010). At 4 and 8 months, the SHR showed decreased spine density in pyramidal neurons from the mPFC and in medium spiny cells from the NAc. At 8 months, the pyramidal neurons from the hippocampus exhibited a reduction in the number of dendritic spines (Sanchez et al., 2010).

It has been suggested that the impaired metabolism (Doroshchuk et al., 2004) and Ca^{2+} regulation (Fellner and Arendshorst, 2002) in SHR could be the basis of many SHR defects, including that of hypertension. Higher intracellular Ca^{2+} levels would decrease the Ca^{2+} gradient across the neuronal membrane and could impair Ca^{2+} dependant neuronal function (Russell, 2007). This hypothesis is interesting, since the SNAP-25 defect in the coloboma mouse impairs its calcium regulated neurotransmission, which has been related to their spontaneously hyperactive behaviour (below).

Considerable concern has been attributed to the possible confounding effect of hypertension on results of the neurochemical studies on the SHR. In fact, more recent studies

have made concerted efforts to study the SHR at the pre-pubertal stage as opposed to the adult stage, since more sever hypertension develops between 6 $\sim$ 10 weeks of age inthe SHR, and only mild hypertension is present in SHR at 4 weeks of age (van den Buuse and de Jong, 1988). An *in vivo* microdialysis study had in fact suggested that increased sensitivity of D2 autoreceptors in SHR but not salt induced-hypertensive rats suggested that the alterations in the nigrostriatal DA system could be involved in the initiation of the development of spontaneous hypertension (Linthorst et al., 1991).
Genetic fingerprinting of SHR in 1991 had shown that SHR stocks between the NIH and the Shimane Institute of Health Science in Japan were substantially different genetically, especially in the substrain of stroke-prone SHR, while the NIH and Charles River SHR stocks differed only slightly (Nabika et al., 1991). Various substrains of both the SHR and WKY have been selectively bred for specific characteristics, e.g the WKY/HA substrain is hyperactive but not hypertensive, while the WKY/HT substrain is hypertensive but not hyperactive (Sagvolden, 2000). Studies between substrains have been useful to determine that the neurobiology of hyperactivity was not a result of hypertension. A more recent comparison of SHR and WKY stock from various sources found that the genetics of the WKY from New Charles River Germany (WKY/NCrl) and WKY from Harlan UK (WKY/NHsd) had diverged by 33.5% and that the WKY/NCrl is a good rat model for ADHD-I when measures of attention were shown to be impared in this substrain compared to WKY/NHsd and Sprague Dawley rats from Taconic, US (SD/NTac). Thus comparisons between the SHR from New Charles River (SHR/NCrl) with the WKY/NCrl as controls would be inappropriate. In addition, the SD/NTac and the WH/HanTac rats show significant genetic and/or behavioural differences from WKY/NHsd rats and appear not to be appropriate controls in studies using the SHR/NCrl (Sagvolden et al., 2008), since comparisons between genetically more similar strains and substrains that have divergent behaviours would provide a narrower focus in determining the origin of the specific behavioural difference (Sagvolden et al., 2009).

The genetic basis of the SHR behavioural deficits are not known. Gene sequencing of the D2, D4 and DAT1 genes showed that the SHR had several variations of the DAT1 gene which was found to be different from the that of the WKY, although no differences in the D2 or D4 receptors were found (Mill et al., 2005a). This finding supports the human linkage and association studies of the DAT gene to ADHD and provides additional support for the model.

Thus, although much disagreement exists in the results of the SHR studies regarding the neural basis of its undisputed hyperactive behaviour, and variably defined inattention and impulsivity, discrepancies due to differences in methodical approach and comparative strain selection between laboratories is possible, but also determinable by further study.

Research since the early 90's had found the SHR to conform to all three validation criteria for a good animal model for ADHD (Sagvolden et al., 1992b), and the SHR is still the strain of choice for the study of ADHD (Russell, 2007), and was utilized in the present study.

1.5.2 The Dopamine Transporter Knock-Out mouse

Since psychostimulant treatment blocks DAT activity, increases striatal DA and decreases locomotive hyperactivity, it serves to study DAT activity, or the effects of the lack thereof, in more detail. The DAT-KO mouse is relevant because it exhibits behavioural characteristics that include hyperactivity and impaired learning and memory (Gainetdinov and Caron, 2001), especially in terms of cognitive inflexibility, shown by their impaired extinction of responses in an operant food reinforcement task (Hironaka et al., 2004). Although, this mouse model also exhibits stereotypic behaviours which are more characteristic of Tourettes syndrome and obsessive compulsive disorder, and its exhibition of hyperactivity occurs predominatly in a novel environment, which contradicts the human behaviour where hyperactivity occurs after environmental habitutation; and is not an ideal animal model for ADHD (Berridge et al., 2005; Russell et al., 2005). However, the DAT-KO mouse provides much valuable insight into the consequences of the extreme loss of DAT and the hyperdopaminergic state, and provides highly relevant comparative data.

In vivo microdialysis of freely moving DAT-KO animals showed that the basal level of DA is at least 5 times higher than in wild type mice, while cyclic voltammetric measures in striatal brain slices showed that these persistently hyperdopaminergic mice had a 300-fold slower clearance rate of DA from the extracellular space and were also found to have a 75% decrease in amplitudes of electrically evoked dopamine release (Jones et al., 1998). Without DAT-mediated dopamine recycling, these mice synthesize new DA continuously, as shown by a complete elimination of DA from the striatum of the mice when tyrosine hydroxylase (TH) was inhibited with use of α-methyl-*para*-tyrosine, and explains the low DA releasing abilities of the striatal neurons (Volkow et al., 1996). Inhibition of of DA synthesis in this experiment also showed that the behavioural hyperactivity exhibited by these mice is mediated by the hyperdopaminergic state in the striatum, since the DA depletion also reversed their hyperactive behaviour (Volkow et al., 1996). The hyperdopaminergic state essentially abolished autoreceptor function, shown by the inability of quinpirole (D2 autoreceptor agonist) to reduce dopamine levels in DAT KO mice, while significantly depleting extracellular dopamine in wild type mice *in vitro* and *in vivo* (Jones et al., 1999). Additionally, D1 and D2 receptors were downregulated by approximately 50% in the ST of the KO mice (Giros et al., 1996). Immunodetection of the the D1 receptor in these mice showed that their trafficking to the plasma membrane and dendrites was reduced by 52% and 40%, respectively, and that the receptors showed

increased accumulation in the endoplasmic reticulum and golgi bodies at 95% and 131% respectively, compared to wild type mice (Dumartin et al., 2000).

The increase of dopamine availability with the use of methylphenidate to block DAT decreases symptoms of hyperactivity in ADHD, and it would seem paradoxical that the higher tonic dopaminergic levels in the DAT-KO mice would exhibit hyperactivity. However, the balance of dopamine in the brain is crucial in the ability to exert motor control (Kuhar, 1992). In cases of psychostimulant abuse where dopaminergic levels increase substantially more than that of treatment dosages of methylphenidate, hyperactivity is also observed (Kuhar et al., 1990; Inada et al., 1992). Hyperdopaminergia caused the expected downregulation of D1 and D2 receptors (Giros et al., 1996; Dumartin et al., 2000), however this compensation appears inadequate for normalizing the behavioural hyperactivity observed (Gainetdinov et al., 1999).

The DAT is a key protein involved in direct dopaminergic regulation, which is likely to be impaired in ADHD. Additionally, human genetic linkage and familial studies have associated various polymorphic alleles of the DAT1 gene to ADHD (Mill et al., 2005b; Bobb et al., 2005b; Dresler et al., 2010), and strongly support further study of the DAT.

1.5.3 The Coloboma (Cm) SNAP-25 mutant mouse

The coloboma mice carry a heterozygous deletion mutation on chromosome 2 that includes a sequence of the SNAP-25 gene, and was shown to exhibit characteristic 'head-bobbing', small eyes and profound spontaneous hyperactivity, compared to their wild type counterparts (C3H/HeSnJ) (Hess et al., 1992). Since the coloboma mutation was induced by neutron irradiation that caused a deletion of approximately $2{\times}10^6$ bp, or between 30 $\sim$ 50 genes, the coloboma phenotype likely arises from several of the affected genes (Hess, 1996). However, the hyperactive behaviour was attributed specifically to the deletion of the SNAP-25 gene by an elegant trangenic study (Hess et al., 1996). Hess *et. al* first showed that the coloboma mutants were 3$\sim$4 times more active than wild type, and that this hyperactivity was ameliorated by low dose (2 or 4 mg/kg, s.c.) *d*-amphetamine treatment, while amphetamine had no effect (2 mg/kg) or increased locomotor activity (4 mg/kg) in wild type mice. Stereotypic behaviour occured in both mutant and wild type mice when higher doses (8 and 16 mg/kg) of amphetamine were used. Methylphenidate treatment (2$\sim$32 mg/kg) however increased locomotor activity in both the mutant and wild type mice. Transgenic mice that over expressed SNAP-25 were then created, and cross bred with the coloboma mice. The results showed that the SNAP-25 expressing transgene normalized the activity in the coloboma mutants, without affecting the head-bobbing or opthalmic deformation (Hess et al., 1996). This work identified the SNAP-25 protein to be involved in the control of motor activity.

SNAP-25 is a peripheral membrane protein involved in the anchoring of vesicles to the

presynaptic terminal membranes of neurons, and assists in the Ca^{2+}-dependant exocytosis of neurotransmitters (Kandel et al., 2000). SNAP-25 appears necessary for neurotransmitter release in all CNS neurons, however highest expression levels appear in the neocortex, piriform cortex, hippocampus, nuclei in the anterior thalamus, pontine nuclei, substantia nigra and cerebellar granule cells (Oyler et al., 1989).

An initial *in vitro* study of cortical, dorsal striatal and ventral striatal slices showed that KCl stimulated release of DA from coloboma tissue slices was inhibited in the dorsal ST but not in the NAc, and was greater in the cortex compared to stimulated wild type slices, although basal release of DA was similar to wild type mice (Raber et al., 1997). The study also showed diminished stimulated release of 5-HT from in the dorsal striatum, but no difference in the NAc or cortex, and similar basal release levels compared to wild type. Additionally, stimulated glutamate release from cortical synaptosomes, as well as total glutamate content were significantly lower than that of wild type (Raber et al., 1997). It was thus demonstrated that the coloboma mutants exhibit region-specific and selective neurotransmitter deficiencies and suggested that there could be differential requirements for SNAP-25 in different neurons for the release of particular neurotransmitters (Raber et al., 1997), since the mutants have 50% less SNAP-25 mRNA expression in the whole brain (Hess et al., 1992). The DA metabolites HVA and DOPAC, measured by HPLC, were significantly lower in the NAc, and showed a trend to be lower in the ST of mutant mice. DA levels also showed trends to be lower in both the ST and NAc, while NE was significantly higher in both the ST and NAC, compared to wild type (Jones et al., 2001). These results supported that this mouse maintained a hypodopaminergic and hypernoradrenergic state.

A later *in vivo* microdialysis study showed that the mutant mice had basal DA concentrations that were at least 80% higher than the controls (Fan and Hess, 2007). This high basal dopaminergic tone was attributed to excess release and not impaired reuptake, indirectly measured by comparisons of the microdialysis extraction fractions between the mutants and controls, which is paradoxical considering the SNAP-25 deletion. Additionally, amphetamine stimulated release in the striatum was higher in the mutants, even after controlling for the basal release levels (Fan and Hess, 2007), contradicting the *in vitro* studies previously performed by the same group (Hess et al., 1992). The 2007 study focused on the ability of raclopride to inhibit the ability of amphetamine to decrease hyperactivity in the mutants, as well as the ability of amphetamine to increase the activity of controls, and showed that D2-like receptors in the mutants were hypersensitive and more effective at inhibiting cAMP levels (Fan and Hess, 2007). The most recent work by this group showed that additional deletion of the D2 gene from the mutant mice normalized both their hyperactivity and basal dopaminergic levels and targets the D2 (but not D3 or D4) receptor for mediating the hyperactivity of this mouse (Fan et al., 2010).

The hypernoradrenergic state was confirmed by an *in vitro* superfusion study that showed the mutants had at least 35% greater NE release from striatal and NAc slices, while DA release in the same region was comparable to that of wild type mice (Jones and Hess, 2003). Depletion of NE with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) (i.c.v.) from mutant mice was then shown to be effective in reducing hyperactivity (Jones and Hess, 2003). Specifically, antagonists to the α_{2C}-adrenergic receptor reduced hyperactivity in mutant mice, while antagonists of other adrenergic receptors had little effect (Bruno et al., 2007). These experiments implicate a strong noradrenergic contribution to the hyperactivity of these mice.

Besides spontaneous hyperactivity, the coloboma mice also display evidence of inattention, as determined by impaired inhibition in a delayed reinforcement task, and implulsivity, as determined through a delayed reinforcement task, of which inattention, but not impulsivity, could be reduced by DSP-4 treatment (Bruno et al., 2007).

Although much research has been performed on the coloboma mouse, the neurochemical basis for its behavioural symptoms have yet to be elucidated. The authors describe the ability of amphetamine but not methylphenidate to decrease hyperactivity in these mice as 'paradoxical' (Hess et al., 1996), however the difference between amphetamine and methylphenidate mechanisms of action in synaptic terminals was not compared. While both methylphenidate and amphetamine inhibit DAT function, amphetamine has the additional ability to cause DA release through reversal of DAT and VMAT2. This difference in drug mechanism is important in a SNAP-25 mutant, since amphetamine would be able to cause a release of DA from terminals through the reversal of DAT, while the inhibition of DAT by methylphenidate would cause a much milder increase in DA levels, by only slowing reuptake. Additionally, methylphenidate, even at low dose, has been shown to increase rodent activity. However, the *in vivo* microdialysis results that show higher basal DA *releasing* levels is paradoxical as compared to the *in vitro* studies, and considering the heterozygot SNAP-25 deletion. The authors hypothesize that the high extracellular dopamine *in vivo* could result from impaired GABA release due to the SNAP-25 defect, which leads to the disinhibition of the dopaminergic neurons that is not relevant in the *in vitro* studies, which show diminished release from striatal slices when measured directly (Fan et al., 2010).

Thus it appears that the coloboma mouse exhibits both hyperdopaminergia, as well as hypernoradrenergia. And while decreasing NE reduces hyperactivity and improves attention, a further (although moderate) increase in DA also decreases hyperactivity. Since the D2 receptor was found to be hypersensitive in the mutants, perhaps the increase in DA from amphetamine treatment decreased hyperactivity because of elevated D2 effects, while the wild type mice showed increased activity due to increased activation of the D1 receptor.

Along with construct validity from the efficacy of amphetamine treatment, genetic associations have linked a microsatellite in the SNAP-25 with hyperactivity in ADHD (Mill et al., 2005b). Although more extensive characterization of the impulsive and inattentive behaviours are required to determine a wider relevance of this model for ADHD aetiology, it is already evident that, further study of this animal model will provide valuable insight into the neural mechanisms that mediate the spontaneous locomotor hyperactivity in this model that is highly relevant for ADHD.

Additional manipulations have also caused presentations of symptoms of ADHD will not be elaborated upon. Briefly, prenatal exposure to alcohol produced defects in dopaminergic neurotransmission, attention deficit and hyperactivity (Hausknecht et al., 2005). Anoxia in the neonatal rat produced alterations in monoaminergic neurotransmission, spatial memory impairment and transient hyperactivity (Lou, 1996). Neonatal 6-hydroxydopamine (6-OHDA) lesioned rats displayed impaired learning in a spatial discrimination task and hyperactivity, but not impulsivity, which could be reduced by dopamine D4 receptor antagonists, as well as NET and 5-HTT inhibitors (Davids et al., 2003). Male transgenic mice that express a human mutant thyroid receptor in the pituitary (TRβ1) displayed symptoms of inattention, hyperactivity and impulsivity, which could be reduced by methylphenidate (Siesser et al., 2006). The deletion of the β2-subunit of the nicotinic acetylcholine receptor caused mice to display inattention, impulsivity and hyperactivity (Granon and Changeux, 2006; Russell, 2007). Mice that were prenatally exposed to nicotine displayed spontaneous locomotion that could be reduced by agonists to the $\alpha_4\beta$2-nicotinic receptor (Granon and Changeux, 2006). Poor performers in the 5-choice serial reaction time (5-CSRT) test selected from Wistar rats have been put forward as a model for impulsiveness and deficient sustained attention, but not hyperactivity (Puumala et al., 1996).

In all, animal models have provided much, although as yet unexplainable contradictions regarding the possible aetiology of ADHD. The dopaminergic, noradrenergic, serotonergic, cholinergic and histaminergic systems have all been implicated, but the mechanisms by which each of these systems participate to cause the various symptoms of the disorder requires further study.

1.6 The Dopaminergic and Noradrenergic Hypotheses for ADHD Aetiology

1.6.1 The Dopaminergic Hypothesis

The ability of stimulant drugs to treat symptoms of ADHD, and the DA increasing effects of these drugs have led to the logical deduction that ADHD should be the result of

a hypofunctioning dopaminergic system. Indeed, early single photon emission computed tomography (SPECT) imaging of [^{23}I]atropane binding to the DAT showed a 73% higher DAT density in the striatum of ADHD subjects (Dougherty et al., 1999). *In vitro* expression of the 10 repeat DAT1 gene in HEK-293 cells showed a 50% greater DAT desity than cells transfected with the 9 repeat allele, with similar affinities to methylphenidate, and possibly indicates increased DAT expression in 10 repeat allele carriers (VanNess et al., 2005). This 10 repeat alle for the DAT VNTR was associated with poorer spatial attention performance (Bellgrove et al., 2008). Taken together with the increased expression of D2 receptors (Vaughan et al., 1999) that correspond with increased D2 autoreceptor response (Russell et al., 1995), this results in a low tonic mesolimbic dopaminergic transmission in the ADHD subjects.

In addition to levels of DA, firing patterns of DA neurons are also highly important in DA signalling. Low frequency tonic background firing of DA neurons is usually maintained, with positive reinforcements causing short-lasting phasic increases in DA release, and error or extinction causing phasic short-lasting decreases in DA release (Hollerman and Schultz, 1998; Schultz, 1998). Thus reduced dopamine tonic firing would require increased phasic activation for adequate postsynaptic stimulation for reward to take place, resulting in their need for more frequent and potent reinforcers in order to maintain attention (Johansen et al., 2002). Likewise, low tonic DA firing would result in decreased sensitivity to the decreased phasic DA release that would prevent the detection of an error, resulting in decreased ability to learn from mistakes (Taylor, 1998). The resultant hyperactivity was hypothesized to be due to an acquisition of various 'intended' and 'unintended' reinforcers where the extinction signals for the unintended reinforcers were ineffective, resulting in an accumulation of responses that becomes viewed as excess motor activity due to reinforcers that cannot be identified (Johansen et al., 2002).

However, the DAT density hypothesis for the low dopaminergic tone has been challenged by more advanced and better controlled PET studies, which have shown that DAT density in the striatum and NAc is in fact *lower* in ADHD subjects than controls (Volkow et al., 2009; Jucaite et al., 2005; Volkow et al., 2007a). Genetic linkage studies have also associated the 9 repeat, and not the 10 repeat, VNTR of the DAT1 gene with ADHD symptoms (Dresler et al., 2010). Decreased DAT density, as well as basal and potassium stimulated DA release was measured in SHR synaptosomes (Simchon et al., 2010). Furthermore, the lack of correlation between the effect of methylphenidate on DAT inhibition and DA release support that the individual difference in response to methylphenidate is more likely due to individual differences in DA release at a given level of DAT inhibition (Ehrlich et al., 1960). DA synthesis was found to be decreased in the striatum of male adolescents with ADHD through PET scans of L-DOPA labelling (Forssberg et al., 2006), supported by decreased TH expression in the striatum of young SHR (Leo et al., 2003).

Thus the decreased DAT availability could have led to the compensatory decrease in DA levels in order to maintain homeostasis, and hyperactivity could result from a preferential activation of the D1 receptors, since D2/D3 receptor density was found to be decreased in the striatum, hypothalamus and NAc of adults with ADHD (Volkow et al., 2009).

Although the cause of low tonic DA availability in ADHD is under dispute, it appears that there is agreement that ADHD is a result of a hypodopaminergic state, although direct study of this assumption has not been possible in humans. Magnetic resonance spectroscopy ($[^1H]$-MRS) is a new and powerful technique that can measure absolute concentrations of various metabolites in the human brain, however DA and its metabolites are not currently included in the list of detectable molecules (Jansen et al., 2006). It has been argued that the increase in extracellular DA to improve symptoms of ADHD is not a sufficient argument for a dopaminergic deficiency hypothesis (Gonon, 2009), since the stimulant treatments such as methylphenidate and amphetamine have been misused frequently by healthy teenagers and young adults for their attention improving effects (Wilens et al., 2008; McCabe et al., 2006). Of course, the dopaminergic system does not operate in isolation, and the variety of animal manipulations have clearly shown that various defects in any of the neurotransmission systems could result in at least one, if not all of the behavioural impairments observed in ADHD. The dopaminergic hypothesis emphasizes the meso-limbic and meso-cortical dopaminergic systems as the most likely aetiological source, since pharmacological intervention at this level has been shown to be effective. It is clear that detailed study of the manner in which the interconnected neural systems interact in ADHD is necessary in order to understand the disorder as whole.

1.6.2 The Noradrenergic Hypothesis

Another convincing hypothesis is focused on an imbalance in the noradrenergic system. Like the success of stimulants that have lead to the proposal of the involvement of the dopaminergic system, noradrenergic drugs have also shown efficacy in improving the symptoms of attention, and support a hypernoradrenergic hypothesis. The α_{2A}-adrenoreceptor agonist guanfacine has been shown to improve visual object discrimination reversal performance in aged rhesus monkeys (Steere and Arnsten, 1997), and improved delayed response performance in young adult rhesus monkeys (Franowicz and Arnsten, 1998). Treatment with the less selective α_{2A}-adrenoreceptor agonist clonidine improved spatial working memory in normal young rhesus monkeys (Franowicz and Arnsten, 1999). In rats, working memory deficits induced by the anxiogenic drug, FG7142, could be reversed with guanfacine treatment (Birnbaum et al., 2000), which also decreased hyperactivity, impulsiveness and improved sustained attention in SHR (Sagvolden, 2006). Atomoxetine, a selective NET inhibitor increased histamine release and improved learning deficits in the SHR (Liu et al., 2008). The β_1-adrenergic antagonist, betaxolol, was shown to improve

working memory performance in rats and monkeys (Ramos et al., 2005).

The locus coeruleus (LC) projects widely to ipsilateral structures of the brain stem, midbrain and all cortical areas (Jones et al., 1977; Arnsten and Goldman-Rakic, 1984). Optimal focused attention occurs during moderate tonic NE discharge rates from the LC. Low rates lead to low arousal and inattentiveness and high rates lead to hyperarousal and distractibility (Foote and Aston-Jones, 1995; Usher et al., 1999). Brain areas most densely populated with α_{2A}-adrenoreceptors include the PFC and the LC (Aston-Jones et al., 1999; Arnsten et al., 1996), and could explain the beneficial effects of α_{2A}-adrenoreceptor agonists such as guanfacine. The activation of α_{2A}-adrenoreceptors in the LC by agonists such as guanfacine should cause a net decrease in its noradrenergic response to excitatory stimuli (Ivanov and Aston-Jones, 1995), and thus modulate LC discharge rates to improve sustained attention of those with ADHD. The noradrenergic hypothesis supports that a hypernoradrenergic system may possibly cause ADHD symptoms (Zametkin and Rapoport, 1987; Biederman and Spencer, 1999).

In vitro superfusion studies in the SHR have shown increased noradrenergic activity and decreased dopaminergic activity (Russell et al., 2000; Russell, 2002). [^{3}H]dopamine release from the nucleus accumbens in SHR was inhibited through the action of α_{2A}-adrenoreceptors (de Villiers et al., 1995). In fact, the beneficial pre frontal cortical cognitive effects of methylphenidate have been shown to be mediated through α_{2A}-adrenoreceptor and dopamine D1 receptor actions in rats (Arnsten and Dudley, 2005).

Genetic studies have also reported associations of ADHD with adrenergic genes (Comings et al., 2000), particularly the NET1 (Bobb et al., 2005a; Kim et al., 2008). Recent genome-wide association scans have additionally implicated the α_{1A}- and β_2-adrenoreceptors (Lasky-Su et al., 2008), while the α_{2A}-adrenoreceptor gene has been linked to specifically to the ADHD-I subtype (da Silva et al., 2008; Schmitz et al., 2006), providing further support for the noradrenergic basis for ADHD.

A catecholaminergic imbalance in ADHD is undisputed, although further studies are required to determine the relative interactions and contributions of each system for a more comprehensive understanding of ADHD aetiology.

1.7 *in vivo* Chronoamperometry

Few *in vivo* studies of the dopaminergic system in the SHR have been performed, all of which made use of microdialysis. The microdialysis technique is able to measure basal metabolite and neurotransmitter concentrations accurately, however the temporal resolution of microdialysis data is poor (tens of minutes). In contrast, *in vivo* chronoamperometry offers the ability to measure changes in dopaminergic concentrations within millisecond time scales, and micrometer spatial resolutions (Kawagoe et al., 1993). Thus, this type of

electrochemical recording can provide dynamic, real-time information of neurotransmitter release and clearance kinetics in discretely localized areas in animals (Gerhardt et al., 1999). When this recording technique is coupled with KCl stimulation and exogenous application of DA (by pressure ejection), it becomes possible to functionally map areas of the rat ST and NAc, which is difficult to achieve using other methods (Kawagoe et al., 1993). *In vivo* chronoamperometry has been used to study DAT activity in the DAT-KO mice (Gainetdinov et al., 1999; Gainetdinov, 2008), but none have yet been published for the SHR.

The use of *in vivo* voltammetric recordings for the analysis of neurotransmitters and neurochemicals in the central nervous system was pioneered by Ralph Adams in the early 1970s (Wightman et al., 1976). The concept of the technique is elegant in its simplicity. When the electropotential of an electrode is made sufficiently positive, it acts as an oxidant to molecules near its surface; electrons transfered to this surface by the molecules can be measured as current, that is directly proportional to the number of molecules oxidized, according to Faraday's Law (Adams and Marsden, 1982). Thus the concentration of the molecules at the electrode tip can be accurately calculated.

Typically, sensing or working electrodes are made with substances with low residual current such as carbon-pastes or graphite-epoxy resin, so that the current measured is a direct result of the molecules in contact with it, and not from the sensor material. Although highly sensitive, the selectivity of electrodes for specific substances of interest has been a major challenge in the field of electrochemistry. For example, DA, NE, 5-HT and ascorbate all oxidize at an applied potential of +0.55V, and the interpretation of the current measured from the cumulative oxidation of these substances is thus limited, since relative contributions of metabolites cannot be calculated. Considering the high amounts of ascorbate present in the brain, and its apparent differential distribution among the regions (Ewing et al., 1982; Stamford et al., 1984), its contribution to the measured signal was a large confounding factor. Various alterations of the electrode surface to improve its selectivity for specific substances have since been developed, and current technology allows for accurate detection of various molecules such as nitric oxide (NO) (Barbosa et al., 2008) and *l*-lactate (Burmeister et al., 2005), as well as neurochemicals such as glutamate, acetylcholine, NE, 5-HT and dopamine (Bruno et al., 2006; Day et al., 2006; David et al., 1998).

Particularly relevant for this project, the coating of the carbon electrode tip with Nafion was found, by Greg Gerhardt, to dramatically improve the selectivity of graphite electrodes for the measurement of cationic neurotransmitters. Nafion is a perfluorosulfonated polymer that renders the electrode practically impermeable to ascorbic acid and other anionic biogenic amine metabolites (Gerhardt et al., 1984). However, the Nafion treated electrodes would sense NE, 5-HT as well as DA; greater selectivity for DA can be

achieved by placing the electrode in an environment, such as the rat striatum, where DA concentrations are high, and other neurotransmitter concentrations are low. In this way, the chemical exclusion of interferents like ascorbate by Nafion, and the locational exclusion of other transmitters by targeting DA rich areas in the brain, affords measurements of high accuracy for DA specifically (Adams and Marsden, 1982; David et al., 1998).

In vivo chronoamperometry is thus a powerful technique for measuring the dopaminergic response to stimulations in real time, and ideal for the study of dopaminergic neurotransmission *in vivo*, in SHR. The generous donation of the equipment required for this technique from Greg Gerhardt allowed for its set-up in this laboratory.

The SHR is the most widely validated animal model for ADHD (Russell, 2007), however data regarding its dopaminergic function are currently contradictory and require further study. The *in vivo* chronoamperometry technique allows for the measurement of dopaminergic concentrations at high temporal and spatial resolutions, and has never yet been studied in the SHR at the time of this dissertation, and has never been set-up in this laboratory. Methylphenidate and guanfacine have exhibited the ability to improve cognitive function and reduce hyperactivity in children with ADHD (Taylor et al., 2004; Minzenberg and Carter, 2008; Biederman et al., 2008b). Thus, the present project elected to set-up the *in vivo* chronoamperometry technique in this laboratory and study potassium-stimulated release and uptake of exogenously applied DA in the striatum of the SHR. Additionally, the behavioural effects of methylphenidate and guanfacine on voluntary running activity and in the open field test were measured.

1.8 Aims

1. Verify strain purity of SHR and WKY stock at UCT.
2. Set-up *in vivo* chronoamperometry technique.
3. Record dopamine release and uptake parameters in anaesthetised SHR, WKY and SD rat strains, using *in vivo* chronoamperometry.
4. Characterise behavioural effects of methylphenidate at various doses on voluntary use of Running Wheels.
5. Characterise behavioural effects of guanfacine at a single dose in the Open Field and in Running Wheels.

2 DNA screening at 3 SNP loci

2.1 Introduction

The SHR, WKY and SD rats are albino strains which appear identical. Cross-breeding and strain contamination was a likely possibility. In December of 2008, samples from the University of Cape Town (UCT) stock of SHR and WKY were sent to Syracuse, New York for genome wide comparisons of 10 000 SNPs with other SHR and WKY stock from around the world. It was found that 1 of our WKY samples was a clear heterozygote between the SHR and WKY strains. Since the studies of the SHR model of ADHD is largely based on comparisons against the WKY strain, genetic screening of the SHR and WKY stock at UCT was required to ensure that the strains were not cross-contaminated prior to further breeding in the Animal Unit.

Dr Frank Middleton, Director, Microarray Core Facility and Center for Neuropsychiatric Genetics, SUNY Upstate Medical University, New York, USA generously provided 3 SNPs that would distinguish between the SHR and WKY strains, and identify mixed strains. These included 'D1RAT47' on chromosome 1, 'D2RAT62' on chromosome 2 and 'D3RAT24' on chromosome 3.

A search on the Rat Genome Database indicated that the expected SNP band sizes for the SHR/NCrl, WKY/NCrl (SHR and WKY from Charles River, USA) and WKY/NHsd (WKY from Harlan, UK) were identical and not polymorphic between the strains; 162 bp for D1RAT47, 166 bp for D2RAT62 and 151 bp for D3RAT24. Strain variations of expected product sizes of these SNPs occur for SHR/OlaHsd rats (SHR from Harlan Spague Dawley Inc, Indianapolis), which were 164, 191 and 151 bp, and for WKY/OlaHsd (WKY from Harlan Spague Dawley Inc, Indianapolis) which were 154, 177 and 144 bp for the D1RAT47, D2RAT62 and D3RAT24 SNPs respectively (http://rgd.mcw.edu, accessed 28-08-2010).

2.2 Methods

2.2.1 Tissue Collection

Animals were born in the main UCT Animal Unit where food and water was available *ad libitum*, temperature was controlled between 22 $\sim$ 24 $^{\circ}C$ and 12 hr light/dark cycle (06:00 lights on) was maintained. Tail snips (approx 3 mm) and ear punch material were collected from a total of 45 SHR and 52 WKY pups between P 21 and P 30 by an animal technician. Rats born from 'SHR' dams were labelled '1' in their first digit of their identification number, and 'WKY' were labelled '2'. Subsequent identification numbers were given in the order of collection. Tissue samples were stored at -20 $^{\circ}C$ until DNA extraction. This procedure was approved by the UCT Faculty of Health Science Animal Research Ethics Committee. Project number: 009/025.

2.2.2 DNA Extraction

Collected tissue samples were incubated in 450 μl Tail Buffer (1% SDS, 0.1 M NaCl, 0.1 M EDTA, 0.05 M Tris pH8) and 15 μl Proteinase K (20 mg/ml) at 50 $^{\circ}C$ between 1 $\sim$ 4 days (until the tissue was dissolved). Potassium acetate (200 μl of 5 M solution) was then added to each sample, shaken vigorously and left to stand for 2 min. Samples were centrifuged at 16 000 g for 3 min to exclude proteins. Each supernatant was placed in a new vial, to which 700 $\sim$ 800 μl of isopropanol (100%) was added. The mixture was shaken, left to stand for 2 min, and centrifuged again at 16 000 g for 3 min to pellet the DNA out of solution. The supernatants were discarded and each pellet was washed with 600 μl ethanol (70%) and centrifuged for the last time at 16 000 g for 3 min. The supernatants were discarded and pellets left to air dry for 20 $\sim$ 30 mins with the vial lids open. Pellets were resuspended in 50 μl of Tris EDTA (TE) Buffer (10 mM Tris-HCL pH 7.5, 1 mM EDTA) and stored at 4 $^{\circ}C$ until determination of DNA concentration and purity with a Nanodrop1000 spectrophotometer. DNA templates were diluted to 200ng/μl as required for polymerase chain reaction (PCR).

2.2.3 Polymerase Chain Reaction

Primer pairs for SNPs D1RAT47, D2RAT62 and D3RAT24 (Sequnces in Appendix A.1) were manufactured by Whitehead Scientific. Pellets were reconstituted in TE buffer in 100 μM stock solutions and diluted to 10 μM working solutions.

For each 25 μl reaction, a primer pair (D1/D2/D3; $\sim$10 ng F and R), template DNA ($\sim$200 ng), 2$\times$ GoTaq® Green Master Mix (Promega Corporation) and dH_2O made up the total, were combined in a 200 μl PCR tube. The thermalcycler was programmed with

an initial 95 $^{\circ}C$ for 5 mins for activation of GoTaq®. Amplification occured through 40 cycles of the following: 95 $^{\circ}C$ - 30 s; 56 $^{\circ}C$ - 30 s; 72 $^{\circ}C$ - 40 s. Finally, the temperature was held at 72 $^{\circ}C$ for 7 mins for elongation of products. Each sample was run at least twice, on different days to ensure accuracy.

2.2.4 Visualization

Agarose was dissolved in TBE buffer (4%), ethidium bromide (EtBr) (1%) was added immediately prior to casting. Once set, gels were placed in horizontal electrophoresis tanks filled with TBE buffer. Each PCR sample was loaded and run at 60 V for 180 mins. GeneRuler Ultra Low Range DNA Ladder (Fermentas) was used in at least one but usually two lanes of each gel. Bands were detected via UV light and images were captured with UVCentral Software.

Band sizes were calculated by the generation of standard curves from distances moved by each band of the ladder vs log of each band size for each gel. Standard curves were fitted with Excel 2007 (Microsoft). The resultant band sizes of D1RAT47, D2RAT62 and D3RAT24 SNPs were calculated from the distance travelled by the band in relation to the standard curve of the gel. Band sizes of replicate gels were averaged to produce one size per band.

2.3 Results

Determination of DNA concentration using a spectrophotometer showed a very high average yield of 1090.7 ng/μl, with a standard deviation of $\pm$420.6 ng/μl. The 260/280 ratios were 1.98 $\pm$ 0.05, and 260/230 ratios were 1.85 $\pm$ 0.19. Of the 97 collected tissue samples, 9 samples did not contain sufficient tissue for successful extraction of DNA templates and were discarded. Discarded samples were random and each had corresponding littermates that produced high yields of DNA templates.
A typical 4% agarose gel following the PCR amplification presented clear amplified bands with expected primer dimers around the 50 bp range (Fig. 2.1).

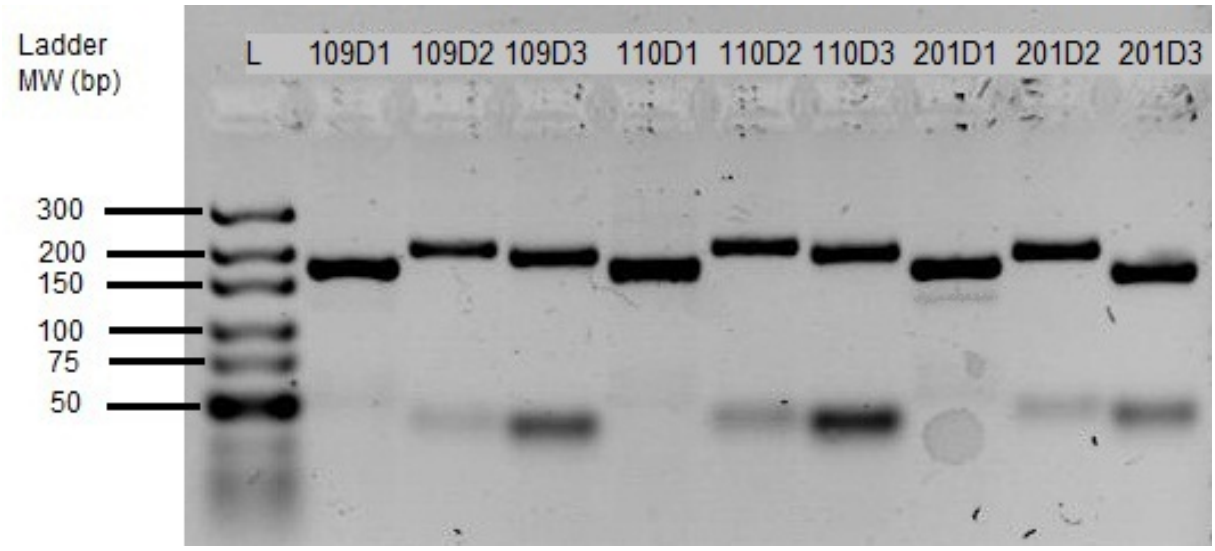


Figure 2.1 – An example gel showing DNA ladder on the far left; SHR is indicated by '1' in the first digit, WKY indicated by '2' in the first digit; Number following the first digit labels the individual rat; D1/D2/D3 indicate the targeted SNP; Bands around 50 bp were primer dimers; Double band was detected in 201D1

An example of a standard curve generated from a gel showed the expected linear relationship between the log of the MW of the ladder vs the distance migrated by the band on the gel (Fig. 2.2).

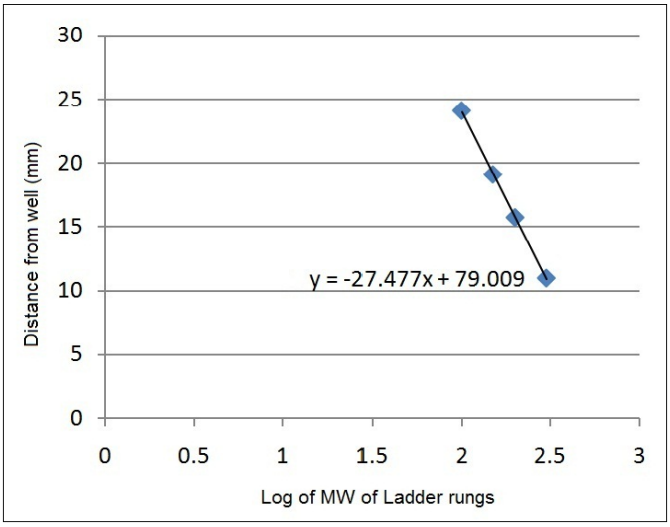


Figure 2.2 – Example of a standard curve generated from the ladder rung of Gel 17 with fitted equation

The median of band sizes of our WKY D1RAT47 D2Rat62 and D3RAT24 band sizes were 5, 8 and 2 bp larger than the WKY/OlaHsd respectively. SNP band size differences between strains showed that the medians of the SHR were 12, 16 and 39 bp larger than the WKY bands for the D1RAT47, D2RAT62 and D3RAT24 SNPs respectively (Table. 2.1). Thus although the D1RAT47 and D2RAT62 SNPs showed the expected approximate 10 bp difference between our strains, the D3RAT24 SNP in the SHR was almost 4 times longer than the expected 10 bp difference suggested by Dr Middleton.

Table 2.1 – Difference in median and mean SNP sizes (bp) between SHR and WKY samples

	SHR-WKY		
SNP	D1RAT47 (bp)	D2RAT62 (bp)	D3RAT24 (bp)
Medians	12	16	39
Mean	17	25	39

An acceptable range of band sizes for each SNP of each strain was calculated by the median± the standard deviation (Fig. 2.2). The rats of all samples that produced bands that did not fall within the ranges were excluded as possible future breeders. In total, 8 SHR and 12 WKY were selected for breeding.

Table 2.2 – Median, mean and standard deviations of SNP sizes (bp) of SHR and WKY samples, with comparison to the expected SNP sizes of rats from Charles River, USA (/NCrl), Harlan, UK (/NHsd), and Harlan Sprague Dawley Inc, Indianapolis (/OlaHsd)

	SHR			WKY		
SNP	D1RAT47	D2RAT62	D3RAT24	D1RAT47	D2RAT62	D3RAT24
	(bp)	(bp)	(bp)	(bp)	(bp)	(bp)
Expected size if from /NCrl or /NHsd	162	166	151	162	166	151
Expected Size if from /OlaHsd	164	191	151	154	177	144
Expected Size if from SD/Rij	170	168	182	170	168	182
Medians of sizes from present study	171	201	185	159	185	146
Difference between Median and expected /NCrl & /NHsd	9	35	34	-3	19	-5
Difference between Median and expected /OlaHsd	7	10	34	5	8	2
Difference between Median and expected SD/Rij	1	33	3	-11	17	-36
Mean of sizes from present study	170	201	181	153	176	142
StdDev of sizes from present study	7	9	8	6	6	5
Acceptable range (Median± StdDev)	164 ~ 177	192 ~ 210	177 ~ 193	153 ~ 164	179 ~ 192	141 ~ 151

Table 2.3 – SNP sizes from all SHR and WKY samples. Highlighted blocks indicate band sizes that did not fall within the acceptable ranges. Rats that were selected for future breeding are marked with (o)

Select	SHR	D1RAT47	D2RAT62	D3RAT24	Select	WKY	D1RAT47	D2RAT62	D3RAT24
(o)	101	173	210	188	(o)	201	170	192	159
		122	188			202	160	178	143
o	104	169	198	188			115		
o	105	169	204	193		203	151	178	143
o	107	176	195	185		204	169	178	155
o	108	166	195	185	o	205	158	185	146
	109	183	217	199	o	206	158	185	146
	110	183	227	208	o	207	158	185	146
o	111	166	206	185	o	208	161	184	150
	112	172	210	196		209	172	184	146
o	113	172	196	184		210	172	184	161
	115	172	210	210		212	160	194	145
	116	170	207	192				125	
				148		213	158	243	151
	117	174	246	184				180	
		154	202			214	167	252	154
	118	172	243	196				188	
			205	145	o	216	165	186	149
	119	177	212	201	o	218	158	181	143
				157	o	219	156	183	146
	120	177	218	200		220	147	237	143
				150				175	
	121	177	209	200	o	221	157	181	145
			184	150		222	157	236	145
o	122	175	207	187				185	
	123	158	181	175		223	163	256	151
		147		138				185	
	124	153	187	181		224	162	245	149
		140	163	138				187	
	125	177	209	192		225	159	240	149
				148				183	
	126	164	196	185		226	159	231	146
			177					183	
	129	170	209	188		227	160	180	150
			177			228	162	234	150
								180	

Table 2.4 – Continuation of previous table. SNP sizes from all SHR and WKY samples. Highlighted blocks indicate band sizes that did not fall within the acceptable ranges Rats that were selected for future breeding are marked with (o)

Select	SHR	D1RAT47	D2RAT62	D3RAT24	Select	WKY	D1RAT47	D2RAT62	D3RAT24
(o)	130	180	251	195	(o)				
			212		o	229	162	185	146
			187		o	230	157	183	143
	131	172	240	187	o	231	157	188	143
			212		o	232	158	180	146
			187			233	159	254	152
	132	172	240	187				189	
			208			234	159	242	148
	133	162	200	185				185	
			180			235	163	189	152
	134	175	205	185		236	159	248	148
		162						187	
	135	171	200	185		237	167	261	152
			185					189	
	136	172	240	188		238	159	248	150
			200					185	
	138	173	240	189		239	158	187	153
			203			240	162	256	153
			178					193	
o	139	162	187	177		241	161	182	148
	140	352	222	179		242	162	193	145
		332	190			243	162	187	149
		159	175			244	157	227	141
	141	158	216	170				172	
			183			245	149	229	141
			162					168	
	142	165	232	183		246	149	168	139
			197			247	153	241	146
	143	161	194	183				179	
			175			248	150	175	145
	144	161	225	179		249	157	175	143
			197			250	153	177	143
			169			251	152	171	140
	145	159	214	175		252	161	169	138
		147	188				147		
			169						

A subsequent RatGenome Database search revealed the strain variations that could be expected from the 3 SNPs that were analysed in the present study (Table. 2.5).

Table 2.5 – Strain variations of the 3 SNPs used in this study according to the Rat Genome Database

Strain Variations for D1RAT47			Strain Variations for D2RAT62			Strain Variations for D3RAT24		
#	Strain Symbol	Allele Size (bp)	#	Strain Symbol	Allele Size (bp)	#	Strain Symbol	Allele Size (bp)
1	ACI/N	162	1	ACI/N	168	1	ACI/N	144
2	AVN/Orl	168	2	AVN/Orl	168	2	BBDP/Rhw	182
3	BBDP/Rhw	179	3	BBDP/Rhw	189	3	BBDR/Rhw	182
4	BBDR/Rhw	162	4	BBDR/Rhw	189	4	BC/CpbU	144
5	BC/CpbU	162	5	BC/CpbU	189	5	BDIX/Han	144
6	BDIX/Han	168	6	BDIX/Han	168	6	BDVII/Cub	180
7	BDVII/Cub	168	7	BDVII/Cub	189	7	BN-Lx/Cub	144
8	BN-Lx/Cub	158	8	BN-Lx/Cub	175	8	BN/SsNHsd	144
9	BN/SsNHsd	158	9	BN/SsNHsd	175	9	BP/Cub	166
10	BP/Cub	168	10	BP/Cub	173	10	BUF/Pit	144
11	BUF/Pit	170	11	BUF/Pit	189	11	COP/OlaHsd	179
12	COP/OlaHsd	162	12	COP/OlaHsd	173	12	DA/PitN	144
13	DA/PitN	162	13	DA/PitN	168	13	DON/Melb	146
14	DON/Melb	158	14	DON/Melb	168	14	F344/Pit	144
15	F344/Pit	158	15	F344/Pit	168	15	FHH/Eur	146
16	FHH/Eur	164	16	FHH/Eur	168	16	GH/Omr	144
17	GH/Omr	166	17	GH/Omr	189	17	GK/KyoSwe	144
18	IS/Kyo	168	18	GK/KyoSwe	177	18	IS/Kyo	179
19	LE/Mol	170	19	IS/Kyo	177	19	LE/Mol	144
20	LH/Mav	162	20	LE/Mol	189	20	LEW/Pit	182
21	LN/Mav	162	21	LEW/Pit	189	21	LH/Mav	144
22	LOU/CHan	162	22	LH/Mav	189	22	LN/Mav	144
23	M520/N	158	23	LN/Mav	189	23	LOU/CHan	148
24	MHS/Gib	170	24	LOU/CHan	168	24	M520/N	144
25	MNR/N	170	25	M520/N	189	25	MHS/Gib	182
26	MNRA/N	170	26	MHS/Gib	189	26	MNR/N	144
27	MNS/Gib	170	27	MNR/N	168	27	MNRA/N	144
28	MR/Pit	170	28	MNS/Gib	163	28	MNS/Gib	182
29	NEDH/K	170	29	MR/Pit	168	29	MR/Pit	144

Table 2.6 – Continued. Strain variations of the 3 SNPs used in this study according to the Rat Genome Database

Strain Variations for D1RAT47			Strain Variations for D2RAT62			Strain Variations for D3RAT24		
#	Strain Symbol	Allele Size (bp)	#	Strain Symbol	Allele Size (bp)	#	Strain Symbol	Allele Size (bp)
30	NP9	162	30	NEDH/K	189	30	NEDH/K	151
31	ODU/N	162	31	NP9	189	31	NP9	144
32	OKA/Wsl	164	32	ODU/N	194	32	ODU/N	166
33	OM/Ztm	162	33	OKA/Wsl	177	33	OKA/Wsl	179
34	P5C	170	34	OM/Ztm	189	34	OM/Ztm	164
35	PVG/Pit	164	35	P5C	163	35	P5C	182
36	SD/Rij	170	36	PVG/Pit	189	36	PVG/Pit	144
37	SHR/OlaHsd	164	37	SD/Rij	168	37	SD/Rij	182
38	SHRSP/Riv	164	38	SHR/OlaHsd	191	38	SHRSP/Riv	182
39	SR/Jr	162	39	SHRSP/Riv	177	39	SR/Jr	144
40	SS/Jr	162	40	SR/Jr	185	40	SS/Jr	144
41	WAG/RijKyo	162	41	SS/Jr	189	41	WAG/RijKyo	144
42	WF/Pit	166	42	WAG/RijKyo	187	42	WF/Pit	144
43	WIST/Nhg	170	43	WF/Pit	189	43	WIST/Nhg	144
44	WKY/OlaHsd	154	44	WIST/Nhg	168	44	WKY/OlaHsd	144
45	WN/N	162	45	WKY/OlaHsd	177	45	WN/N	164
46	WTC/Kyo	162	46	WN/N	189	46	WTC/Kyo	144
			47	WTC/Kyo	177			

2.4 Discussion

The yields achieved from the DNA extractions were high. Each PCR reaction required only only 200 ng/μl, and at least 500 μl of high concentration genomic DNA template was extracted from the samples that had sufficient tissue. Those samples that did not contain sufficient tissue were discarded, however each discarded sample had other littermates that should have had similar genetic profiles. Residual ethanol content was possibly high as reflected by the 260/230 ratios, and 260/280 ratios suggested that samples were devoid of protein contamination, but may contain some level of RNA, however neither ethanol or RNA interfered with the PCR amplifications, since the high yield of template DNA required very low volumes of the extracted templates to be used (typically 1 μl), and thus the level of ethanol and RNA in the reaction samples would be negligible. Since the expected difference in SNP band sizes between the strains were so small ($\sim$10 bp), a high percentage agarose gel (4%) was required in order to differentiate the bands. Gels were run at very low voltage over a long period of time in order to minimize the amount of heat and shearing of the bands through the agarose medium.

The SHR and WKY of this study were reportedly purchased from Charles River Laboratories, USA (Personal communication with UCT Faculty of Health Sciences Animal Unit manager), However the calculated medians of the SNPs for each strain showed smaller differences with the expected SNP sizes of SHR and WKY from Harlan, Indianapolis, and much larger differences when compared with the expected band sized of rats from Charles River, USA or Harlan, UK. The present results suggest that the rats in the Animal Unit were more likely to have been purchased from Indianapolis, and not Charles River. The reported expected band sizes from the Rat Genome Database are exact sequenced sizes, and the slight differences in band sizes between the calculated band sizes of our SHR and WKY compared to the exact band sizes of the SHR/OlaHsd and WKY/OlaHsd was not surprising. However, this assumes that the UCT rats were in fact purchased from Indianapolis.

In the present study, the SNP band sizes were calculated according to the distance migrated compared to ladder bands of known sizes. The sizes of expected bands were unknown in this study because should both strains have originated from Charles River USA, there would have been no expected variability in these 3 SNP loci according to the Rat Genome Database, however polymorphisms were found at the loci. Thus the medians of all of the generated bands for each SNP for each strain were taken to be the 'correct' size to expect for our SHR and WKY, and an 'acceptable range' was calculated from the median$\pm$standard deviation to account for gel, measurement and standard curve variabilities.

There is a possibility that rats that were of pure strain were excluded from breeding

as a result of this selection method, since there were rats that did not show double bands, but were exluded due to band sizes that did not fall within the calculated acceptable size range. However, calculated band sizes could be variable because of different brands of agarose, electrophoresis tanks and powerpacks used on the various experimental days, in different laboratories. These difference could have changed the rate at which the bands were pushed through the gel and changed the slope of the standard curve and thus the calculated band size, and without known pure strain SNP samples for each strain to run on the gels as controls, this type of variation could not be accounted for. However, it was decided that it would be more prudent to maintain a more stringent selection process, since the number of generations that had passed since the possible cross breeding was unknown, and whether it was possible that with chromosomal crossing over during fertilizations, some SHR could have bred to have homozygous SNP alleles that were characteristic of WKY size, and vice versa.

The bands that were larger than the median+standard deviation were suspected to have been due to the binding of primers onto the amplified SNPs, since some samples were kept at $^{\circ}C$ overnight prior to gel electrophoresis the subsequent day. However, these bands occured repeatedly in the D2RAT62 SNP. When PCRs were repeated without overnight incubation of products, the same results were observed. Non-specific amplification could also explain bands that were too small or too large. This appeared likely, since it occured mainly for the D2RAT62 and D3RAT24 SNPs. Perhaps the *Taq* or primers were not sufficiently specific, and caused an amplification of other bands around 250 bp in the D2RAT62 SNP.

Post selection, it was observed that the median size for the D3RAT24 SNP in the SHR was 34 bp larger than the expected size at this locus for SHR of any origin. Bands around the expected 151 bp occured only 7 times and were always accompanied by the larger bands that were of similar size to the calculated median. This observation suggests that all of the SHR that produced an unexpected band of around 185 bp were selected for breeding, while rats that produced the expected SNP sizes of around 151 bp were not selected for further breeding, although double bands occurred for every $\sim$151 bp band that appeared, which would have excluded the same rats from selection. However, the origin of the 185 bp bands that were so numerous that it was selected as the 'correct' band is unknown. Possible candidates of the origin of this band size at this locus according to the Rat Genome Database are the Stroke Prone SHR of the Riv substrain, SD of the Rij substrain or diabetes prone rats BBDP/Rhw and BBDR/Rhw where the D3RAT24 expected SNP size is 182 bp. The diabetes prone rats are less likely, since their darker, piebold/grey fur and much larger body mass render them easily distinguishable from

the SHR, however the SHRSP and SDs appear identical to our SHR and cross breeding between these strains could have been possible. In fact, the median band sizes of D1RAT47 and D3RAT24 our SHR were more similar to the SD/Rij than to SHR/OlaHsd, while the D2RAT62 SNP was more similar to the SHR/OlaHsd. These results suggest that although 'pure' SHR were selected on the basis that they were homozygous for our calculated expected band sizes, there were no SHR that were homozygous for all 3 of the SNPs of the size expected from a pure SHR/OlaHsd strain, and that all of the sampled SHR were possible crossed with SD/Rij. This interpretation would suggest that a separate strain crossing had occured since the initial 10 000 SNP screening in Syracuse, New York, which had revealed only a direct SHR and WKY cross, and no involvement of the SD strain.

Only 3 SNP loci were examined per rat in this screening, and it was possible that during chromosomal crossing over and breeding over an unknown number of generations, mixed strains could become homozygous for some of these alleles but not others. Thus some loci could produce single bands, however they could have been single bands of a different rat strain. If this were the case, the optimal solution would be to purchase new stock from Charles River or Indianapolis, since this laboratory is not equipped for whole genome screening.

Recent advances in genetic technologies and the focus that the SHR receives due to its validated importance for the study of hypertension has led to full genome sequencing of the SHR/Ola (NIH decendants), as well of the progenitor normotensive strains, now available on SHRBase (http://shr.csc.mrc.ac.uk/index.cgi) (Pravenec and Kurtz, 2010). Embryonic stem cells have also been developed from rat blastocysts in the rat (Buehr et al., 2008), and the phenomenal technology of targeted gene KO with the use of zinc-finger nucleases (ZFNs) has been shown to reliably knock out genes of choice with between 25% to 100 % efficiency, that are also faithfully transmitted through the germline, via a single microinjection (Geurts et al., 2009). The technologies that were available for the manupulations of the mouse genome since 1981 (Buehr et al., 2008) are finally available for the rat. These powerful new technologies will provide unprecedented opportunities to identify gene functions of each of the variants systematically (Pravenec and Kurtz, 2010) and will not only advance the study of SHR for hypertension, but also allow the study of direct contributions of each implicated gene in ADHD on behavioural phenotypes. The variants between the impulsive and non-impulsive subpopulations of the SHR (Adriani et al., 2003) could lead to the identification of the genetic basis of impulsiveness (at least in the SHR, but could have wider implications). Finding overlapping variants between the SHR/NCrl, WKY/HA and WKY/HT would have the potential to identify the genetic basis of hyperactivty. Further, heterogeneity of the SHR can be even more stringently controlled, and aid conclusions that can be drawn from comparative neurochemical and

behavioural studies. With the assistance of genetic technologies, further research on the SHR as an animal model for ADHD is highly promising and exciting.

3 *In vivo* Chronoamperometry

3.1 Introduction

As introduced in section 1.7, the measurement of accurate dopamine concentrations in the striatum of the SHR in real time and high spatial resolution is possible with the use of *in vivo* chronoamperometry and Nafion coated carbon fibre electrodes (Day et al., 2006; Bruno et al., 2006). Much research has been performed *in vivo* in adult Fischer-344 (Friedemann and Gerhardt, 1992; Diao et al., 1997; Cass and Gerhardt, 1995) and SD (Gerhardt et al., 1987) rats, as well as rhesus monkeys (Cass et al., 1995; Gerhardt et al., 1996) with this technique, and the parameters for the measurement of dopamine *in vivo* have been thoroughly optimized, especially in anaesthetized animals. Studies utilizing the carbon fibre electrode in freely moving rats have also been performed (Sabeti et al., 2002), however, since the technique had never been set-up in this laboratory, it was not recommended to attempt to record from freely moving SHR at this stage (personal communication with Gregory Gerhardt).

The present study utilized well established parameters that could detect dopamine most reliably in the rat striatum, as published by the Gerhardt laboratory (Friedemann and Gerhardt, 1992; Diao et al., 1997; Cass and Gerhardt, 1995; Gerhardt et al., 1987) in order to assess the success of the set-up and acquire comparable data. An applied potential of -0.55 V between the working electrode and the reference electrode was used to cause the oxidation of a narrow range of molecules, of which NE, 5-HT and DA were most relevant. Measurements were taken from the SHR ST and NAc firstly due to its regional relevance of the ADHD hypotheses, and additionally to minimize the contributions of NE and 5-HT from recordings. Animals were deeply anaesthetized with urethane as it was shown to minimally interfere with DA neurotransmission (Sabeti et al., 2003).

Potassium stimulated release of endogenous DA aimed to measure and compare possible differences in the amplitudes of DA released between the SHR, WKY and SD rat strains, thus the volume of KCl applied was controlled. DA clearance rates were measured by the application of exogenous DA of varying volumes such that the amplitudes attained were controlled, and served as an indication of DAT activity. Each application of either potassium or exogenous DA increased the regional DA concentration until the peak amplitude was reached. The subsequent decline of the DA concentrations was measured as the clearance rate. The T_{80} and T_{50} parameters were the time (s) for the concentration of DA to decrease to 80% or 50% of the peak amplitude respectively, while T_c, was the time

of clearance between 60% to 20% of the peak amplitude.

Conclusions that can be drawn from the results recorded from anaesthetized animals are limited and cannot be extrapolated directly to freely moving animals, or humans. However information obtained in this manner serves to characterise the neurochemistry of SHR under these conditions, which would be comparable with other studies (Sabeti et al., 2003; Gerhardt and Burmeister, 2000).

3.2 Methods

3.2.1 Animals

Animals were tranferred to the satellite Animal Unit at P 21. Food and water was available *ad libitum*, temperature was controlled between $22 \sim 24\ ^{\circ}C$ and a 12 hr light/dark cycle (06:00 lights on) was maintained. Rats were kept with littermates, between $2 \sim 5$ per cage until surgery. Rats from 75 g $\sim$ 160 g were used between P 32 $\sim$ P 37. Including initial tests that do not contribute to the analyzed data, a total of 20 SHR, 15 WKY and 13 SD were utilized in this study. In the portion of the experiments where KCl was used to stimulate endogenous DA release, the results from 8 SHR, 8 WKY and 7 SD were used; in the portion of the experiment where exogenous DA was applied, the results from 9 SHR, 9 WKY and 5 SD were used, however some rats were involved in both the KCl and DA experiments. 4 SHR and 2 WKY were used to test the accuracy of the stereotaxic apparatus and the histology technique. This experiment was approved by the UCT Animal Research Ethics Committee. Project numbers: 009/005 and 009/065.

3.2.2 Equipment Set-Up

Immediately after the arrival of the core components of the FAST equipment in March 2009, our laboratory began the attempt to set it up. Although there was much support from the Lexington labs through Skype and email contacts, and previously published papers of this technique were consulted thoroughly, many practical details of the set-up remained elusive. Fortunately, I was able to visit the Gerhardt laboratory for two weeks in July 2009 where I obtained all of the critical details required to set-up the equipment and received practical training to use this technique effectively.

3.2.2.1 Equipment Components

The hardware components include the Fast Analytical Sensing Technology (FAST) mkI Control Box (black), a picospritzer (white) and a headstage (Fig. 3.1), which communicate with the FAST v2.3 computer program (Fig. 3.2). In addition, a pulled and bumped glass micropipette, a carbon fibre working electrode and silver chloride reference electrode were

required for each experiment. The preparation methods for each of these are detailed below. Briefly, the sensing tip of the electrodes were inserted into defined brain areas, while the opposite end was connected to the headstage, which connected to the FAST control box, and finally the FAST program. The FAST v2.3 program, along with the appropriate drivers were installed on a dedicated computer, with the anti-virus programs removed. The program was set to detect signals from a ×50 headstage (20 pA/mV) and the FAST mkI Control Box. Gain settings for Channel 1 on the control box were set at '2×' and corresponded to the settings on the software.

Settings were verified in the Amperometry environment in the program where the Applied Potential was set to -0.5V to produce a +0.55V against the Ag/AgCl reference electrode. The current measured when a 10 MΩ resistor connected between the 'Ref' and 'E1' jacks was confirmed to be 5 nA.

Figure 3.1 – Headstage connects electrodes to FAST Control Box. A 10 MΩ resistor connecting the 'Ref' and 'E1' jacks should produce 5 nA reading in the amperometry environment (Applied Potential = - 0.5V) once the correct settings had been selected.

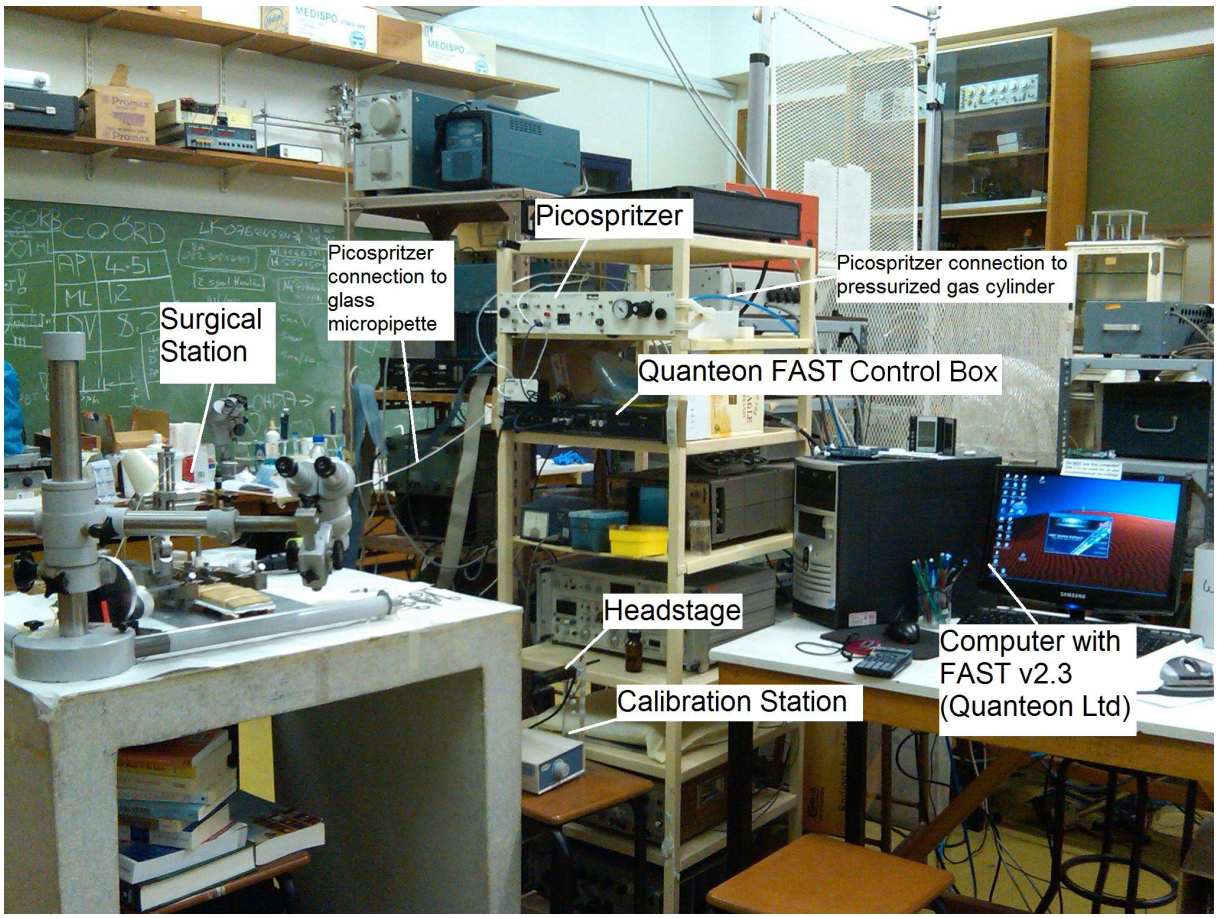
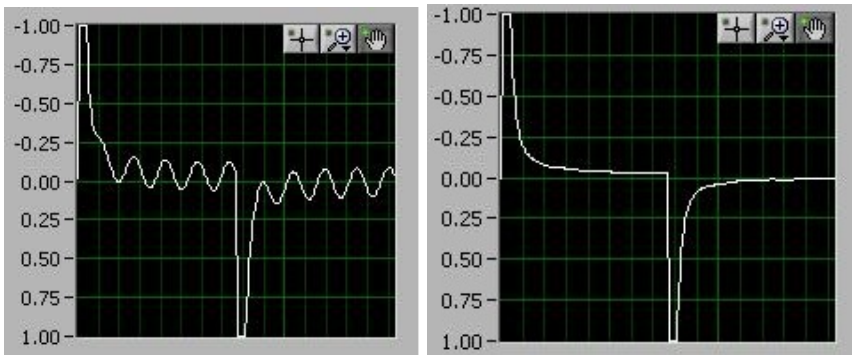


Figure 3.2 – Photograph of FAST set-up in our laboratory. FAST Control Box connects the Headstage (at the Calibration Station in this photograph) to the computer program. Picospritzer connects to the top of a glass micropipette in the Surgical Station via a plastic tube.

3.2.2.2 Trouble-shooting of Electrical Noise

Electrical noise is a notorious problem in all electrophysiological studies, and this study was no exception. The laboratory was fortunately previously re-wired so that certain plugs were earthed to a single location in the building. All equipment involved in the FAST recordings (FAST Control Box, picospritzer and computer) were attached to this lead *only*. A Faraday cage did not decrease the amount of noise detected in the system, and it was suggested by the Lexington team to exclude the cage. Additionally, *every* piece of electrical equipment in the laboratory was checked for whether it influenced the shape of the oscilloscope in the FAST program (Fig. 3.3). Eventually it was determined that virtually no other equipment, especially the drill and lamps, could be plugged in to any socket in the laboratory during chronoamperometry recordings. The standard magnetic stirrer was exchanged for a battery operated stirrer to prevent electrical noise during calibration.



(a) Oscilloscope with noise **(b)** Oscilloscope without noise

Figure 3.3 – a) Oscilloscope detected much electrical noise when the drill and lamps were plugged in. b) Noise was eliminated when other equipment in the laboratory were not plugged in.

3.2.2.3 Stereotaxic Station

A stereotaxic frame was dedicated to the set-up. This frame required the addition of a vertical microdrive. Adjustments were also made so that all planes moved correctly, i.e. the microdrive moved vertically, perpendicular to the floor, and not at any angle, and that the AP and ML planes moved perpendicularly to each other, parallell to the floor.

A dissection microscope was required for the surgery, and a reticule for the microscope was required for measuring the volume ejected from the glass micropipette. A dissection microscope that was able to receive a reticule in its eyepiece was eventually found, and the the reticule successfully installed.

A heating pad capable of controlling the temperature at 37 $^{\circ}C$ was required to maintain rat body temperature under anaesthesia. Several of these were tested and the one which caused the least electrical noise in the system was selected for use.

3.2.2.4 Micropipette Puller Adjustments

The were several micropipette pullers in the laboratory, however none were successful at producing the required uniform taper that is 1 cm in length at *any* combination of heat vs solonoid settings. It was finally concluded that the length of the coil on our puller was too short to produce the parameters that we require. A new, longer coil was fashioned and fitted, and we were finally able to produce micropipettes of ideal taper and length as stipulated by the Gerhardt laboratory.

The pulled micropipettes were successfully bumped to inner diameters of $\sim$10 μm and stored in dust-free containers until they were 'waxed' to working electrodes.

3.2.2.5 Calibration of Volume of Liquid in the Glass Micropipettes

Minute volumes of approximately 50 $\sim$ 250 nl of KCl and DA needed to be delivered from the pulled and bumped glass micropipettes, through pressure ejection via the picospritzer. This volume could only be measured via the distance of liquid displaced along the tube of the micropipette, as viewed through the dissection microscope with fitted reticule.

Although it was previously published by the Gerhardt laboratory, we performed our own measurements to determine the amount of liquid in 1 cm of the tube. The masses of 5 empty micropipettes were taken. Micropipettes were each filled with distilled H_2O, the lengths of the fluid in the tubes were measured with vernier calipers, and the masses of each tube including the water were recorded. Some fluid was then expelled from each tube, the new lengths of the fluid were measured, along with the new masses. Since 1 ml of dH_2O is 1 g, we were able to determine that 1 cm of these micropipettes held 250$\pm$6 nl.

3.2.3 Preparation of the Working Electrode

3.2.3.1 Description of Working Electrode

Working electrodes were purchased from CenMet (Quanteon Ltd.). The tip of a carbon fiber working electrode consisted of a carbon fibre (30 μm in diameter) supported by a micro-glass pipette ($\sim$30 μm inner diameter) such that a length of 150$\sim$200 μm protruded from the glass support. The carbon fibre was connected to a copper wire via compacted Graphpoxy in the funnel portion of the micro-glass pipette support (Fig. 3.4). Upon arrival, working electrodes were stored at -20 $^{\circ}C$ until Nafion coating.

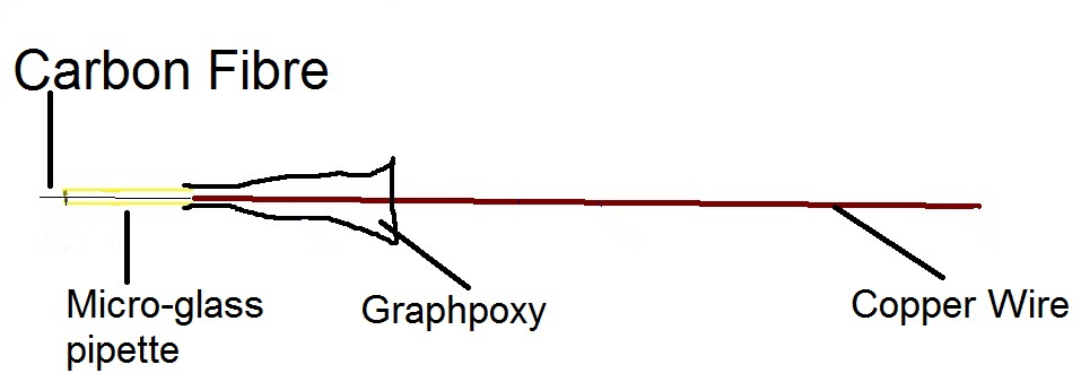


Figure 3.4 – Diagram of a carbon fibre working electrode.

3.2.3.2 Nafion Coating

Nafion (Sigma-Aldrich) was aliquoted into 2 ml, light-proofed, air-tight glass vials upon opening of the original bottle. Each aliquoted vial was used for a maximum of 2 weeks once it had been opened.

Working electrodes were removed from the -20 $°C$ freezer. The tip of each electrode was rinsed briefly in distilled H_2O, and immediately baked at 200 $°C$ for 5 mins to dry. The electrodes were carefully removed from the oven and the tips were dipped into the Nafion vial and swirled 3 times around the inner circumference of the vial, without touching the glass. The dipped electrodes were baked at 200 $°C$ for another 5 mins to bind the Nafion coating. All electrodes were used within 2 weeks of Nafion coating.

Each coated working electrode was 'waxed' together with a pulled and bumped glass micropipette. Literally, the electrode and pipette were manipulated under a reticuled microscope until their tips were flush, parallell, in the same plane, with the inner diameters 180 $\sim$ 220 μm apart, and finally held together in this position by sticky wax (Fig. 3.5).

The end of the copper wire was stripped and soldered to an amphenol that would connect the working electrode to the the headstage.

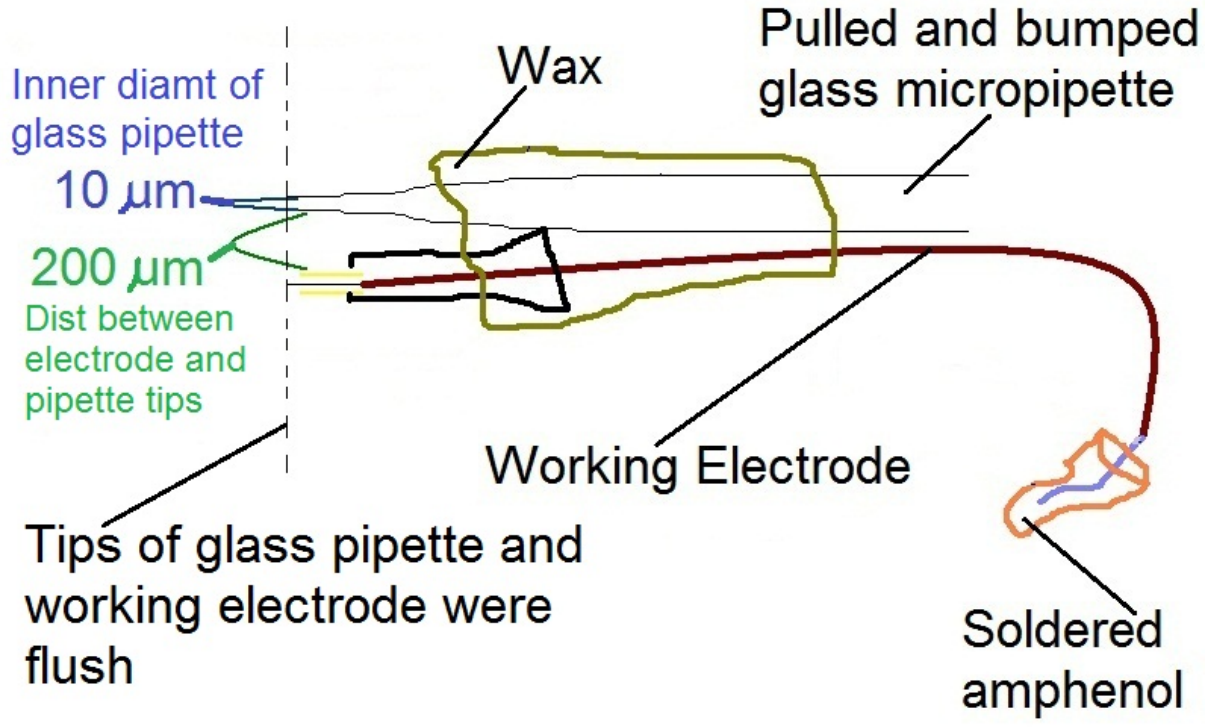


Figure 3.5 – Diagram of working electrode waxed to pulled and bumped glass micropipette. Micropipette inner diameter was bumped to $\sim$10 μm. Working electrode was flush, parallel and in the same plane as micropipette, and between 180 $\sim$ 220 μm apart, then held together by sticky wax. Amphenol was soldered to stripped end of working electrode.

3.2.4 Construction of the Reference Electrode

Each reference electrode was constructed with approximately 10 cm of teflon coated silver wire (AM-Systems). Both ends of the wire were stripped (approx 7 mm) and one end was be soldered to an amphenol. The other end of the wire was chlorided at approximately 10 V with a DC adapter in a bath containing 1 M HCl saturated with NaCl.

3.2.5 Calibration

Each electrode was calibrated immediately prior to use in the rat brain. An RE5b glass reference electrode (BASi) was used for the calibrations as it is more sensitive and stable than those constructed for *in vivo* experiments. It cannot be used for *in vivo* work as it is too large and cannot be manipulated to fit in the rat skull.

In the calibration window of the FAST program set for square wave chronoamperometric recording, The applied voltage was set at -0.55 V, and resting voltage at 0 V. The pulse period was set to 0.2 s, which was the total duration of the voltage pulse. The sweep time was set to 1 s, which was the interval between the start of voltage pulses. The amount of current (nA) in a 40 ml of gently stirred phosphate buffered saline (PBS) lite (0.01 M NaH_2PO_4; 0.04 M Na_2HPO_4; 0.1 M NaCl) was measured and marked as 'baseline'. Then, 500 μl of freshly made ascorbic acid (20 mM) was added to the 40 ml beaker, and the current for the final concentration of 250 μM of ascorbic acid was measured and marked as 'interferent'. 40 μl of DA (2 mM) was added to the beaker and the current for the final concentration of DA (2 μM) was marked as 'analyte 1', 'analyte 2' was measured with a further addition of 40 μl that increased the total concentraiton of DA to 4 μM, and a final 'analyte 3' was measured in the same manner. These measurements allowed for the calculation of the Limit of Detection (LOD), electrode selectivity of DA over ascorbic acid, linearity of the relationship between current and DA concentration (R^2) and slope. Essentially, the current sensed by the electrode was equated to the known concentrations of DA in the beaker to enable the calculation of DA concentrations when *in vivo*. Parameters for useable electrode included an LOD of <0.1 μM, selectivity in the oxidative channel >100 and R^2 >0.98.

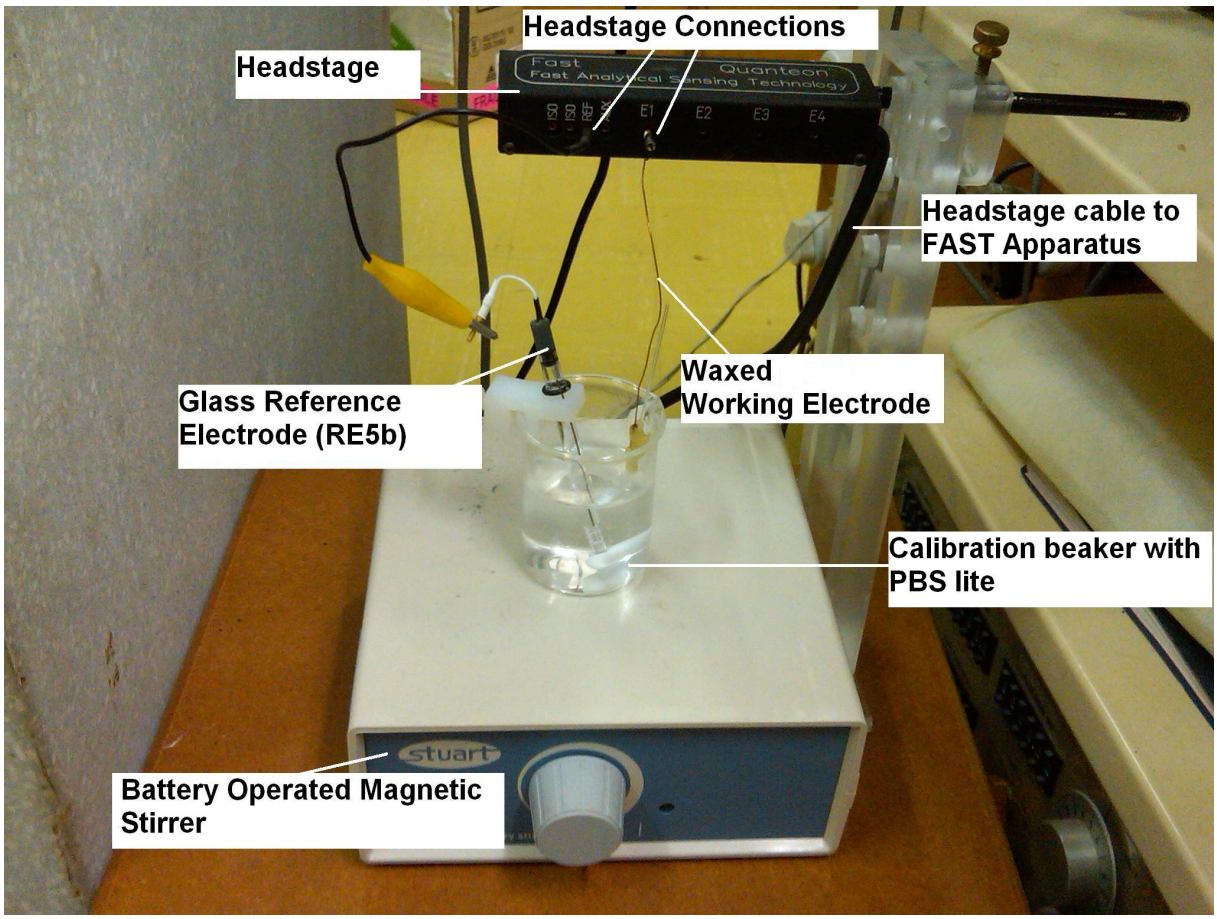


Figure 3.6 – Photograph of calibration set-up. Glass reference electrode and waxed working electrode are connected to the headstage at the non-sensing end. The sensing ends of the electrodes are in the 40 ml glass beaker inititally containing PBS lite. Magnetic stirrer was battery operated to prevent electrical noise

3.2.6 Surgery

Each rat was weighed and anaesthetized with 25% urethane (i.p. 1.25 g/kg with boosters to 2.5 g/kg) given over approximately 2 hrs. The rat was determined to be stably anaesthetized when it no longer exhibited any pain (toe pinch) or eye blink reflexes. Subsequently, the hair on the head was removed, the rat was placed on the heating pad and secured with the ear and incisor bars in the stereotaxic frame. The skin of the head was reflected and the part of the skull over the striatum was removed bilaterally (approx AP -1 $\sim$ +2 mm; ML $\pm$1.5 $\sim$ $\pm$3 mm) (Paxinos and Watson, 2005). A small hole was drilled in the posterior, lateral part of the skull for the placement of the reference electrode (Fig. 3.7). A saline soaked cotton ball was placed over the left side of the brain to prevent dessication while experiments were conducted on the right side of the brain. The meninges over the right side of the brain were removed and the area cleared of blood. The glass micropipette of the working electrode construction was filled with KCl (120 mM) and the

construct was lowered to DV 3.0 of the right side of the brain at co-ordinates AP +1; ML -2.5 mm. The working electrode was always oriented posterior to the micropipette. The Ag/AgCl reference electrode was hooked into the hole in the posterior of the skull, between the skull and the meninges. Saline soaked cottons balls were placed around the working electrode to prevent dessication of the brain. The picospritzer was connected to the end of the glass micropipette and the drill and lights were unplugged. The electrodes and rat were allowed to equillibrate for 1 hr to reach baseline. A setting of 20 psi and

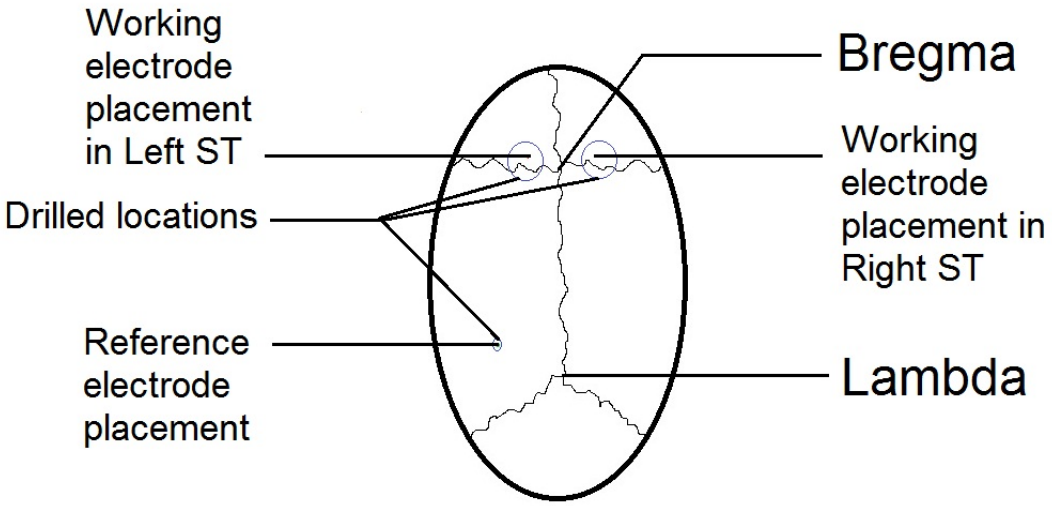


Figure 3.7 – Diagram of stereotaxic surgeries with working and reference electrode placements.

500 ms on the picospritzer was used to check that the micropipette was not obstructed. The duration and pressure of the ejection would be adjusted until the volume of KCl ejected was between 100 $\sim$ 250 nl. After ejection of the correct volume at DV 3.0 mm, the stimulated peak was recorded and when the DA levels had returned to baseline, the working electrode would be lowered 500 μm DV 3.5 mm, where a similar volume would be ejected. This continued every 500 μm until DV 7.5 mm. The working electrode was then retracted from the brain, the pipette emptied of KCl and replaced with DA (200 μM in 100 μM AA). The working electrode was then moved to ML +2.5 mm, the meninges were removed over the left side of the brain and the area cleared of blood. The working electrode was lowered to DV 3.0 mm and baseline was usually obtained within 20 mins. A setting of 10 psi and 500 ms was used to eject a small volume of DA to check the

pipette was not obstructed. The duration and pressure of DA ejection was varied until the amplitude of DA recorded on the screen was between 1 $\sim$ 2 μM. After such a peak was obtained, the working electrode was lowered a further 500 μm, and the performance was repeated until DV 7.5 mm.

3.2.7 Tissue collection and preservation

After the completion of the recordings, rats were removed from the stereotaxic frame and sacrificed via cervical dislocation. The head was removed, skull was reflected and the anterior of the brain was sectioned within 1 min, and placed in a glass vial containing 20 ml 30% sucrose 10% formaldehyde solution at 4 $^{\circ}C$ until sunk. The tissue was then immersed in tissue freezing medium and frozen at -20 $^{\circ}C$ until histological processing.

3.2.8 Histology

Frozen brains were mounted onto chucks and sliced at 60 μm with a cryostat (CM1850, Leica). Sections from the striatum were collected in ice trays containing 1 $\times$ PBS. Each slice of interest was mounted onto glass slides and dried overnight. Slices were dehydrated and stained with cresyl fast violet (CFV), and dehydrated further, with a final immersion in xylol prior to being cover slipped. Photographs of each slide were taken with a Canon *Power Shot* G10 camera (15 MP) and enlarged on a screen for better tract visibillity.

Since the atlas depths were determined from approx 360 g rats and my rats were between 75 $\sim$ 160 g, it was necessary to check that the depth of the different sized rat brains were similar. Thus 2 fresh brains were harvested from 120 g rats and immediately chopped with a McIlwane Tissue Chopper (The Mickle Laboratory Engineering Co. Ltd) at 0.9 mm. Each slice was measured with vernier calipers and photographs were taken with a Canon *Power Shot* G10 camera (15 MP) (Fig. 3.8). Fresh brain distances were scaled to the atlas at 5 AP locations and the location (ST or NAc) of each DV co-ordinate was determined (Table. B.1 and B.2).

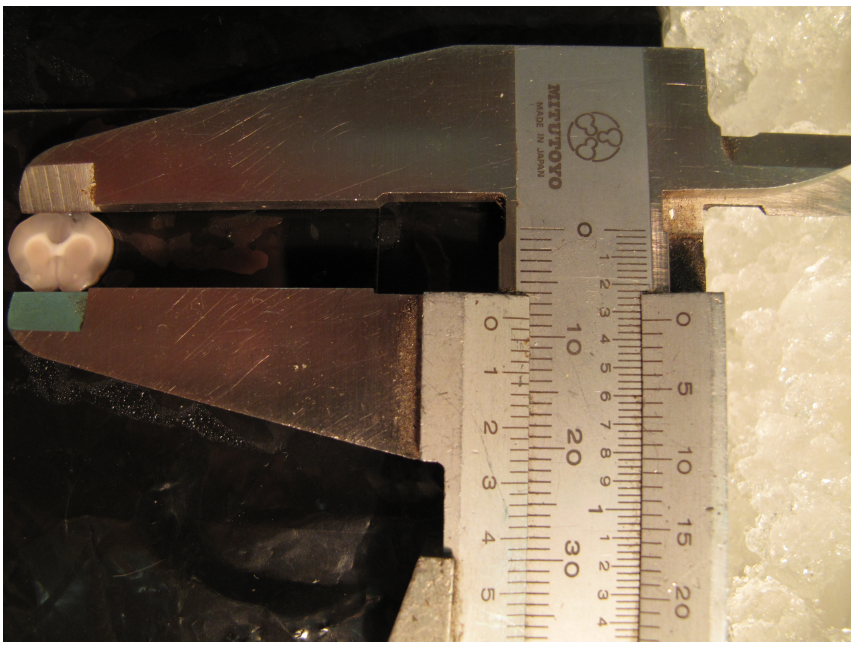


Figure 3.8 – Measurement of total DV depth from brain of a 120 g rat with vernier calipers

3.2.9 Data Processing

3.2.9.1 Data Exclusion Criteria

The tip of each micropipette was 10 μm in inner diameter, while the working electrodes tips were 30 μm in diameter. Neither of these tips were clearly visible in the histological sections. The large tract marks as seen in the histological slices were made by the larger, tapered portion of the glass micropipette. Since the working electrode was oriented ~200 μm posterior to the micropipette in each case, the location of measurement was determined to be 2 frames (Paxinos & Watson, 2005) posterior to the visible tract. The AP location of each experiment was determined and each depth was grouped to 'ST' or 'NAc' depending on its corresponding scaled location on the atlas (Fig. B.3).

The first 8 recordings were not used for analysis, additionally the rats for recordings 11 and 33 died during the baselining period prior to recording. Data points where no response occurred were discarded. For KCl stimulation experiments, volumes smaller than 100 nl and larger tha 250 nl were not analysed. For DA uptake experiments, amplitudes smaller than 0.8 μM and larger than 1.99 μM were not analysed.

The FAST Analysis program was used to calculate the amplitude, T_{100}, T_{80}, T_{50}, T_c, and T_{rise} parameters.

3.2.9.2 Statistical Analysis

Statistica 9 was used for all analyses. Shapiro-Wilks' test for normality performed on raw data showed that the data were non-parametric. After log transformation the data

remained non-parametric. The Kruskal-Wallis test was used on the original data for strain comparisons of all parameters. Scatterplots were drawn to determine possible correlations between rat mass, volume of KCl ejected, amplitude of DA selected and T_{80}. Calibration slopes of all the electrodes used were found to be parametric and 1-way ANOVA was performed for strain comparison.

3.3 Results

3.3.1 A typical DA peak

Although parameters of DA peaks differ between strains and method of stimulation, a typical DA peak, whether as a result from KCl stimulation of application of exogenous DA began at 'baseline'. Stimulation with either KCl or DA lead to a rise in striatal DA concentration until peak 'Amplitude' was obtained. The time (s) from stimulation to peak amplitude was termed 'T_{rise}'. From the peak amplitude, the level of DA decreased to 20%, 50%, 60%, 80% and 100% over time. The 'T_{80}' was the time (s) for the DA to decrease to 80% of the peak amplitude, the 'T_{50}' was the time (s) for the DA to decrease to 50% of the peak amplitude, and the 'T_c' was the time (s) for the DA to decrease from to 20% to 60% of the peak amplitude (Fig. 3.9).

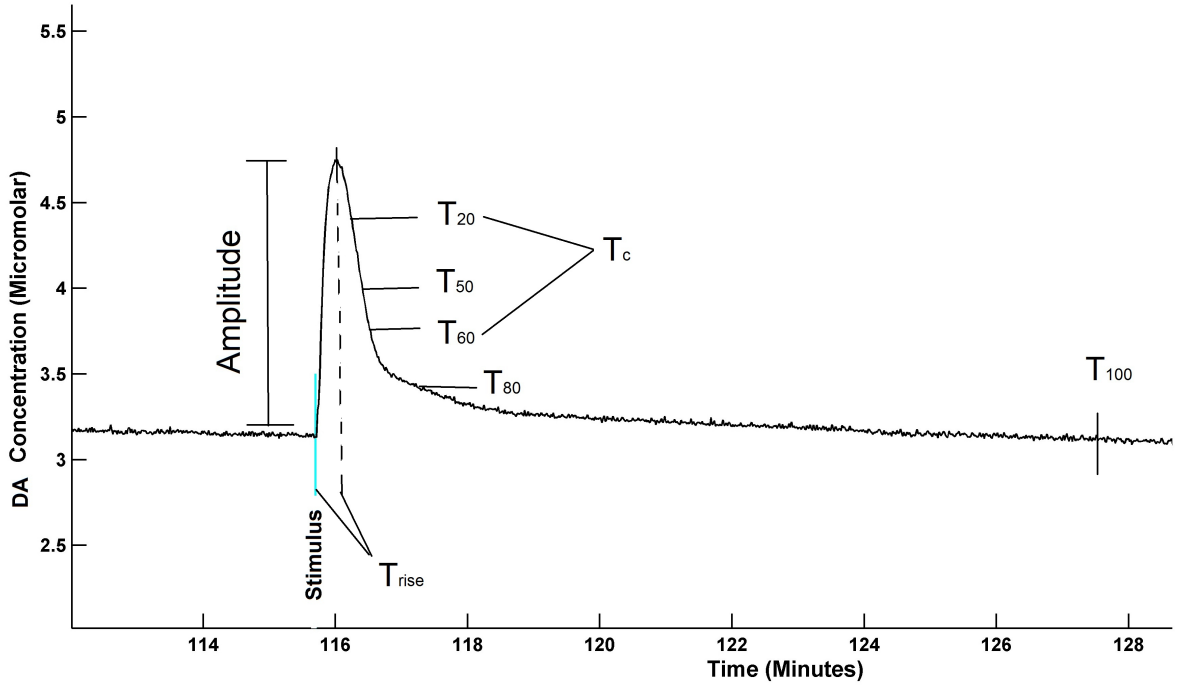


Figure 3.9 – Example of a typical DA peak with amplitude, T_{80}, T_{50}, T_c, T_{100} and T_{rise} parameters marked in approximate locations

3.3.2 Potassium Stimulated release of endogenous dopamine

3.3.2.1 Verification of data

3.3.2.1.1 Analysis of calibration slopes There was no significant strain difference in the calibration slopes used in the KCl stimulation experiments (Fig. 3.10). This indicated that electrode calibration factors were not significantly different between strains (Appendix Fig. B.8 ∼ Fig. B.9).

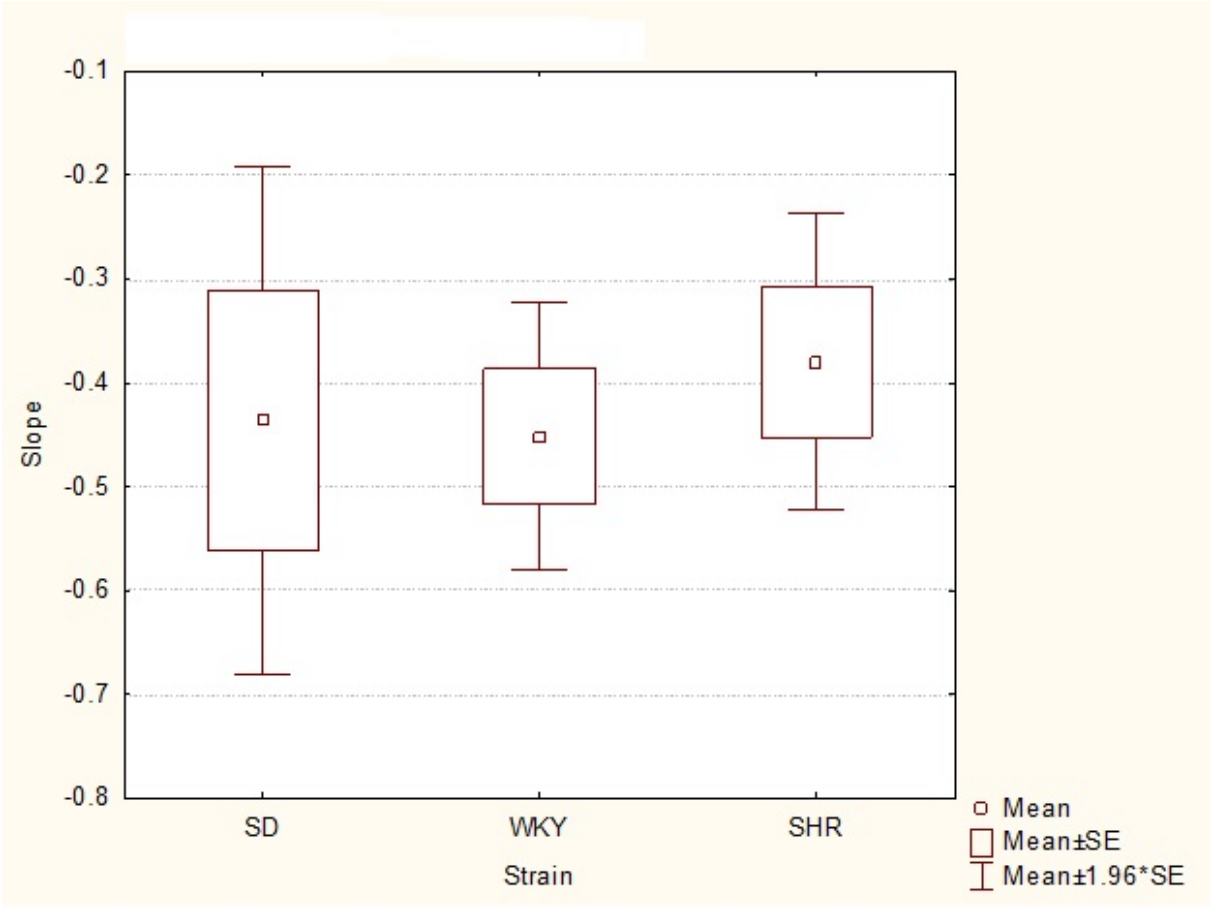


Figure 3.10 – Strain comparison of calibration slopes used in the KCl stimulation experiments; p=0.8281

3.3.2.1.2 Analysis of rat mass There was significant strain difference in rat mass in the KCl stimulated release experiments. SHR and WKY masses were similar and significantly lighter than the SDs (Fig. 3.11).

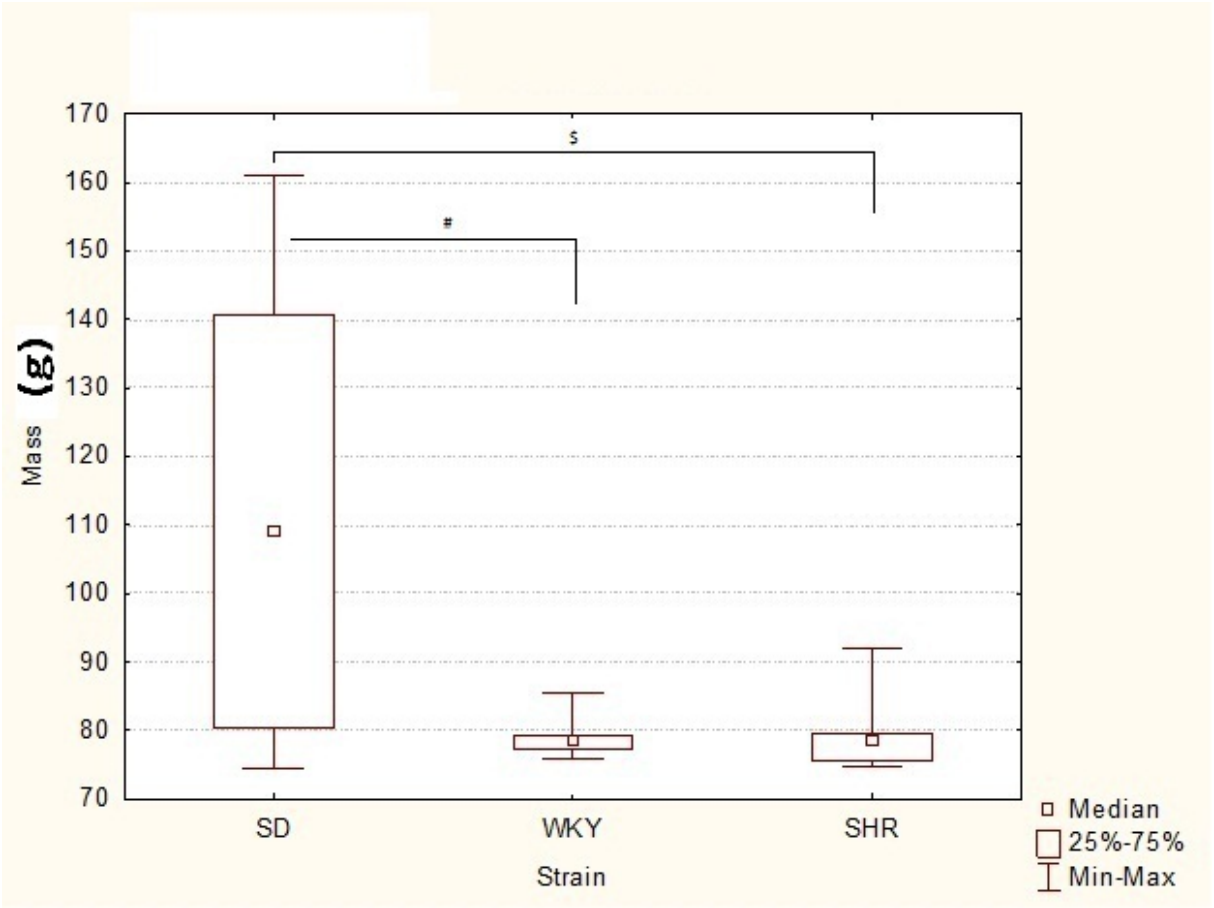


Figure 3.11 – Strain difference in rat mass. SHR and WKY were significantly smaller than that of SDs, $ p=0.0000, # p=0.0000; Overall: H(2, N=215)=36.2518 p=0.0000

However, scatterplots showed that the amplitude and T_{80} parameters of KCl stimulated release of DA were not dependent on rat mass (Fig. 3.12 and Fig. B.7), and that mass was not a confounding factor in subsequent analyses.

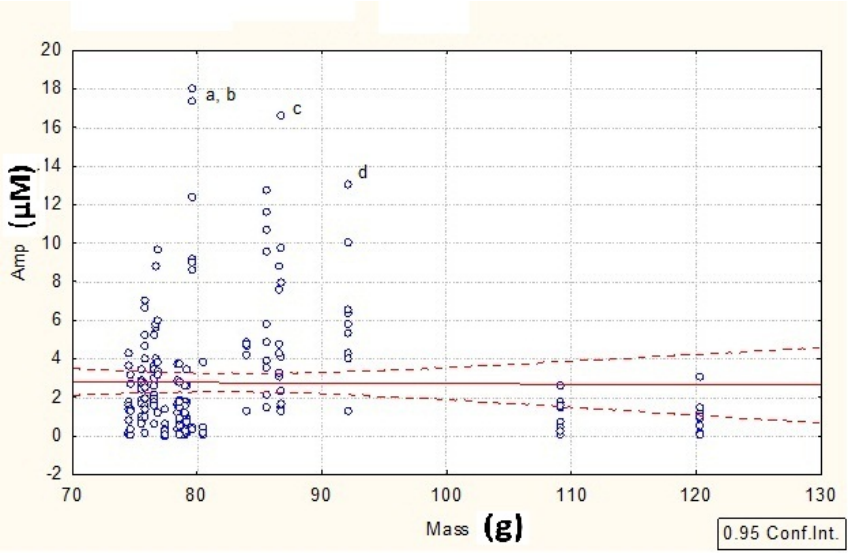


Figure 3.12 – Scatterplot of KCl stimulated release amplitudes vs rat mass. Outliers 'a' and 'b' were contributions from Rec 38, SHR; outlier 'c' was a contribution from Rec 28, SD; outliers 'd' was a contribution from Rec 39, SHR

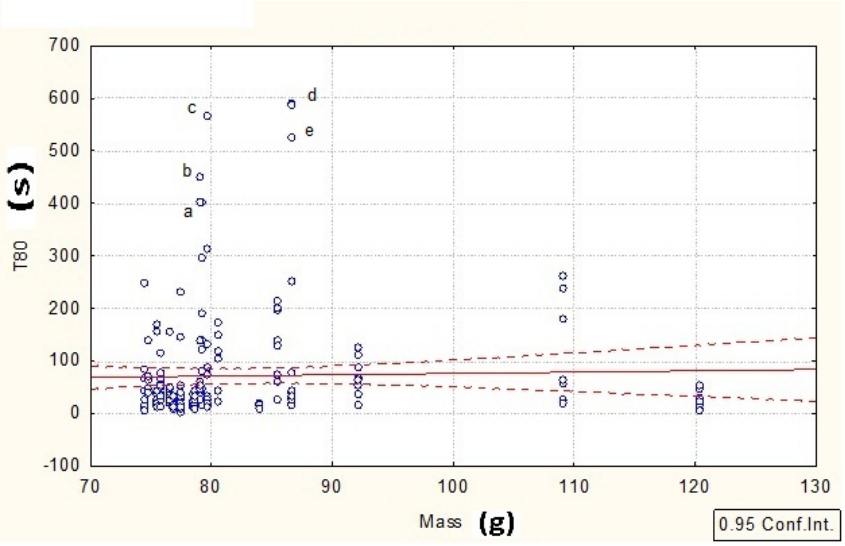


Figure 3.13 – Scatterplot of T_{80} vs rat mass. Outlier 'a' was a single contribution from Rec 20, WKY; outlier 'b' was a contribution from Rec 30, WKY; outlier 'c' was a contribution from Rec 38, SHR; outliers 'd' and 'e' were 2 contributions from Rec 28, SD

3.3.2.1.3 Analysis of KCl ejection volumes The volume of KCl ejection was limited to between 100 $\sim$ 250 nl (12 $\sim$ 30 nmol KCl), but a strain difference was detected in the ejected volumes (H(2,N=215)=13.6405 p=0.0011), where SHRs received amounts of KCl on the larger end of the range than SD (Fig. 3.14). This was an anomaly which should not have occured, however, the amplitudes that were measured were not dependent on the KCl volumes within this range (Fig. 3.15), which indicated that higher amplitudes were not solely due to higher volumes of KCl ejections and that the strain difference in ejection volumes did not compromise subsequent strain comparisons of amplitudes.

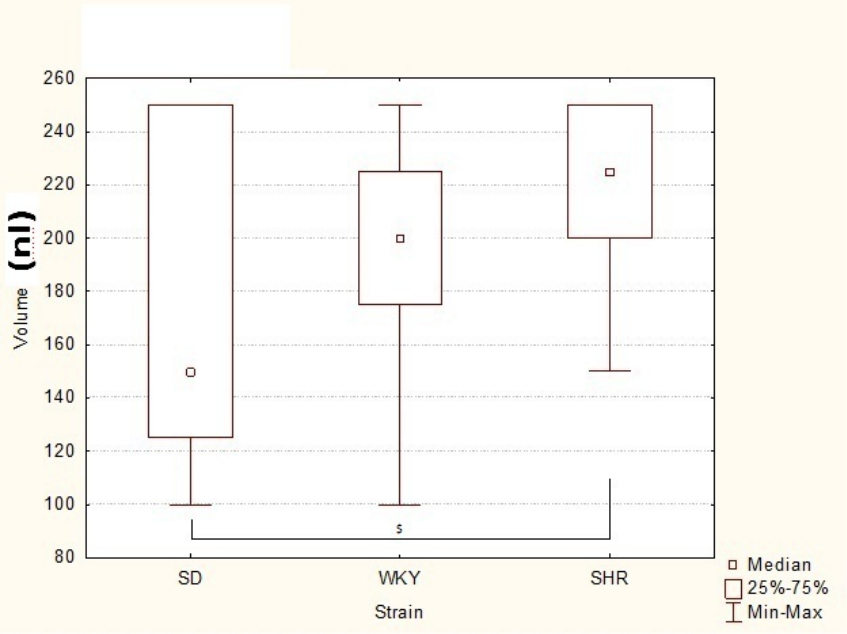


Figure 3.14 – Strain difference of KCl ejection volumes. SHR received significantly higher volumes of KCl than SDs, $ p=0.0009, Overall: H(2, N=215)=13.6405 p=0.0011

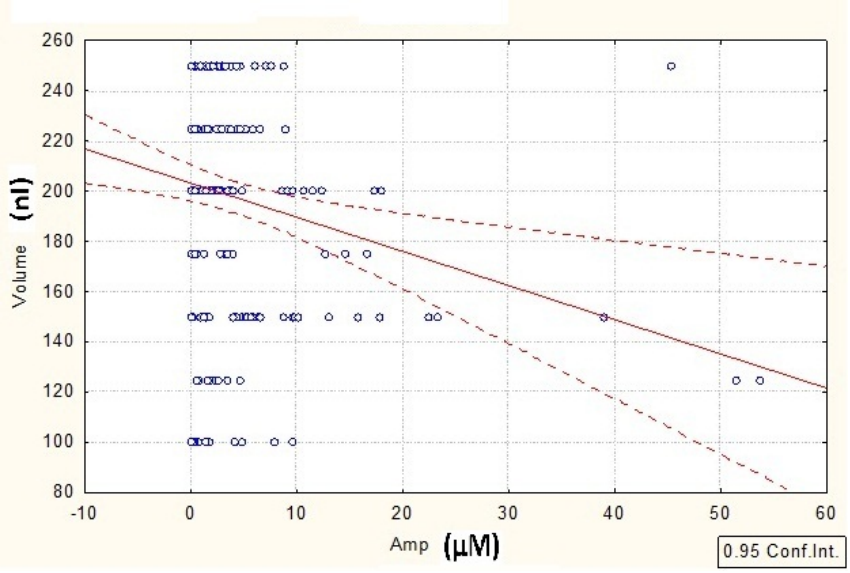


Figure 3.15 – Scatterplot of KCl volumes used vs the amplitude of DA released

3.3.2.2 Strain difference in the amplitude (μM) of stimulated dopamine release

Analysis of strain difference of KCl stimulated release amplitudes showed that the SHR released similar amounts of DA to SD, and both strains released significantly larger amounts of DA than the WKY (Fig. 3.16). When the ST and NAc areas were analysed separately, the effect was significant in the ST (H(2, N=157)=17.1049 p=0.0002), but not in the NAc (H(2, N=30)=5.7272 p=0.0571).

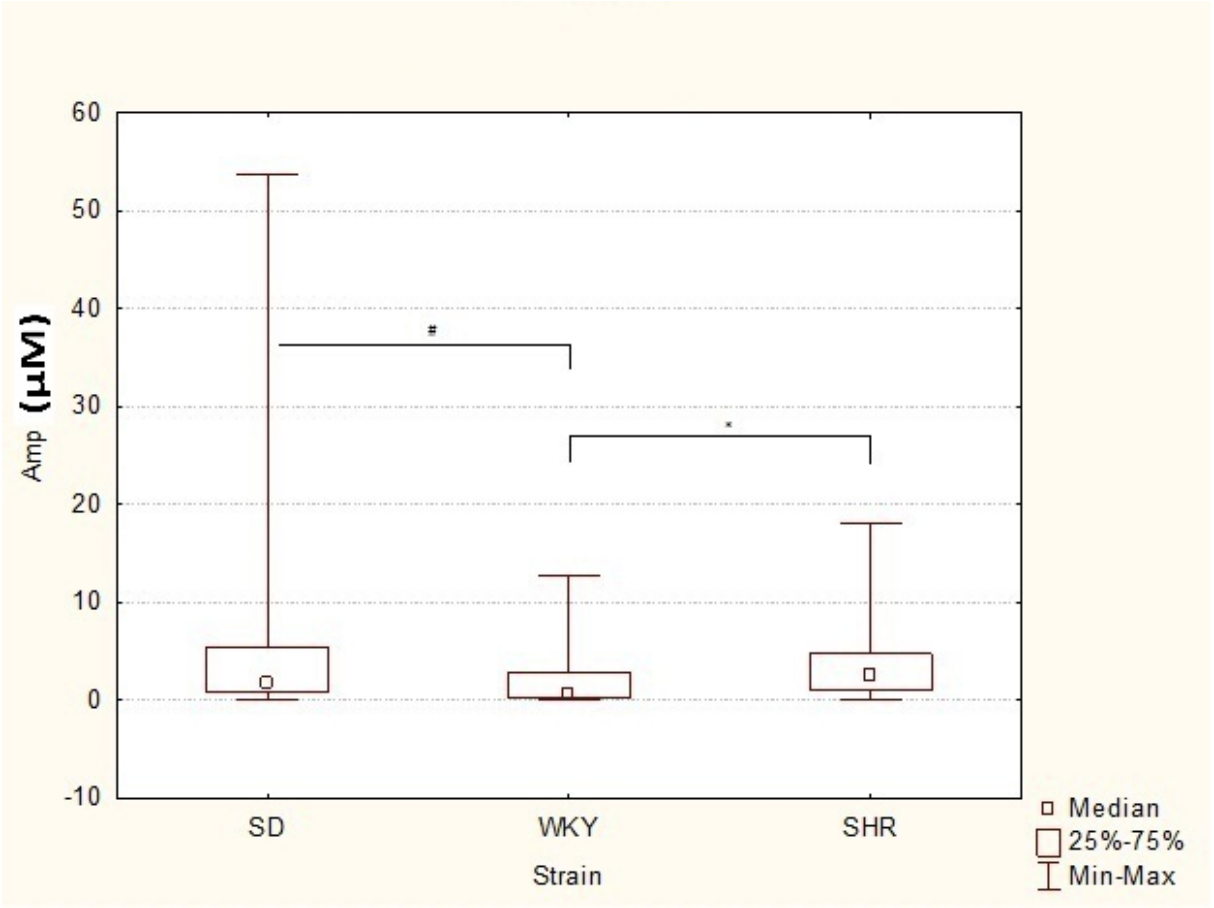


Figure 3.16 – Strain difference in DA peak amplitudes where the SHR and SD released significantly larger amounts of DA compared to WKY, ∗p=0.0005; # p=0.0031; Overall: H(2, N=215)=17.4160 p=0.0002; n = 70 datapoints recorded from 8 SHR, n = 81 datapoints recorded from 8 WKY, n = 64 data points recorded from 7 SD

3.3.2.3 Strain difference in T_{80} of KCl stimulated release of endogenous DA

The T_{80} parameter was not dependent on amplitude size (Fig. 3.17) and was thus comparable across strains.

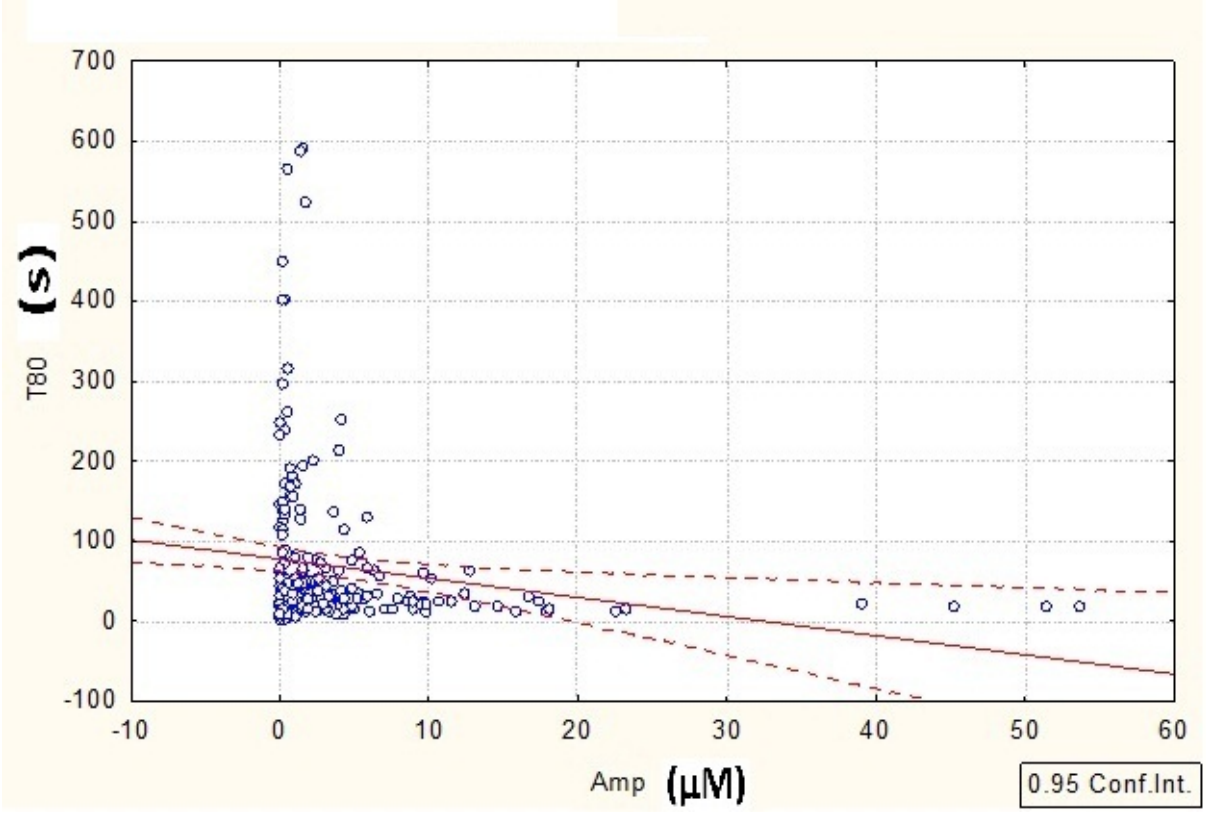


Figure 3.17 – No correlation between T_{80} and amplitudes, r=-0.1754; n = 70 datapoints recorded from 8 SHR, n = 81 datapoints recorded from 8 WKY, n = 64 data points recorded from 7 SD

Strain comparison of T_{80} showed that SHR and WKY had similar T_{80} values, that were both significantly longer than that of SDs (Fig. 3.18). When the ST and NAc were analysed separately, this pattern was significant in the ST (Fig. 3.19) but not in the NAc (H(2, N=30)=2.8921 p=0.2355).

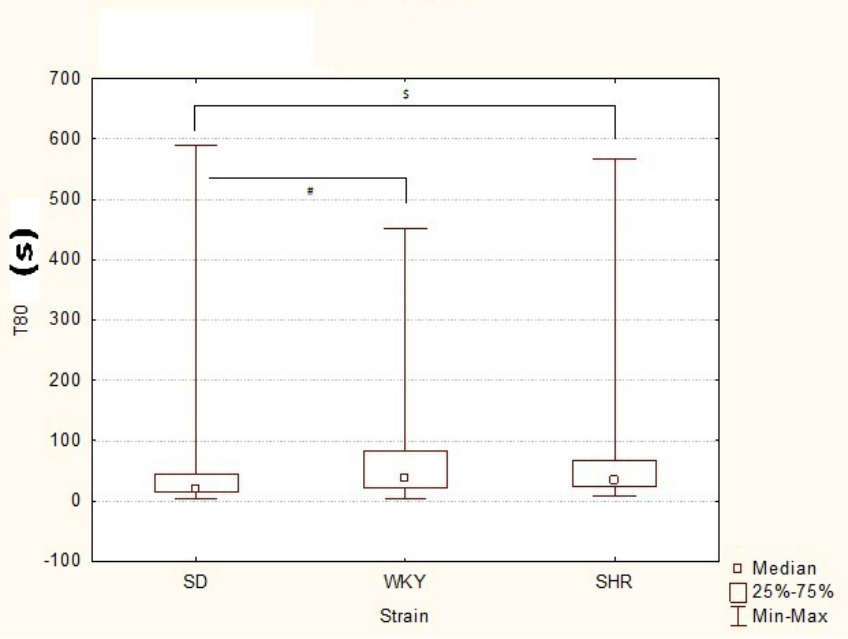


Figure 3.18 – Strain difference in T_{80} of KCl stimulated release of DA. $ p=0.0099, # p=0.0065; Overall: H(2, N=215)=11.7372 p=0.0028; n = 70 datapoints recorded from 8 SHR, n = 81 datapoints recorded from 8 WKY, n = 64 data points recorded from 7 SD

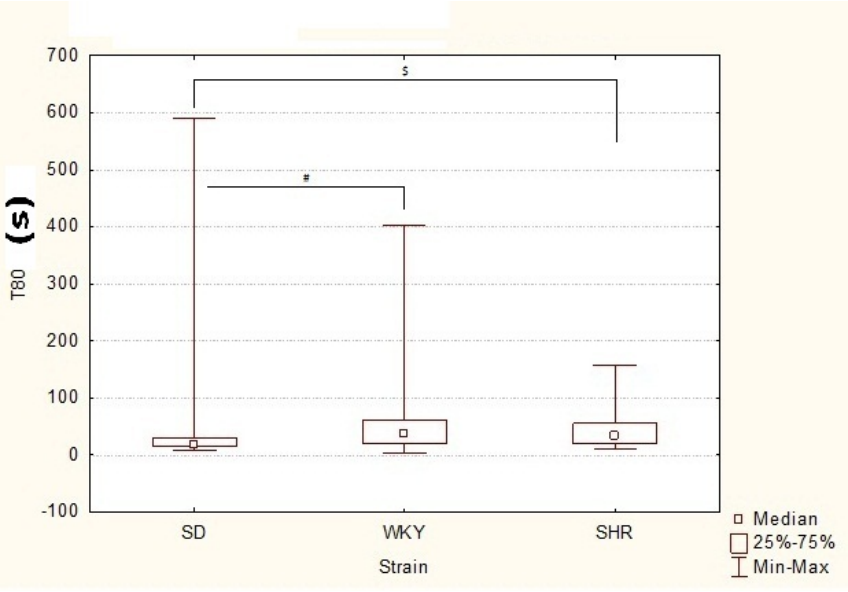


Figure 3.19 – Strain difference in T_{80} of KCl stimulated release of DA in the ST region only. $ p=0.0215, # p=0.0031; H(2, N=157)=11.8774 p=0.0026

3.3.2.4 Strain comparison of T_{rise}

Strain comparison of T_{rise} showed no significant difference, even though the peak amplitudes were signifcantly different between strains.

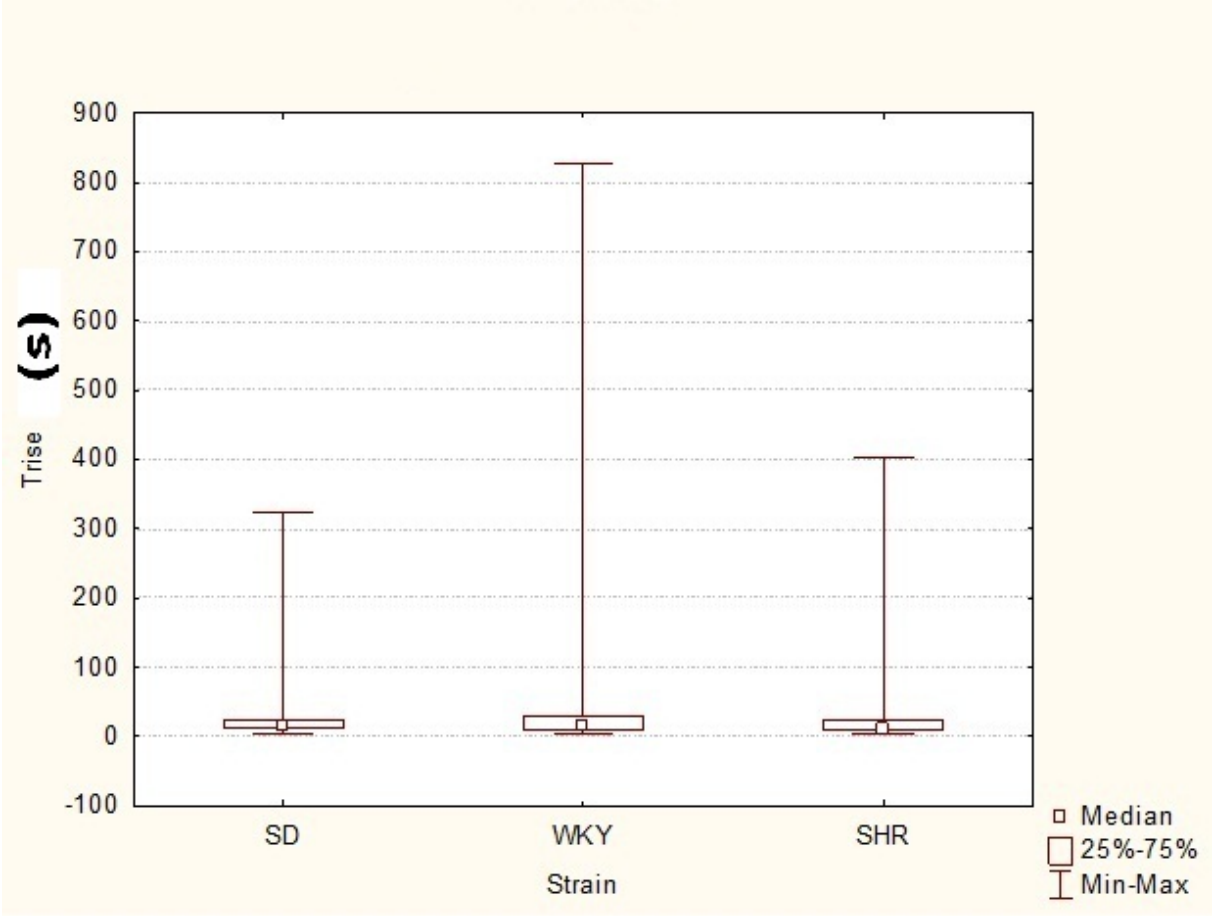


Figure 3.20 – Strain comparison of T_{rise}; H(2, N=215)=1.643204 p=0.4397; n = 70 datapoints recorded from 8 SHR, n = 81 datapoints recorded from 8 WKY, n = 64 data points recorded from 7 SD

3.3.2.5 Strain comparison of parameters when results were averaged per rat

In the striatum, when all ST points of each rat was averaged to a single mean value, no significant strain differences were detected for any parameter: Amplitude H(2, N=23)=1.9216 p=0.3826; T_{80} H(2, N=23)=3.1150 p=0.2107; Volume H(2, N=23) =1.6066 p=0.4479.

Similar results were observed in the NAc: Amplitude H (2, N=15)=3.2600 p=0.1959; T_{80} H(2, N=15)=0.7200 p=0.6977; Volume H(2, N=15)=0.9916 p=0.6091.

3.3.3 Clearance of exogenously applied dopamine

3.3.3.1 Verification of data

3.3.3.1.1 Analysis of working electrode calibration slopes A comparison of calibration slopes showed no significant differences between strains (p=0.1098). This indicated that electrode calibration factors were not significantly different between strains (Appendix Tables B.36 and B.37).

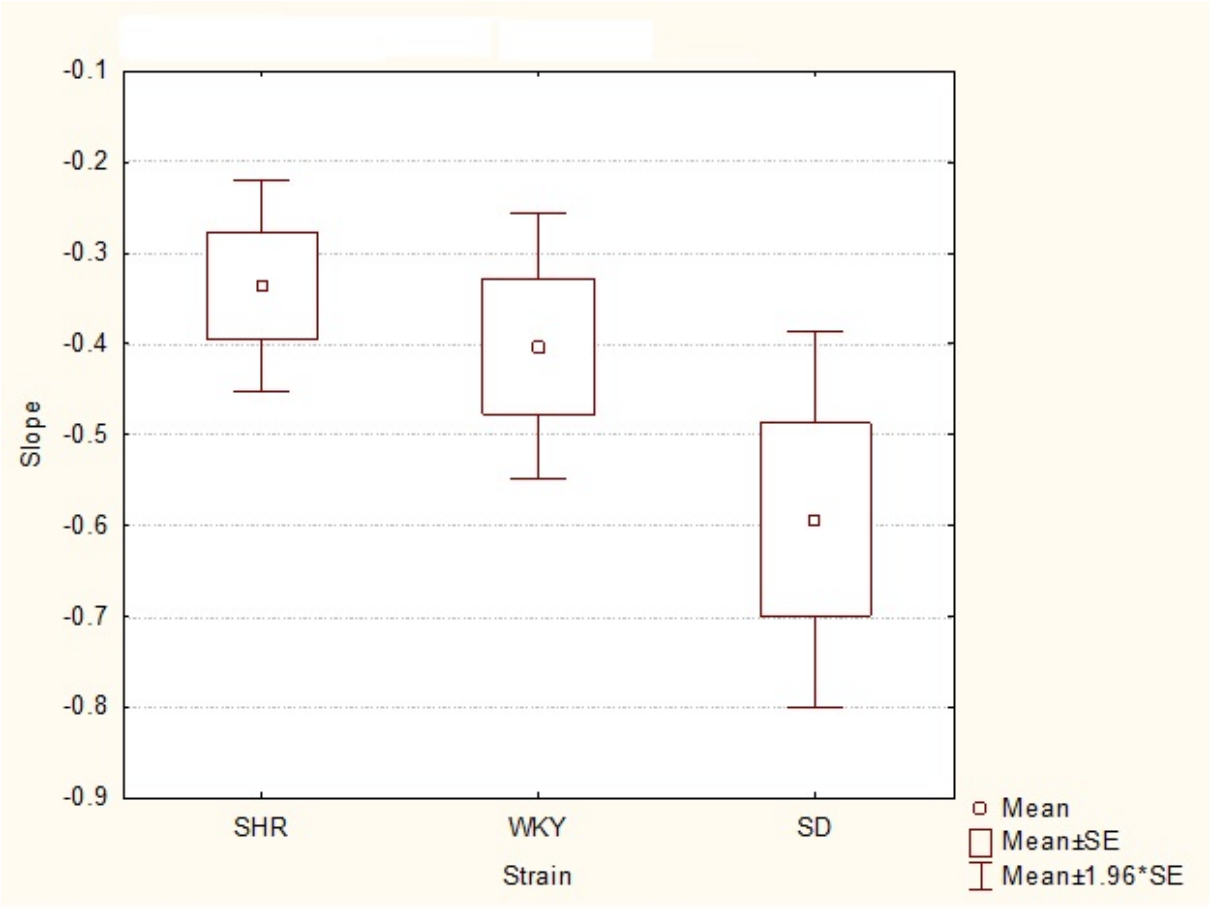


Figure 3.21 – Strain comparison of calibration slopes in the DA clearance experiments; p=0.1098

3.3.3.1.2 Analysis of rat mass A comparison of rat mass across strains showed no differences between strains in the experiments where exogenous DA was applied (Fig. 3.22).

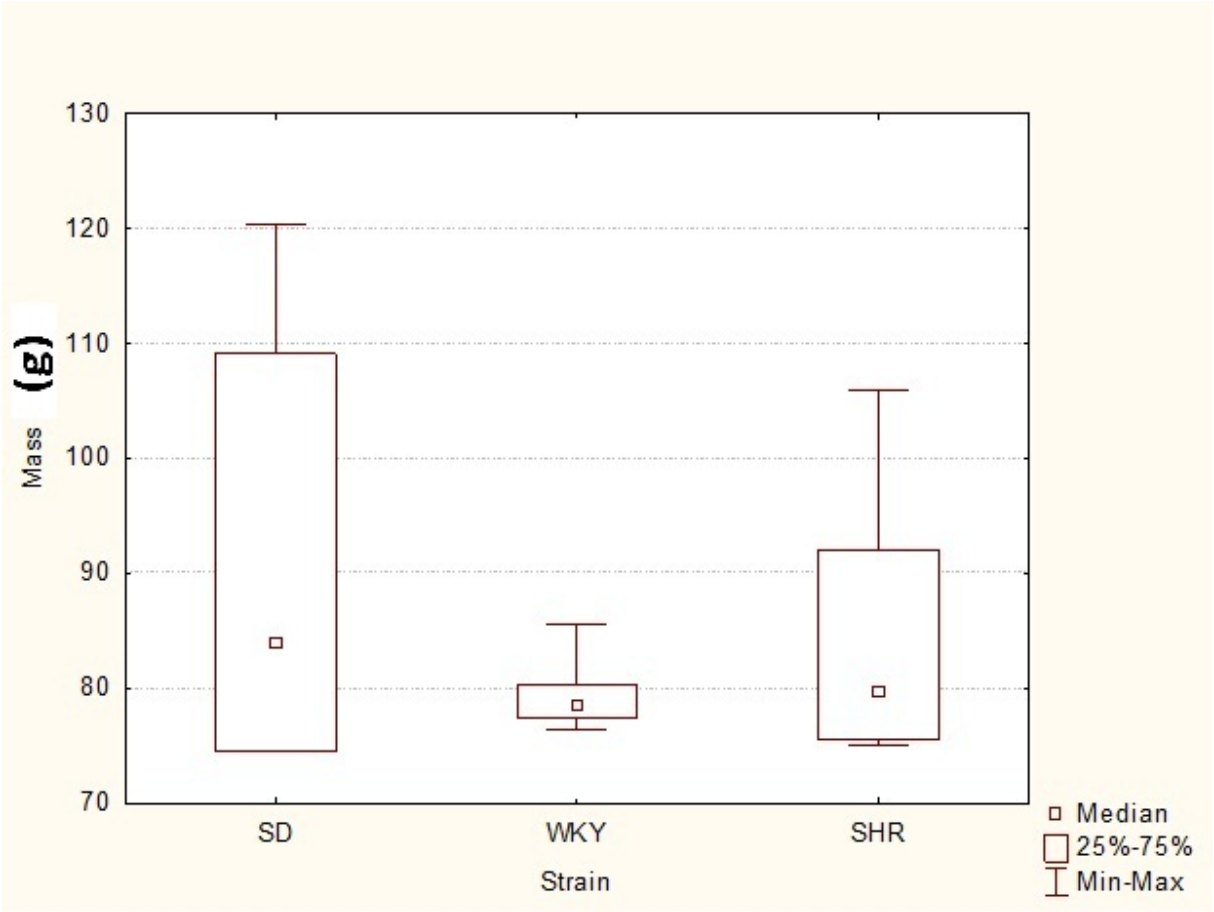


Figure 3.22 – Strain comparison of rat mass in the DA clearance experiments; H(2, N=306)=1.5009 p=0.4721

3.3.3.1.3 Analysis of controlled amplitudes of exogenously applied DA Amplitudes of exogenously applied DA were controlled between 0.8 μM $\sim$ 1.99 μM and no strain differences were observed (Fig. 3.23), indicating that amplitudes were well controlled across strains.

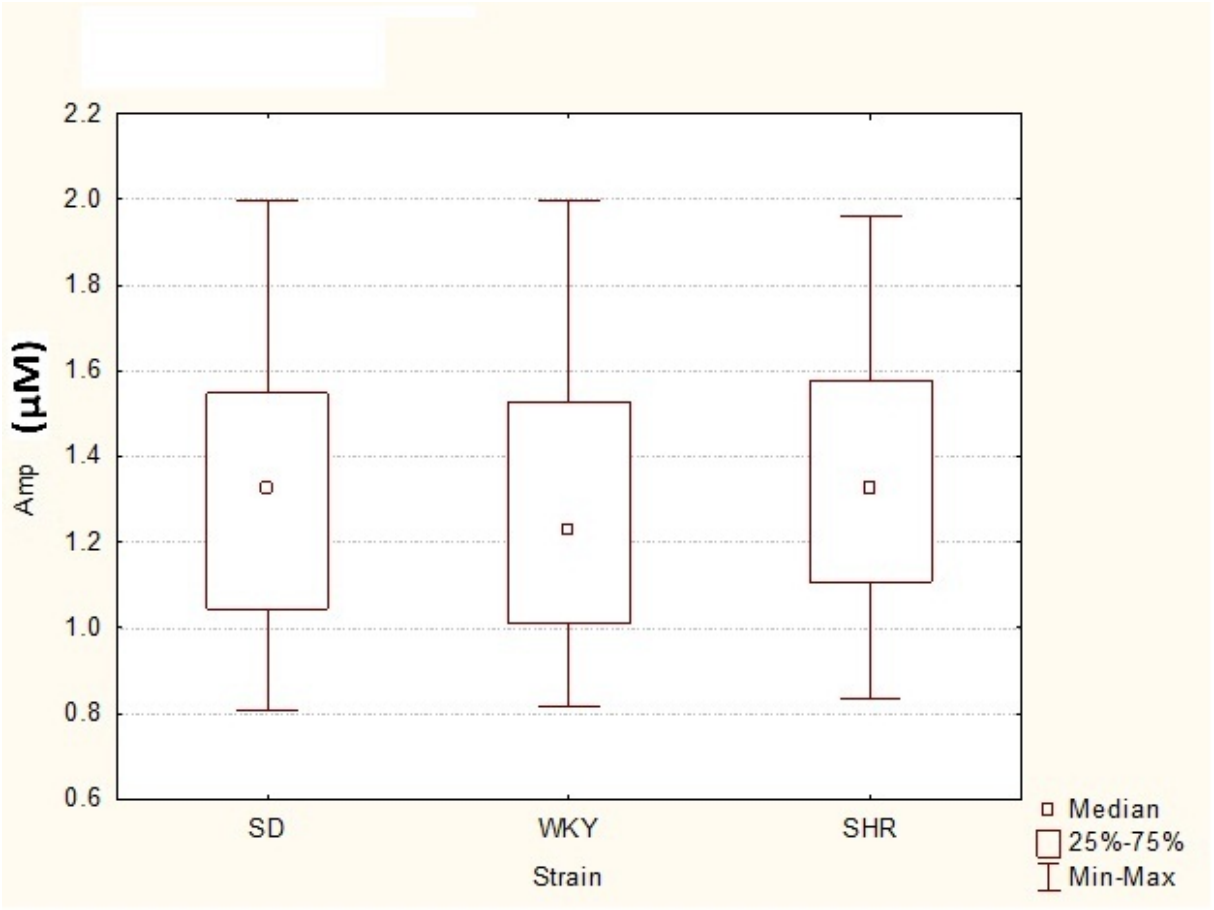


Figure 3.23 – Strain comparison of controlled amplitudes of exogenously applied DA; H(2, N=306)=2.0867 p=0.3523; n = 95 datapoints recorded from 9 SHR, n = 116 datapoints recorded from 9 WKY, n = 95 data points recorded from 5 SD

3.3.3.1.4 Analysis of T_{80} comparability Scatterplots indicated that the T_{80} parameter was not dependent on the rat mass (Fig. 3.25), or volumes of DA ejected (Fig. 3.24). Lack of correlations for these factors showed that parameters were comparable across strains.

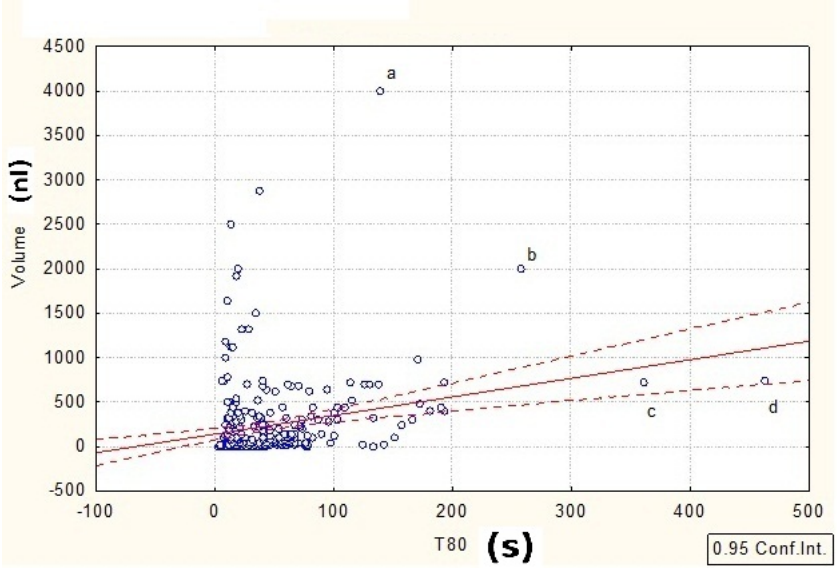


Figure 3.24 – Scatterplot of volume vs T_{80} of responses to exogenously applied dopamine. Outlier 'a' was a single contribution of Rec 15, SD; outliers 'b' and 'c' were two contributions from Rec 22, WKY; outlier 'd' was a single contribution from Rec 38, SHR

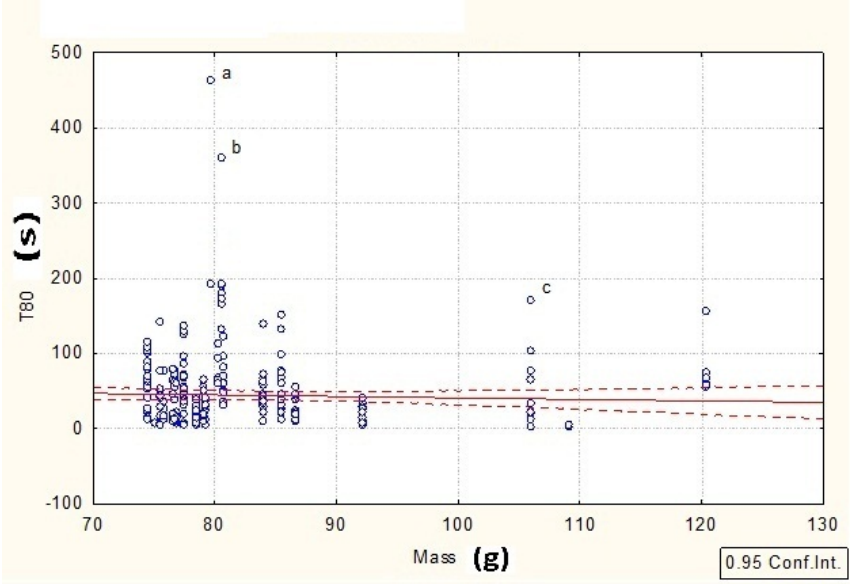


Figure 3.25 – Scatterplot of T_{80} vs mass of rats used in this experiment. Outlier 'a' was a single contribution from Rec 38, SHR; outlier 'b' was a single contribution from Rec 22, WKY; outlier 'c' was a single contribution from Rec 34, SHR

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3.3.3.2 Strain comparison of T_{80}

The T_{80} parameter was the time (s) for the concentration of DA to decrease to 80% of the peak amplitudes that were limited between 0.8 μM $\sim$ 1.99 μM. A comparison of T_{80} where the entire range of amplitudes showed no significant differences between strains (H(2, N=306)=3.5961 p=0.1656). However when the range of amplitudes was separated into 3 groups for more stringent analysis, strain differences became apparent. Amplitudes from 0.8 $\sim$ 0.99 μM were labeled 'Small', 1 $\sim$ 1.5 μM were labeled 'Medium' and 1.6 $\sim$ 1.99 μM were labeled 'Large'.

No strain difference was seen in T_{80} in the 'Small' (Appendix Fig. B.41) or 'Medium' amplitude groups (Appendix Fig. B.42), however, significant differences occured in the the 'Large' amplitude group. The SHR T_{80} was significantly shorter than that of WKY (p=0.0301) (Fig. 3.26).

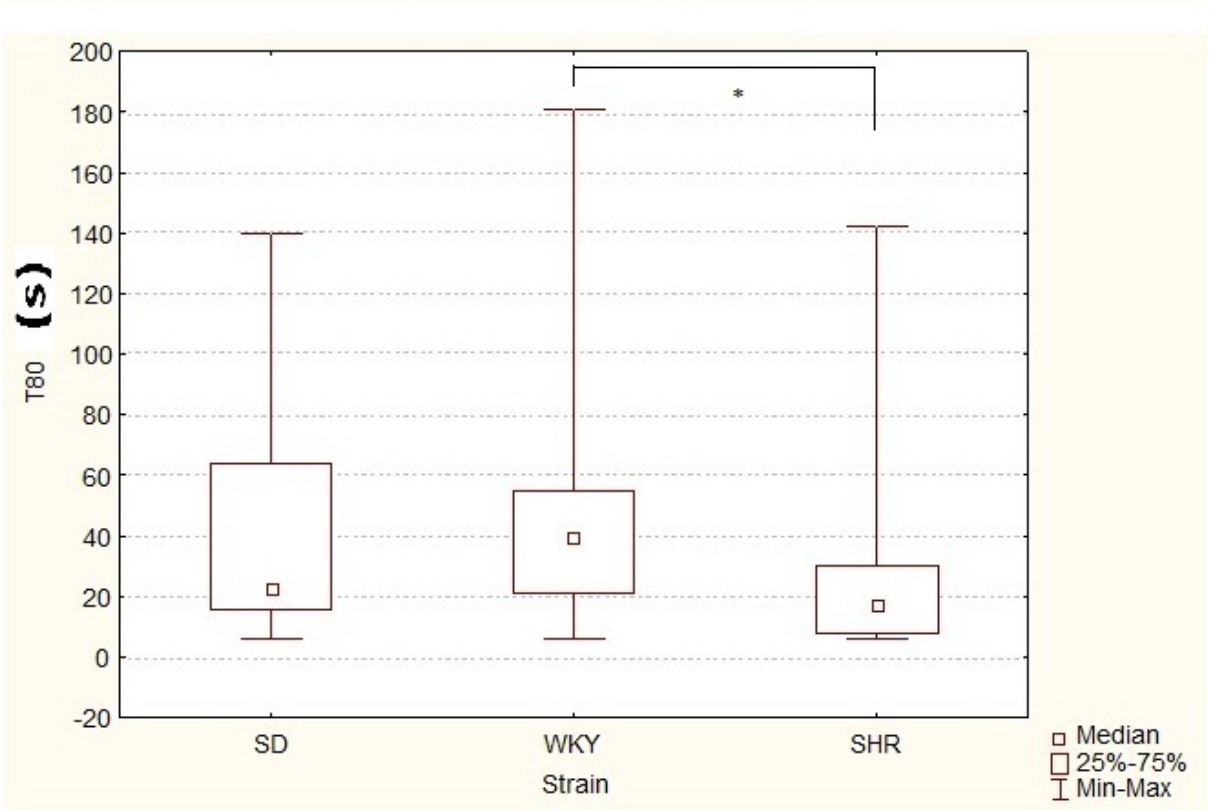

Figure 3.26 – Strain difference in T_{80} in the 'Large' amplitude group; H(2, N=61)=6.6896 p=0.0353; $*$p=0.0301; n = 23 datapoints recorded from 9 SHR, n = 23 datapoints recorded from 9 WKY, n = 15 data points recorded from 5 SD

3.3.3.3 Strain comparison of T_{50}

No strain difference in T_{50} was observed when the entire range of amplitudes was analysed together (H(2,N=306)=3.8139 p=0.1485), however singnificant strain difference was observed in 'Large' amplitude group where SHR had the shortest T_{50} and WKY the longest (H(2, N=61)=6.4571 p=0.0396) (Fig. 3.27).

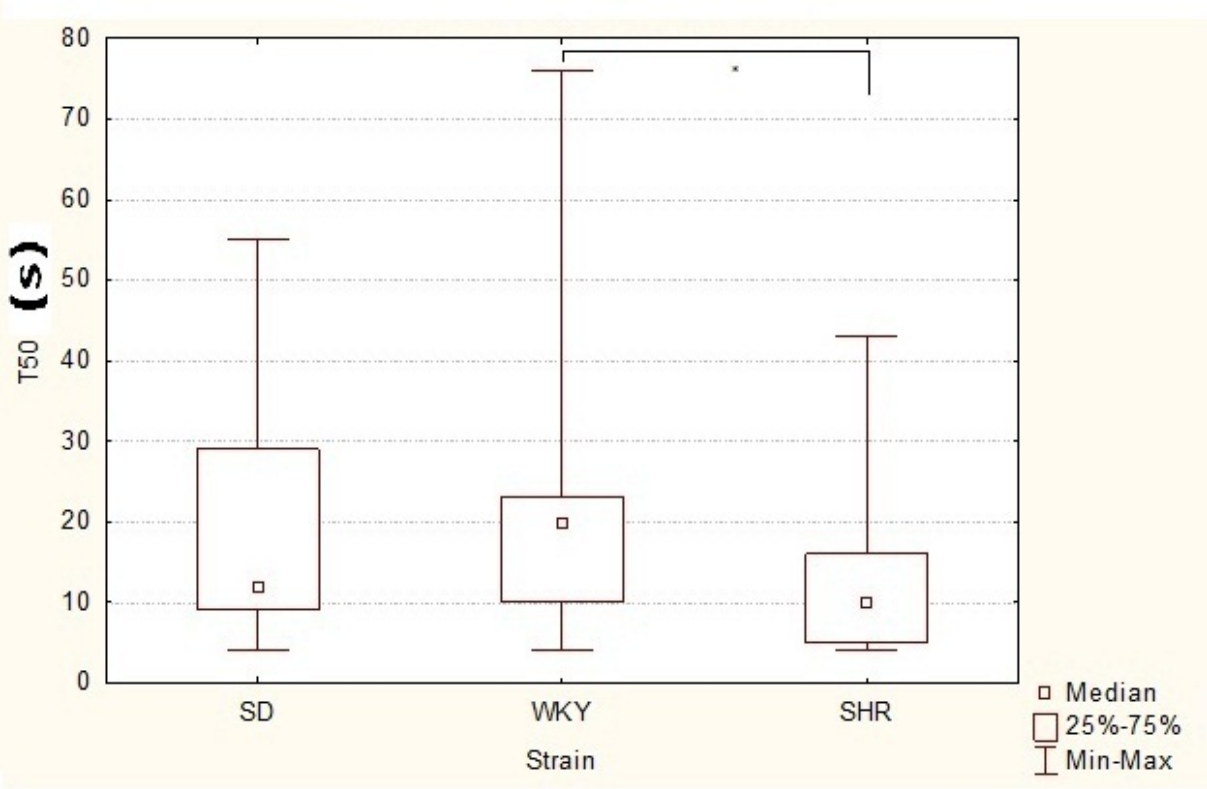

Figure 3.27 – Strain difference in T_{50} in the 'Large' amplitude group; H(2, N=61)=6.4571 p=0.0396; ∗p=0.0395; n = 23 datapoints recorded from 9 SHR, n = 23 datapoints recorded from 9 WKY, n = 15 data points recorded from 5 SD

3.3.3.4 Strain comparison of T_c

Similar to the findings for T_{80} and T_{50}, the T_c showed no difference when the entire range of amplitudes was analysed together (H(2, N=306)=4.4758 p=0.1067), however a significant strain difference between SHR and WKY was found in the 'Large' amplitude group where SHR had the shortest and WKY the longest T_c (H(2, N=61)=6.5305 p=0.0382) (Fig. 3.28).

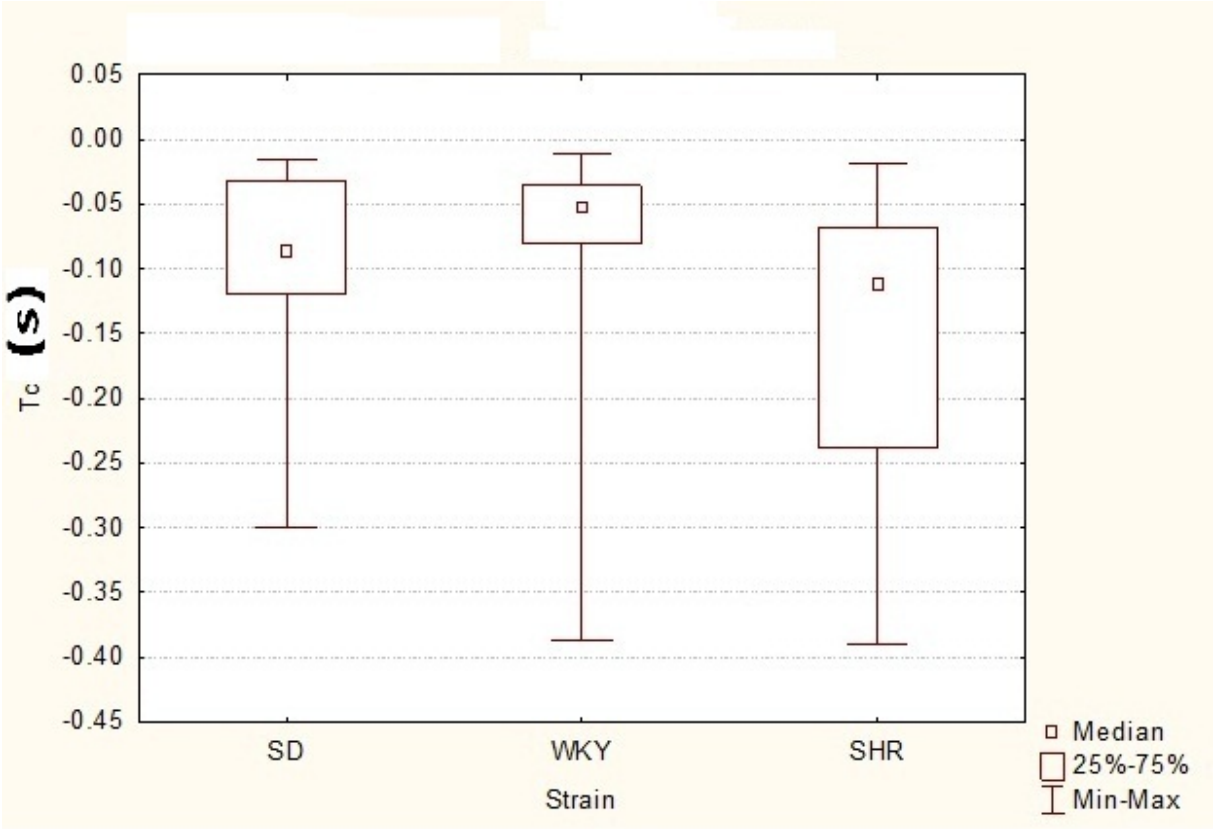

Figure 3.28 – Strain difference in T_c in the 'Large' amplitude group; H(2,N=61)=6.5305 p=0.0382; ∗p=0.0331; n = 23 datapoints recorded from 9 SHR, n = 23 datapoints recorded from 9 WKY, n = 15 data points recorded from 5 SD

3.3.3.5 Strain comparison for T_{100}

A significant strain effect was seen in T_{100} where SHR and SD times were similar and both were smaller than that of WKY (Fig. 3.29).

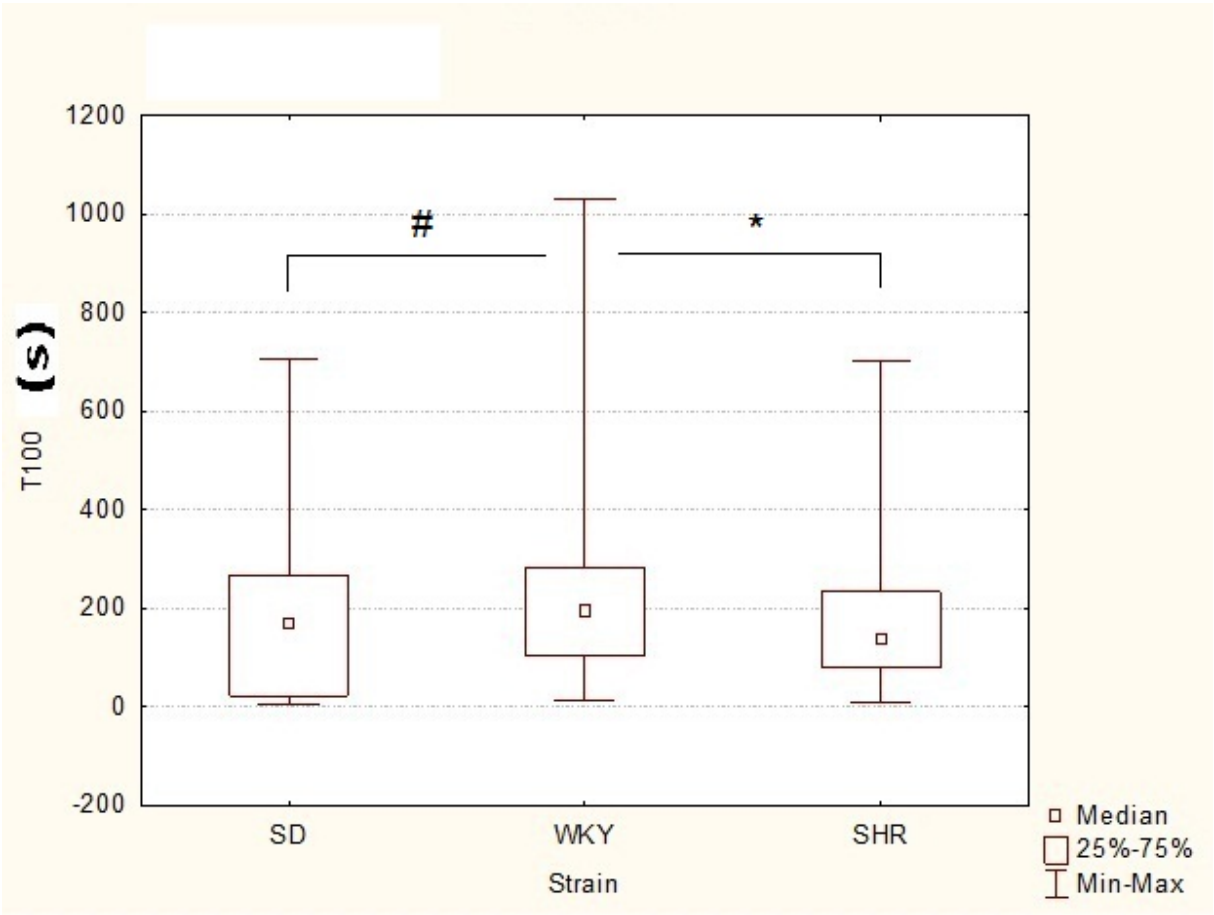

Figure 3.29 – Strain difference in T_{100}; SHR and SDs had T_{100}s that were similarly shorter than the WKY ; Overall H(2, N=306)=6.6654 p=0.0357; n = 95 datapoints recorded from 9 SHR, n = 116 datapoints recorded from 9 WKY, n = 95 data points recorded from 5 SD

3.3.3.6 Strain comparison in the amount of DA required to generate amplitudes within the specified range

A large range of volumes (∼3 nl ∼ >4000 nl) of DA was ejected in order to attain the required range of DA amplitudes, however a scatterplot showed that the amplitude sizes were not dependent on the amount of dopamine applied (Fig. 3.30). Thus it was queried whether there was any strain difference in the amount of DA required to generate the specified amplitudes.

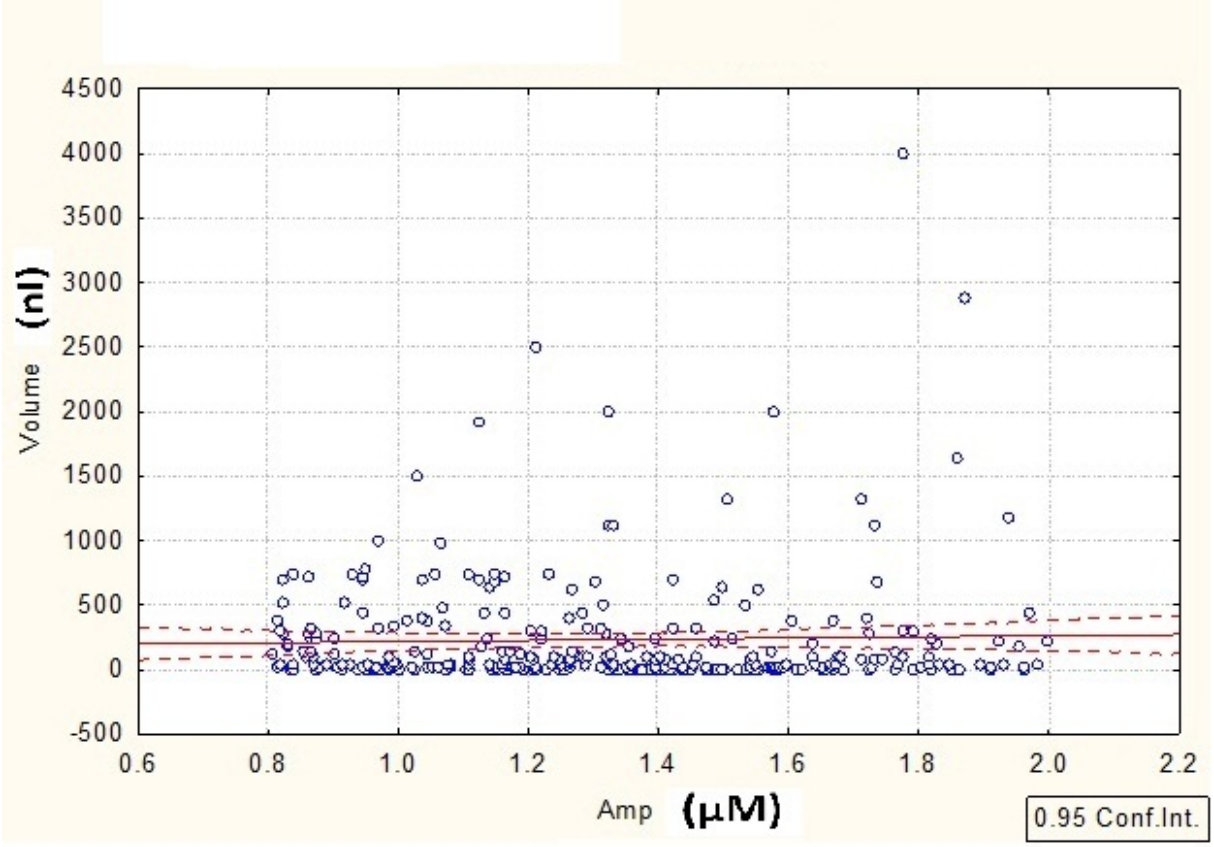

Figure 3.30 – Scatterplot of volume of DA applied and amplitudes obtained; r=0.0325

Strain comparison of the volumes that were ejected in order to achieve the required amplitudes (0.8 $\sim$ 1.99 μM) showed that the SHR required significantly smaller amounts of DA than the other strains (Fig. 3.31). This pattern was robust. When the amplitude groups were analysed individually, all amplitude groups showed significant strain differences in the same pattern with p <0.001. Specifically, 'Small' amplitudes H(2,N=66)=14.0906 p=0.0009, 'Medium' amplitudes H(2, N=179)=20.3880 p=0.0000 and 'Large' amplitudes H(2, N=61)=22.8645 p=0.0000. When the ST and NAc areas were analysed separately, this pattern remained consistent and significant in both the ST and NAc (ST: H (2, N=254)=37.8390 p=0.0000; NAc: H(2, N=34)=8.9469 p=0.0114).

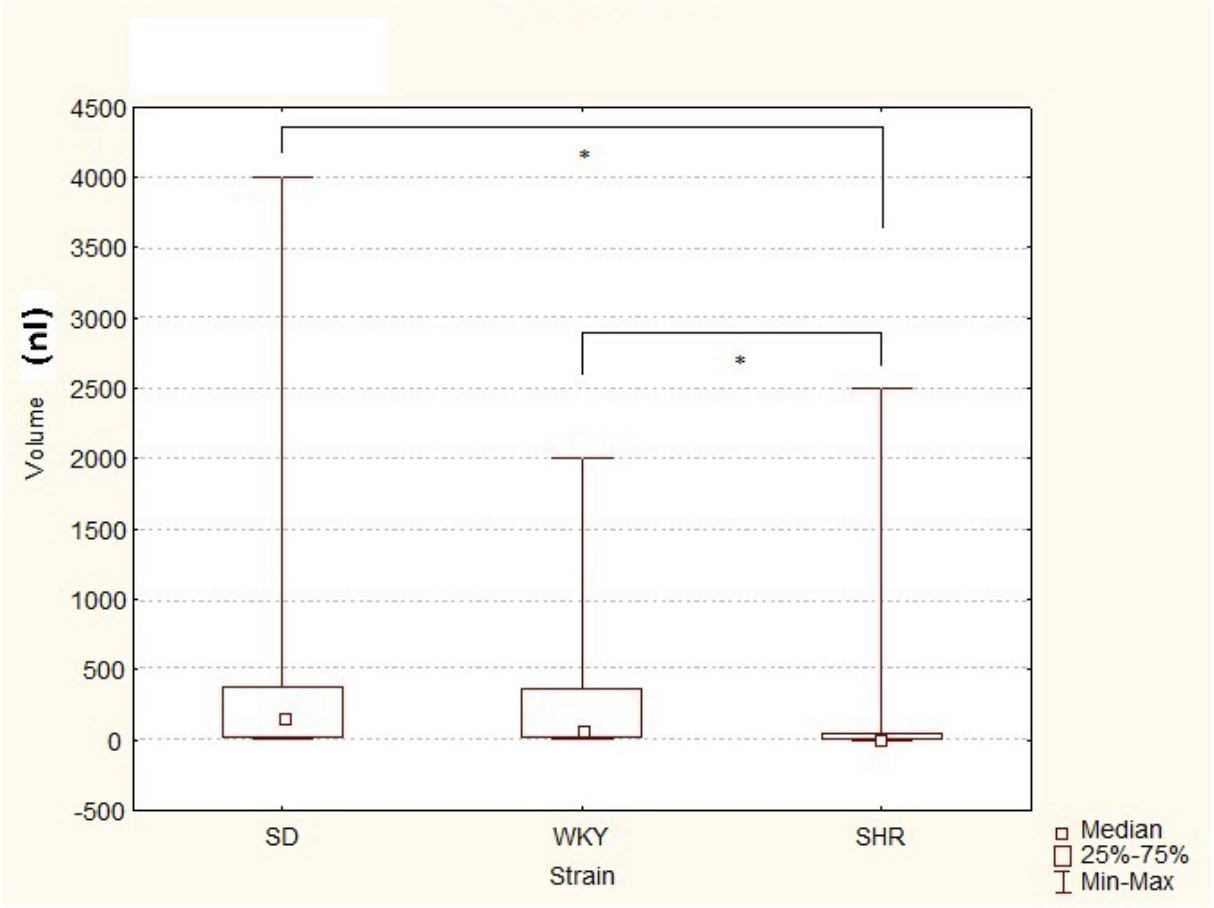

Figure 3.31 – Strain difference in the volume required to reach amplitudes between 0.8 $\sim$ 1.99 μM; H(2, N=306)=47.6532; $*$p=0.0000; n = 95 datapoints recorded from 9 SHR, n = 116 datapoints recorded from 9 WKY, n = 95 data points recorded from 5 SD

3.3.3.7 Strain comparison of T_{rise}

Strain comparison of T_{rise} showed that SHR had the shortest and WKY the longest T_{rise}, with SDs in between (H(2, N=306)=9.8539 p=0.0072). This pattern was seen most clearly in the ST (Fig. 3.32, but not in the NAc (H (2,N=34)=0.8531). More specifically, a strain difference was detected in the ST of the 'Large' amplitude group (H(2, N=53)=10.3834 p=0.0056), and not in the smaller amplitude groups ('Small': H(2, N=66)=3.6728 p=0.1594; 'Medium': H(2, N=179)=4.0079 p=0.1348).

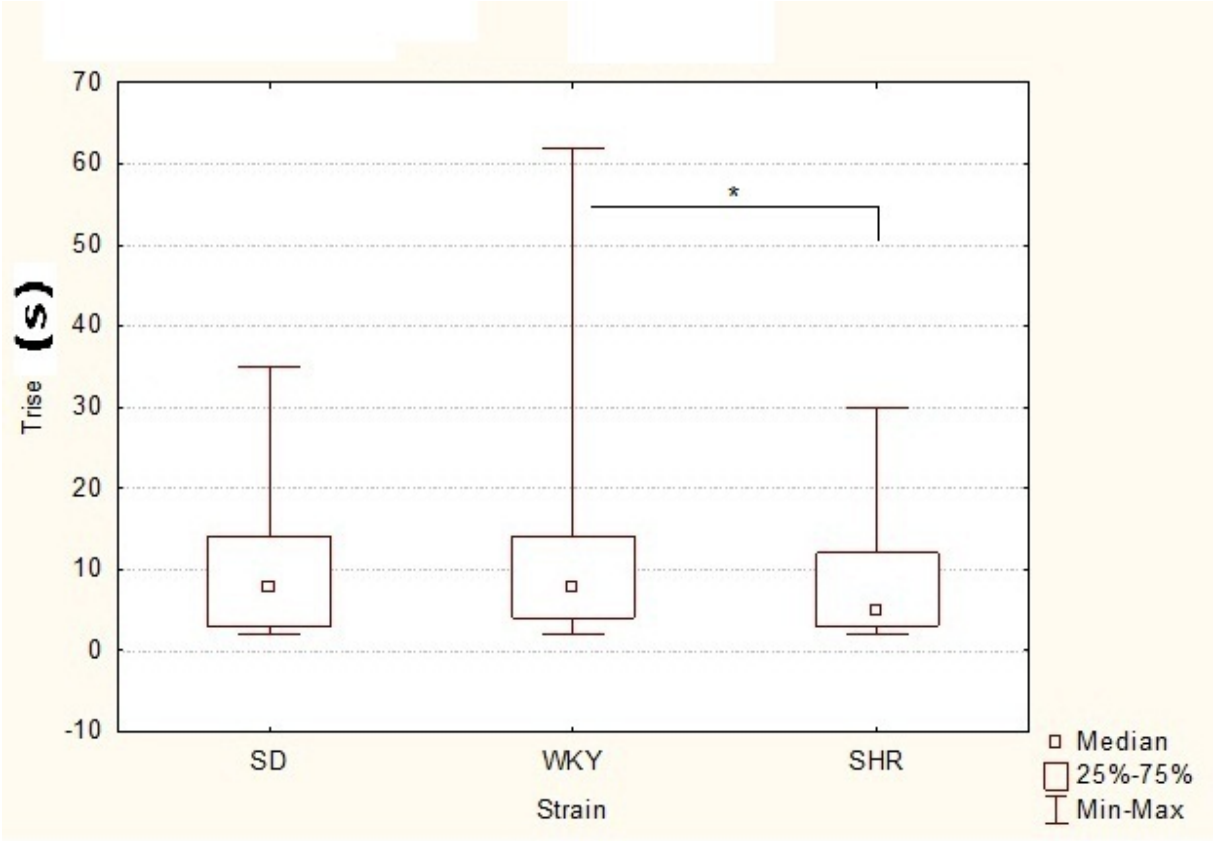

Figure 3.32 – Strain difference in T_{rise} in the ST; H(2, N=53)=10.3834 p=0.0056

3.3.3.8 Strain comparison of parameters when results were averaged per rat

In the striatum, when all ST points of each rat were averaged to a single mean value, no significant strain differences were detected for any parameter: T_{80} H (2, N=23)=0.0773 p=0.9621; T_{50} H (2, N=23)=0.0773 p=0.9621; T_c H(2, N=23)=0.5382 p=0.7641; T_{rise} H (2, N=23)=1.4850 p=0.4759.

Similar results were observed in the NAc:T_{80} H(2, N=14)=0.7020 p=0.7040 ; T_{50} H(2, N=14)=1.2900 p=0.5247; T_c H(2, N=14)=1.4748 p=0.4783; T_{rise} H(2, N=14)=0.0808 p=0.9604.

3.3.4 Analysis of calibrations

Analysis of 2 calibrations taken prior to and after recordings Rec 39 and Rec 40 showed that the calibration factors deteriorated slightly during the experiments (Table. 3.1). However, the electrode parameters after the recordings were still adequate for recordings, since the LODs were <0.1 μM and selectivities were >100.

Recording	LOD before	LOD After	Selectivity Before	Selectivity After	Slope Before	Slope After
39	0.0092	0.0407	1748.5833	334.7100	-0.1303	-0.0326
40	0.0037	0.0111	2122.1268	238.6563	-0.3931	-0.1014

Table 3.1 – Calibration parameters prior to and after rat recording numbers 39 and 40

3.4 Discussion

3.4.1 SHR vs WKY

Compared with the WKY, the SHR released significantly larger amounts of DA from similar KCl ejection volumes, while the T_{80} clearance times of these stimulated peaks were similar, and suggested that the WKY clear DA slower than that of SHR. This was confirmed with the DA clearance of exogenously applied DA experiments, when the amplitudes were controlled, SHR T_{80} was significantly shorter than that of WKY, and contradicts a previous *in vitro* study that suggested that DAT activity was not impaired in the SHR (Simchon et al., 2010). The present results suggest that DAT activity is impaired in the WKY as compared to the SHR in the falling phase of the DA peak. The volume of exogenously applied DA required to reach the specified amplitude was significantly lower in the SHR than WKY, and the T_{rise} to the peak amplitudes were significantly shorter in the SHR. This suggested that the clearance of DA in the SHR is delayed in the rising phase of the DA application, compared to WKY.

The SHR released significantly larger amounts of DA than WKY and contradicts previous *in vitro* superfusion studies that had showed decreased KCl stimulated release of DA (Russell et al., 1995), and no difference between the strains by *in vitro* measurement of synaptosomal release of DA (Simchon et al., 2010). Additionally, these data also contradict previous *in vivo* microdialysis results, where KCl stimulated release of DA was lower in SHR than in WKY (Carboni et al., 2003).

The potassium stimulated results of the present study was similar to only one previous Japanese *in vivo* voltammetric study from 1984, which had found that the release of DA and 5-HT in juvenile SHR ST was more prominent than in WKY, under stress (Ikeda

et al., 1984). Unfortunately this paper was not accessible via any means from UCT, and the authors were not contactable in order to obtain further details of the study. It cannot be determined from the abstract whether the 'release' was basal, electrically or KCl stimulated, although it was mentioned to be 'stress' stimulated, however the form of stress is not clear from the abstract. The DA and 5-HT were mentioned together, although it cannot be determined whether the attempts were to measure each neurotransmitter seperately, or whether the authors were accounting for the lack of selectivity of the electrodes at the time - however, current electrodes still do not differentiate between DA and 5-HT adequately. Finally, 'under stress' implied that the study was performed on freely moving animals, while the present study was performed on urethane anaesthetized animals. The Japanese study suggested that the SHR were more susceptible to stress in the central monoaminergic systems due to this increased release response in the SHR (Ikeda et al., 1984). Perhaps stimulated release is larger in SHR in the juvenile stage as seen in the present study and in the Japanese study, which causes a developmental compensation in the system that leads to decreased release in the adult stage, as found by the *in vivo* and microdialysis studies. Alternatively, the timescale of the voltammetric experiments measure the effects of the stimulus within seconds, while microdialysis results are an accumulation of minutes. It is possible that the increased immediate release of DA could cause decreased further release by action of autoreceptors and resulting in lower release data from SHR in the microdialysis studies.

3.4.2 SHR vs SD

Compared with the SD, the SHR released similar amounts of DA from KCl stimulations, while the SD had shorter T_{80} clearance times from these stimulated peaks. Comparison of T_{80} when the amplitudes were controlled showed that the T_{80}s were similar in these strains. The volume of exogenously applied DA required to reach the required amplitude was significantly lower in the SHR than SD, while the T_{rise} to the amplitudes were similar. These results support that although the SHR and SD have similar release and uptake parameters, the SDs reached the same amplitude with much larger amounts of applied DA, but in the same amount of time as SHR, suggesting that the clearance of DA in the SHR could be delayed in the rising phase of the DA application.

No studies have compared KCl stimulated release responses between SHR and SD. One *in vivo* microdialysis study had shown that basal DA levels of SHR were lower than of SD, and that amphetamine stimulated release of DA was higher in SHR than SD (Heal et al., 2008). However, amphetamine and KCl stimulations are not comparable, since amphetamine induces vesicular leakage of DA into the presynaptic cytoplasm and non-vesicular release of DA, while KCl mainly stimulates vesicular release.

3.4.3 WKY vs SD

Compared with the SD, the WKY released significantly lower levels of DA from KCl stimulated release, and T_{80} of these peaks were significantly longer than SD. Comparison of T_{80} when the amplitudes were controlled showed that the T_{80}s were in fact longer in the WKY. The volume of exogenously applied DA required to reach the specified amplitude was significantly lower in the WKY than SD, while the T_{rise} to the amplitudes were significantly longer than the SDs. These results indicate that the WKY release less DA and have significantly slower uptake rates than SD, and that the slow uptake rates occurs both in the rising and in the diminishing phase of the DA response.

Taken together, the present results support that in the rising phase of the DA response, DAT activity in the SHR is slower compared to both SD and WKY, although the WKY were slower than the SDs. SHR and SD release similar amounts of DA, while the WKY released the least. T_{80}s showed that the WKY had the longest clearance times in the falling phase of the response compared to the other strains.

Strain difference in clearance times were most detectable in the 'large' amplitude group when amplitudes were $1.6 \sim 1.99$ μM. Although strain difference did not reach significance in the 'small' and 'medium' size amplitude groups, these groups showed the same patterns of lowest T_{80}, T_{50} and T_c times in the SHR, highest in the WKY and SDs in between. It appears that when DA amplitudes are low, all three strains clear DA similarly, however at higher amplitudes of DA, the defect in DAT function in the falling phase of the response becomes more evident in the WKY.

3.4.4 Considerations and controls for possible confounding factors

3.4.4.1 Lack of correlation beween Mass, volume, amplitude and T_{80} for exogenouly applied DA

Scatterplots showed that ejection volumes and rat mass were not correlated with amplitude or T_{80}, enforcing that rat mass and volume of exogenous DA applied were not confounding factors and significant differences observed were strain dependent. Scatterplots showed various outliers that were the results from various peaks from various rats and not attributed to a single rat recording or electrode. Histological examination did not warrant the exclusion of these points from the analysis.

3.4.4.2 Analysis of KCl ejection volumes

Although SHR received volumes of KCl on the larger end of the range limit (100 $\sim$ 250 nl) compared to the SDs, the KCl volumes within this range were not correlated with amplitudes, which indicated that higher amplitudes were not solely due to higher volumes of KCl and that the slightly higher volumes of KCl received by the SHR did not compromise subsequent strain comparisons of amplitudes or T_{80}. Additionally, the SDs that received the lowest volumes of KCl but achieved peak amplitudes that were more similar to that of the SHR, and the WKY had received similar amounts of KCl compared to both of these strains, had released the least amount of stimulated DA further indicating that the strain difference in ejection volumes was not a confounding factor in these experiments.

3.4.4.3 Rat mass did not influence amplitude and T_{80} parameters in KCl experiments

Rat mass was found to be significantly larger in the SDs compared to the SHR and WKY in the KCl stimulate release experiments which was not found in the DA clearance experiments. Rat mass was however not correlated with either amplitude or T_{80}, which indicated that it was not a confounding factor in the analyses.

3.4.5 Analyss of averaged data in the ST and NAc

When individual points from the ST and NAc were averaged for each rat, so that each rat contributed a single ST and NAc point, no significant difference was observed for any comparison. One could argue that the data should be averged, since the multiple points taken from each site are technically dependent samples in that they were taken from the same rat. However, if this logic is employed to its fullest, the ST and NAc data should in fact be reduced to a single point, with each rat contributing only one point to the dataset. However, it is known that different areas in the ST and NAc respond differently, and the averaging of data points could render the results physiologically meaningless.

3.4.6 Electrode Sensitivity and Selectivity

Electrode calibrations before and after 2 experiments showed that the selectivity had decreased slightly. This is could have been due to adsorption of the electrode sites from previously oxidized material, which gradually decreased the available sites on the electrode that could cause oxidation and conduct signal, also termed bio-fouling. Decreasing sensitivity and selectivity of the electrodes would be a cause for concern, since it could cause latter points in the recording to be due to less selective signals, although, since it is impossible to determine a point at which the bio-fouling occurs, or the gradient of severity

at which it occurs, it is not possible to correct for the changes in calibration parameters during or after the experiment. However, the calibration parameters that were observed after these experiments remained adequate for recordings, and even though the slope of Rec 40 had increased, the slope after the Rec 40 experiment was similar to that of the start of the Rec 39 experiment. It appears that the variation between calibrations could in fact balance the decrease in electrode sensitivity within each experiment.

3.5 Limitations

3.5.1 Non-parametric data

The present data was not parametric, and thus statistical analyses of lower power had to be employed for comparisons, however the non parametric data could not be avoided, since amplitude and time parameters were all >0. For clearance time parameters, the majority of peaks returned to baseline within the first 100 s, although there were some peaks that took up to 600 s, these could not be justifiably excluded on basis of histology, or other factors noted on the experimental day. For the amplitude parameter, the majority of peaks resulting from KCl stimulations were <10 μM, although observations ranged to ∼60 μM, these outliers could also not be justifiably excluded. Thus, the data was inevitably skewed toward the left side.

3.5.2 Histological determination of recording location

Histological determination of the exact placement of the electrodes were difficult, since the glass micropipette diameter leading up to the tip was quite large in comparison to the small 30 μm tips of the working electrodes and the ∼10 μm tips of the glass electrodes. A previous study that had compared the difference in DA clearance rates between the ST and the NAc had passed current was at the end of the experiment to mark the deepest point of the measurements (David et al., 1998). However this procedure required the use of a different headstage and was not suggested during training.

3.5.3 Lack of significance in NAc recordings compared to ST recordings

The majority of comparisons within the NAc showed a lack of significance between strains, while recordings from the ST tended to follow patterns of the general strain comparisons that included the NAc. This could have been due to a genuine lack of differences in NAc responses between strains. However it could have also been due to lower sample sizes of recordings obtained from the NAc. Histological determination of tracts typically showed

that the aim was often too lateral and recordings often taken from outside the striatum or in the anterior commisure and there was no response, which resulted in the exclusion of these points from the analyses.

Previous studies had only compared differences in responses between the ST and NAc within strains. Rat ST and NAc DAT were found to have different molecular weights (Lew et al., 1991, 1992), and differences in molecular weights (and thus possibly function) between the ST and NAc could explain the differences in clearance rates between the ST and NAc that were previously observed (David et al., 1998).

3.5.4 Rats surviving under anaesthesia

In some rats, only the KCl or DA recordings could be performed, while others were used for both types of recordings. This was dependent on whether the rat survived the recording. Rat body masses did not differ for the DA application experiments, while SDs in the KCl experiments were heavier than the SHR and WKY. Since the DA application recordings typically occured after the KCl stimulations, one would assume that the smaller rats that were not able to survive past the KCl recording thus did not contribute to the DA recordings. However, the descriptive statistics of rat masses showed that the SD rat masses were smaller in the DA experiments than in the KCl, suggesting that it was the larger rats that did not survive the experiments - which was not expected.

Some rats died suddenly during the recordings without any apparent reasons, which is a cause for concern, since brain responses in the rat could have become errant prior to death. However observations of rat breathing saw no change in rate or depth, and deaths were unpredicatable. Thus the last point from these recordings were excluded from analyses.

Results taken from anaesthetized animals cannot be extrapolated to occurrences in conscious animals, and further to conscious humans. However data obtained in this manner increases the comparability of the data with other *in vivo* chronoamperometry studies performed on anaesthetized rats (Sabeti et al., 2003; Gerhardt and Burmeister, 2000), and allow assessment of whether this initial set-up was successful. Additionally, it was necessary to show competence in conducting experiments with anaesthetized rats prior to setting up the technique to perform recordings in awake animals due to increased complexity of the latter. More importantly, it is not possible to perform mapping experiments on awake animals, since the locations of electrodes implanted into awake animals cannot be manipulated.

3.6 Conclusion

The set-up of the *in vivo* chronoamperometry technique in the Russell laboratory was successful. This technique, although with certain limitations, was able to detect neurochemical differences between the SHR, WKY and SD rat strains. The present results showed that the WKY released the least amount of DA and also had the slowest clearance times compared to the other two strains, while the SHR showed similarities with the SD, although with a delayed DAT function in the rising phase of the responses.

4 Behavioural Responses to Methylphenidate Treatment

4.1 Introduction

Methylphenidate (MPH) has been shown to be useful in the treatment of symptoms of ADHD. In the SHR model however, MPH produced increased activity in SHR and WKY in terms of lever presses (Sagvolden et al., 1993b), as well as exploratory behaviour in the open field as well as in the operant chamber during a test of visual spatial attention (Wultz et al., 1990; Thanos et al., 2010). Although sub-chronic methylphenidate treatment decreased rearing activity of the SHR in the Làt-maze (Aspide et al., 2000).

Currently no known studies have been published regarding SHR activity in voluntary running wheels. Measurement of wheel revolutions produced by rats housed in cages with attached running wheels could allow accurate measurement of voluntary activity and possibly provide a valuable strain comparative measure.

Since low dose MPH appears to ameliorate while high doses potentiates ADHD symptoms, the present study tested low oral doses of MPH from 0.5 to 5 mg/kg in the SHR for 4 days each in order to determine the optimum dose at which MPH affects SHR behaviour.

4.2 Methods

4.2.1 Animals

Animals were born in the main UCT Animal Unit where food and water was available *ad libitum*, temperature was controlled between $22 \sim 24\ ^{\circ}C$ and a 12 hr light/dark cycle (06:00 lights on) was maintained. At P 21 pups were weaned and transferred to the satellite Animal Facility in the Anatomy Building where temperature and food availability were controlled as above, but the 12 hr light/dark cycle was altered (09:00 lights off). Rats were kept with littermates and allowed to acclimatize to the new light/dark cycle for 1 week prior to commencement of experiments at P 28. From P $24 \sim$ P 27 rats were placed in individual cages for 30 mins each day and were given condensed milk vehicle (CM) in petri-dishes. All rats readily consumed vehicle by P 27. A total of 11 SHR and 7 WKY were used. This experiment was approved by the UCT Faculty of Health Sciences Animal Research Ethics Committee. Project number: 008/005.

4.2.2 Running Wheels

SHRs were randomly assigned into treated (SHRM) and non-treated (SHR) groups, while WKY served as strain comparison controls and were not treated. At P 28, rats were placed into individual cages with attached running wheels. In dosing period 1 (P 28 $\sim$ P 31), vehicle was administered to all groups. In dosing periods 2 $\sim$ 5 (P 32 $\sim$ P 35; P 36 $\sim$ P 39; P 40 $\sim$ P 43; P 44 $\sim$ P 47) SHRM received 0.5, 1.0, 2.0 and 5.0 mg/kg MPH respectively, while SHR and WKY groups received vehicle. Vehicle or drug was administered twice a day, at the start and 6 hrs into the dark cycle (09:00 and 15:00), since the half-life of MPH is approximately 2.5 hours. In dosing period 6 (P 48 $\sim$ P 51) no vehicle or drug was administered to any groups of rats.

Revolutions were recorded via an automated computer system (Exercise Wheel Data Capturer, RRE v1.2). Each revolution was equivalent to a distance of 1 m.

4.2.3 Statistical Analysis

Dark and light cycle revolutions over each dosage period were averaged for each rat. Shapiro-Wilks's Normality tests (Statistica 9) revealed that the data were not parametric (Fig. **??**). Kruskal-Wallis and Dunn's Multiple Comparison tests were used for all group comparisons (Prism 5, and verified with Statistica 9). Results are reported as median and interquartile range.

4.3 Results

4.3.1 Running revolutions during dosage period 1: Vehicle administration only

In the first dosage period when vehicle was administered to all groups, there was no significant difference in running revolutions between groups during the dark cycle (Fig. 4.1). However during the light cycle, the SHR and SHRM groups ran significantly more revolutions than the WKY (Fig. 4.2).

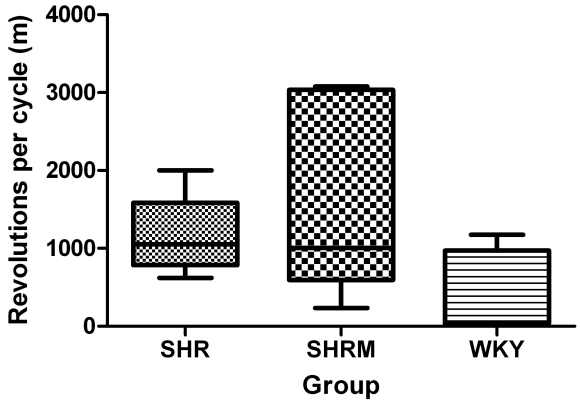

Figure 4.1 – Average number of revolutions of running wheels during dark cycles when vehicle was administered to all groups; No significant difference between groups was observed; H(2, N=18)=5.913617 p=0.0520; SHR n=6, SHRM n=5, WKY n=7

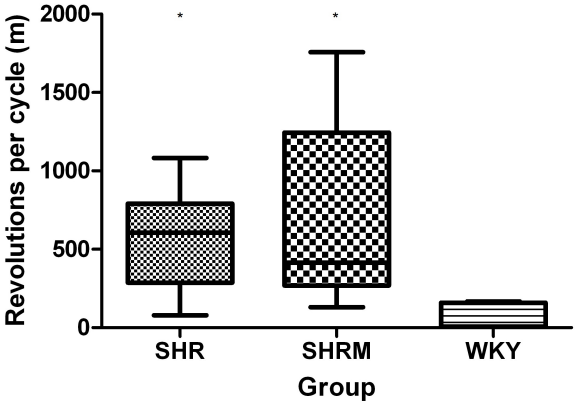

Figure 4.2 – Average number of revolutions of running wheels during light cycles when vehicle was administered to all groups; WKY ran significantly fewer revolutions than SHR (p=0.0309) and SHRM (p=0.0291); H(2, N=18)=9.225898 p=0.0099; SHR n=6, SHRM n=5, WKY n=7

4.3.2 Running revolutions during dosage period 2: 0.5 mg MPH/kg for treated group

During dosage period 2, MPH was administered at 0.5 mg/kg to the SHRM. The WKY ran significantly fewer revolutions than SHRM in both dark and light cycles, and also ran less than SHR during the light cycle (Fig. 4.3 and Fig. 4.4). No difference in running distance was detected between SHRM and SHR groups, indicating no effect of MPH treatment.

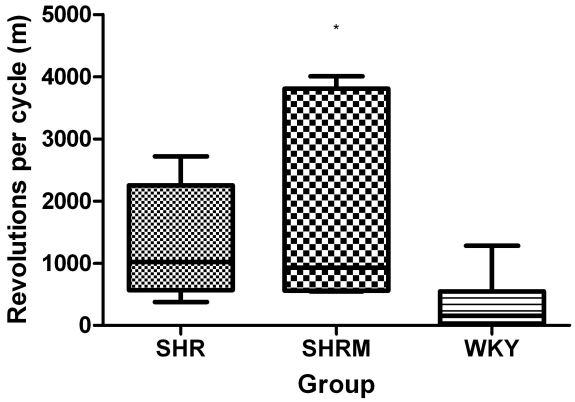

Figure 4.3 – Average number of revolutions of running wheels during the dark cycle when SHRM received 0.5 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0487), and had a tendency to run fewer revolutions than SHR (p=0.0950); H(2, N=18)=7.2610 p=0.0265; SHR n=6, SHRM n=5, WKY n=7

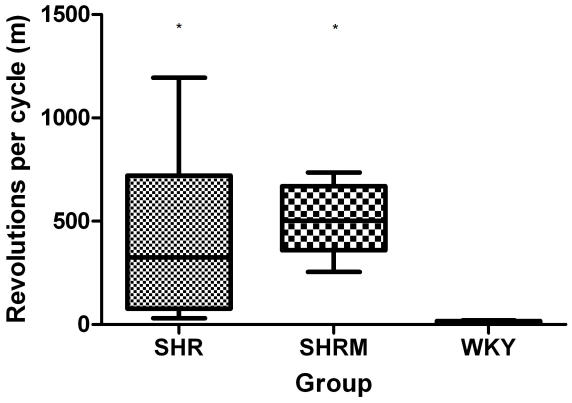

Figure 4.4 – Average number of revolutions of running wheels during the light cycle when SHRM received 0.5 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0041) and SHR (p=0.0179); H(2, N=18)=12.4924 p=0.0019; SHR n=6, SHRM n=5, WKY n=7

4.3.3 Running revolutions during dosage period 3: 1.0 mg MPH/kg for treated group

Dosage period 3 when MPH was administered at 1.0 mg/kg showed the same pattern as dosage period 2. The WKY ran fewer revolutions than SHRM in the dark cycle, and ran fewer revolutions than both SHR and SHRM in the light cycle (Fig. 4.5 and Fig. 4.6). No effect of MPH treatment was detected.

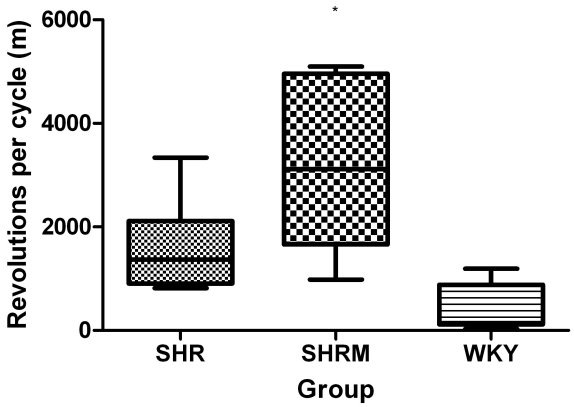

Figure 4.5 – Average number of revolutions of running wheels during the dark cycle when SHRM received 1.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0047); H(2, N=18)=10.5627 p=0.0051; SHR n=6, SHRM n=5, WKY n=7

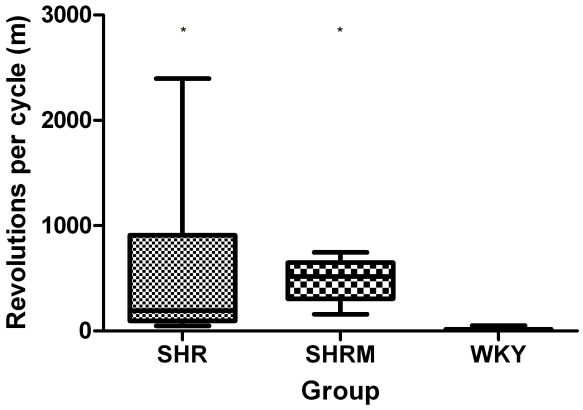

Figure 4.6 – Average number of revolutions of running wheels during the light cycle when SHRM received 1.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0031) and SHR (p=0.0339); H(2, N=18)=12.2621 p=0.0022; SHR n=6, SHRM n=5, WKY n=7

4.3.4 Running revolutions during dosage period 4: 2.0 mg MPH/kg for treated group

During dosage period 4 when MPH was administered at 2.0 mg MPH/kg, WKY ran significantly fewer revolutions than SHRM in both dark and light cycles, and also ran less than SHR during the light cycle (Fig. 4.7 and Fig. 4.8). No difference in running distance was detected between SHRM and SHR groups, indicating no effect of MPH treatment.

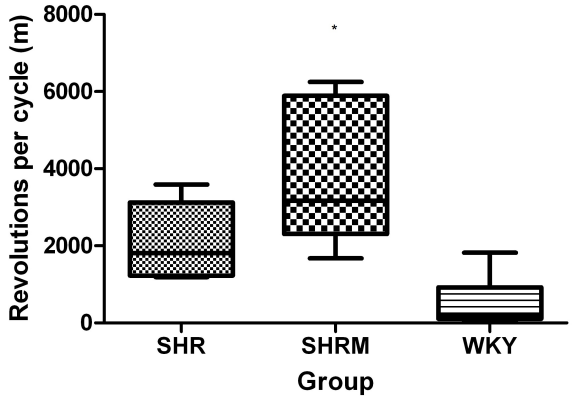

Figure 4.7 – Average number of revolutions of running wheels during the dark cycle when SHRM received 2.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0040), and had a tendency to run fewer revolutions than SHR (p=0.0913); H(2, N=18)=11.00301 p=0.0041; SHR n=6, SHRM n=5, WKY n=7

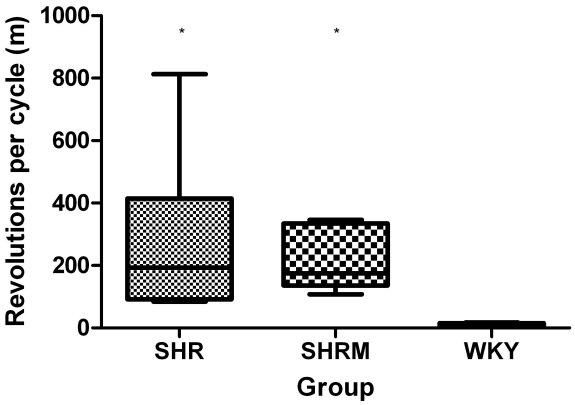

Figure 4.8 – Average number of revolutions of running wheels during the light cycle when SHRM received 2.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0064) and SHR (p=0.0126); H(2, N=18)=12.2737 p=0.0022; SHR n=6, SHRM n=5, WKY n=7

4.3.5 Running revolutions during dosage period 5: 5.0 mg MPH/kg for treated group

Group comparison of revolutions run during dosage period 5, 5 mg MPH/kg, was similar to that of previous dosage periods. WKY ran significantly fewer revolutions than SHRM, and had a tendency to run fewer revolutions than SHR in the dark cycle (Fig. 4.9). WKY ran less than both SHR and SHRM in the light cycle (Fig. 4.10). No effect of MPH was found.

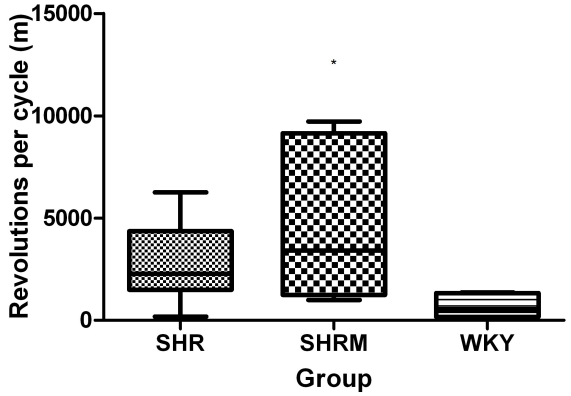

Figure 4.9 – Average number of revolutions of running wheels during the dark cycle when SHRM received 5.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0359), and had a tendency to run fewer revolutions than SHR (p=0.0842); H(2,N=18)=7.800 p=0.0202; SHR n=6, SHRM n=5, WKY n=7

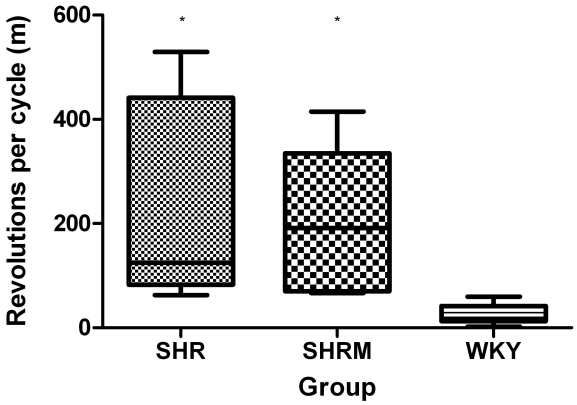

Figure 4.10 – Average number of revolutions of running wheels during the light cycle when SHRM received 5.0 mg MPH/kg; WKY ran significantly fewer revolutions than SHRM (p=0.0120) and SHR (p=0.0073); H(2,N=18)=12.1579 p=0.0023; SHR n=6, SHRM n=5, WKY n=7

4.3.6 Running revolutions during dosage period 6: No vehicle or drug administration for all groups

When no substances were administered to any group during dosage period 6, no group difference in running was found during the dark cycle (Fig. 4.11). A strain difference in running was found between the SHR and WKY controls in the light cycle, where the WKY ran significantly less than the SHR, and had a tendency to run less than the SHRM group (Fig. 4.12).

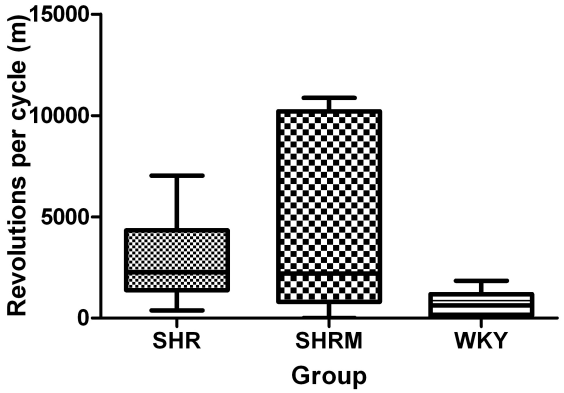

Figure 4.11 – Average number of revolutions of running wheels during the dark cycle when no substances were administered to all groups; No group differences in running distances were observed; H(2,N=18)=5.3489 p=0.0689; SHR n=6, SHRM n=5, WKY n=7

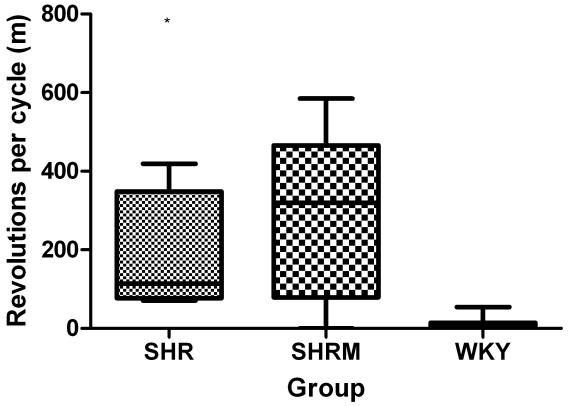

Figure 4.12 – Average number of revolutions of running wheels during the light cycle when no substances were administered to all groups; WKY ran significantly less than SHR (p=0.0406) and had a tendency to run less than SHRM (p=0.0538); H(2,N=18)=8.1392 p=0.0171; SHR n=6, SHRM n=5, WKY n=7

4.4 Discussion

The purpose of this experiment was to determine whether the use of cages with attached running wheels could be useful as a strain comparative measure, and to find a dose of MPH that could decrease the running activity in SHR to a level that is more similar to WKY.

Significant difference in running revolutions were consistently observed between the SHR and WKY during the light cycles but not the dark cycles. This suggests that voluntary running during the light cycle, in cages with attached running wheels, could be used to differentiate between SHR and WKY strains.

During the dark cycle, the SHRM ran significantly more than WKY at every dosage period where MPH was administered, and not in the vehicle and non-treated periods. Although no significant differences in running was measured between the SHR and SHRM, when compared to the WKY, the SHRM ran more while the SHR did not, suggesting that MPH could be increasing voluntary activity levels in the SHRM group that was not detectable in direct comparison with the SHR at the present sample sizes. These results are in line with studies that have shown increased activity levels as a result of MPH treatment. In the open field test, MPH increased exploratory behaviour of both SHR and WKY (Wultz et al., 1990; Barron et al., 2009). Locomotor activity in the operant chamber of a visual stimulus position discrimination task increased in both SHR and WKY (Thanos et al., 2010), and number of lever presses increased as a response to MPH treatment in a delay-of-enforcement task for both SHR and WKY (Sagvolden et al., 1993b). Thus is terms of general motor activity, MPH has an apparent stimulatory effect in SHR and no dose was found to decrease their activity levels to that of the WKY.

The effects of MPH on reducing hyperactivity in children could be due to improvements in top-down regulation of motor control. MPH has been shown to inhibit both DAT and NET (Markowitz et al., 2006) and the increase in NE levels in the PFC (Kuczenski and Segal, 1997, 2001) was also shown to increase the intrinsic excitability of PFC neurons, that was mediated through the α_2-adrenoreceptor (Andrews and Lavin, 2006; Arnsten and Dudley, 2005). The effect of MPH on rat cognitive function appears more promising in the literature. MPH treatment was shown to increase the effectiveness of a delayed enforcer in both the SHR and WKY, although the response was less prominent in the SHR (Sagvolden et al., 1993b), and sub-chronic MPH treatment was shown to decrease rearing activity of the SHR in the Làt-maze, a behaviour that is considered as a reflection on impulsivity (Aspide et al., 2000). Thus perhaps MPH decreases elements of motor impulsivity that can be measured with specific tasks when reinforcements are clear, however general activity in the SHR that are elicited by unintended

stimuli (Johansen et al., 2002) may not be affected. In children with ADHD, excessive activity is discouraged while lower levels of activity are reinforced socially, thus reinforcers for motor activity are clear. However, 'social appropriateness' of locomotor activity in rats cannot be determined, thus, if MPH functions by increasing the effectiveness of reinforcers (Johansen et al., 2002), it could be reasonably expected that voluntary activity in the running wheels would not decrease as a result of MPH treatment.

4.5 Limitations

Condensed milk was used as the vehicle in this experiment. Even with dilution with dH_2O, the sugar content was likely to still be quite high. It was possible that sucrose could have different effects in SHR and WKY and induced the increase in activity in the SHR more than WKY. However, the SHRM only ran significantly more than WKY during dosage periods where MPH was administered, and not in the vehicle and non-treated periods, the SHR ran significantly more than the WKY during the light cycle when vehicle was not administered and the pattern of activity in dosage period 6 when no vehicle was administered to any of the groups was identical to that seen in dosage period 1 when condensed milk was administered to all groups. These results suggest that condensed milk did not cause differential effects on the two strains, although groups of SHR and WKY without CM/high sucrose administration are required to confirm this assumption.

Isolation stress could impact on voluntary running behaviour. All the rats were kept in identical individual cages, which relatively controls for isolations stress, however the SHR and WKY have been shown to have differential levels of responses to stress, which may have impacted on the amount of voluntary running between the strains. Unfortunately, it is difficult to measure accurate activities of individual animals if they were housed in groups. Perhaps an alternative protocol could be used in which the rats were housed in groups for the majority of the day and only placed in the wheels for measurement of voluntary activity for a few hours every night. However this protocol could potentially enhance the experience of isolation, since the rats never becomes accustomed to it, and additional handling (since rats would be removed from the wheels each day) could induce a different form of unquantifiable stress.

The light/dark cycle of animals was changed from 06:00 lights on to 09:00 lights off from P 21, since the short half life of MPH warrants dosing twice during the dark cycle, and it was not feasible to administer the 2nd dose of MPH at midnight for each experimental day. The change in light cycle could have been a potential stressor, however the rats were given 7 days to acclimatize to the new light/dark cycle prior to commencement

of experimentation, and residual effects of this stress was minimized.

Different doses of MPH were given to the same rat at increasing ages and duration in the wheels. The previous administrations of MPH could possibly influence the effects of the subsequent doses due to drug sensitization, which has been shown to occur in 0.6 mg MPH/kg in adult rats and 2 mg MPH/kg (i.p.) for adolescent as well as adult SHR (Barron et al., 2009). However, i.p. drug administration has been shown to cause sensitization more readily than low oral doses of MPH that are therapeutically relevant to those given to children (Kuczenski and Segal, 2002; Arnsten and Dudley, 2005).

5 Behavioural Responses to Guanfacine Treatment

5.1 Introduction

As reviewed in the introduction, guanfacine's positive effects in the treatment of ADHD symptoms and low side effect profile led to FDA approval for its use in treating ADHD in September, 2009 (May and Kratochvil, 2010). In the SHR, guanfacine treatment at 0.6 mg/kg i.p. showed improvements in sustained attention and reduced hyperactivity and impulsiveness (Sagvolden, 2006).

This project elected to study the effect of guanfacine on voluntary running in order to compare drug effects with that of methylphenidate. In addition, activity in the open field was analysed for the effect of isolation stress in the running wheels. The vehicle for drug administration was changed from CM to raspberry flavoured jelly to minimize possible effects of the high sucrose content in the CM.

5.2 Methods

5.2.1 Animals

Animals were trasnferred to the satellite Animal Facility in the Anatomy Building at P 21. Food and water was available *ad libitum*, temperature was controlled between 22 $\sim$ 24 $^{\circ}C$ and a 12 hr light/dark cycle (06:00 lights on) was maintained. Rats were kept with littermates when not in running wheels. Rats of each strain were randomly assigned to control (SHRC, WKYC or SDC) or guanfacine treated groups (SHRG, WKYG or SDG). On P 21 and P 22 rats were placed in individual cages for 30 mins and were given raspberry flavoured jelly vehicle in small petri dishes at 18:00. From P 23 $\sim$ P 27 Control animals received vehicle and dH_2O while Treated animals received vehicle and guanfacine (HCl salt, Sigma-Aldrich) at 0.69 mg/kg (0.6 mg/kg free base (Sagvolden, 2006)) once a day at 18:00, since the half life of guanfacine is approximately 17 hrs. Rats were placed in individual cages for the brief period of dosing and returned immediately to the home cage. An open field test was conducted on P 28, after which rats were placed in cages with attached running wheels from P 28 $\sim$ P 32. Dosing continued once a day while rats were in cages with attached running wheels. Rats were removed from

running wheels on P 33 and housed with original litter mates until P 35 when another open field test was performed. Rats were kept with littermates for 2 more days prior to dissection of PFC, ST, dorsal HIP and ventral HIP for future proteomics studies. A total of 18 SHR, 13 WKY and 11 SDs were used. This experiment was approved by the UCT Faculty of Health Sciences Animal Research Ethics Committee. Project number: 009/005.

5.2.2 Running Wheels

Animals were placed into individual cages with attached running wheels from P 28 $\sim$ P 32 where vehicle and dH_2O or guanfacine was administered in petri dishes once a day at 18:00. Running revolutions were recorded via an automated computer system (Exercise Wheel Data Capturer, RRE v1.2). Each revolution was equivalent to a distance of 1 m.

5.2.3 Open Field

Rat behaviour in the open field was measured on P 28 prior to being placed into running wheels and on P 35, 2 days after rats were removed from running wheels and rehabituated with littermates for 2 days prior to open field recordings. On recording days, rats were brought to the open field room and allowed to habituate to the new environment for 1 hr prior to recordings. The open field apparatus consisted of a 1 m $\times$ 1 m black box. The 'inner zone' was the 70 cm $\times$ 70 cm area in the middle of the box starting 15 cm away from the aparatus walls. The 'outer zone' was the area between the outer walls of the apparatus and the 'inner zone'. After the 1 hr habituation period, each rat was placed in the outer zone of the open field, facing the right wall and activity was recorded for 15 min in the light cycle using mounted HD cameras (Sony). Faecal pellets of each rat were counted after each recording, the field was first cleaned with paper towelling and then wiped with 70% EtOH and left to dry for 20 s prior to recording the activity of the next rat.

5.2.4 Statistical Analysis

Running Wheel data. Dark and light cycle wheel revolutions were averaged over the 4 running days for each rat. Shapiro-Wilks' nomality test was performed and non-parametric statistics were used (Fig. D.1). Kruskal-Wallis tests were used for comparison of multiple groups. Mann-Whitney U tests were performed for comparisons within each strain or treated and non-treated animals. Results are reported in median and quartile range.

Open Field data. Behavioural videos were analysed with Ethovision XT7 software (Noldus). Unfortunately several videos were corrupted during data transfer, resulting in very small sample sizes for the SD and WKY strains (SDC n=2, SDG n=1; WKYG n=6;

WKYC n=3; SHRG n=10; SHRC n=8). Total Distance travelled, average velocity and times spent in the inner zones before and after placement in running wheels were compared. Data were not parametric. The Wilcoxon matched pairs test was used to compare before and after placement in the running wheels. Mann-Whitney U test was used to compare the effect of guanfacine treatment on open field parameters in SHR. Results are reported in median and quartile range.

5.3 Results

5.3.1 Running Wheels

5.3.1.1 Strain comparison of running activity in the dark and light cycles

Strain comparison of running differences in the vehicle treated groups showed that the SHR ran significantly more than both the WKY and SD strains in both the dark and light cycles (Fig. 5.1 and Fig. 5.2).

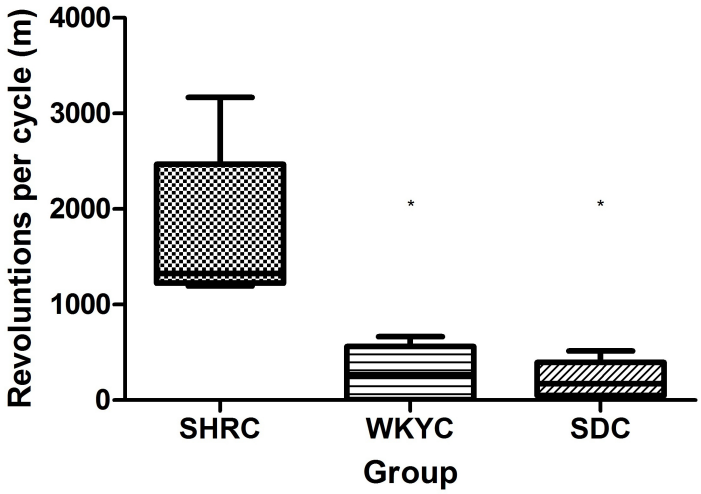

Figure 5.1 – Average number of revolutions of running wheels during dark cycles in control groups; SHRC ran significantly more than WKYC (p=0.0064) and SDC (p=0.0075); H(2, N=19)=13.2116 p=0.0014; SHRC n=8, WKYC n=6, SDC n=5

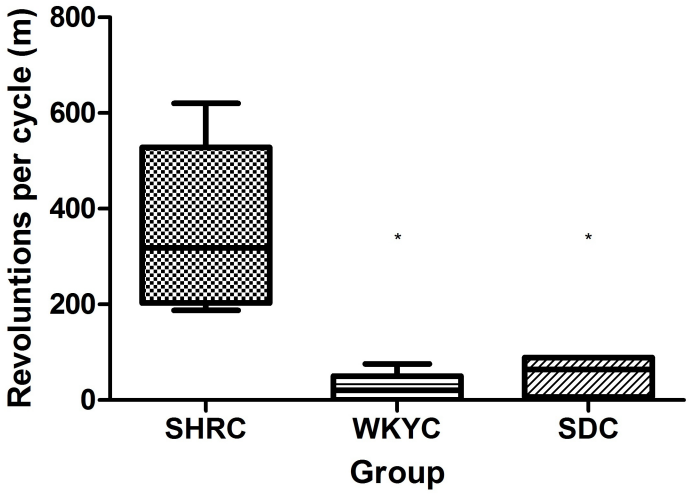

Figure 5.2 – Average number of revolutions of running wheels during light cycles in control groups; SHRC ran significantly more than WKYC (p=0.0017) and SDC (p=0.0290); H(2, N=19)=13.6289 p=0.0011; SHRC n=8, WKYC n=6, SDC n=5

5.3.1.2 Strain comparison of effect of guanfacine treatment on running activity in the dark and light cycles

The SHRG ran significantly more than WKYG and SDG during the dark cycle (Fig. 5.3). During the light cycle however, SHRG ran significantly more than SDG, but not WKYG (Fig. 5.4). This suggests that guanfacine treatment may have increased WKY activity, although WKYG running levels was not significantly different from that of SDG (Fig. 5.4).

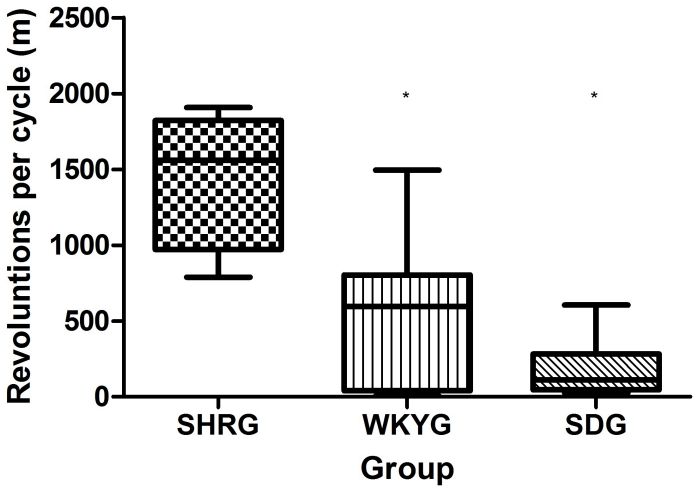

Figure 5.3 – Average number of revolutions of running wheels during dark cycles in treated groups; SHRG ran significantly more than WKYG (p=0.0158) and SDG (p=0.0024); H(2, N=23)=13.7946 p=0.0010; SHRG n=10, WKYG n=7, SDG n=6

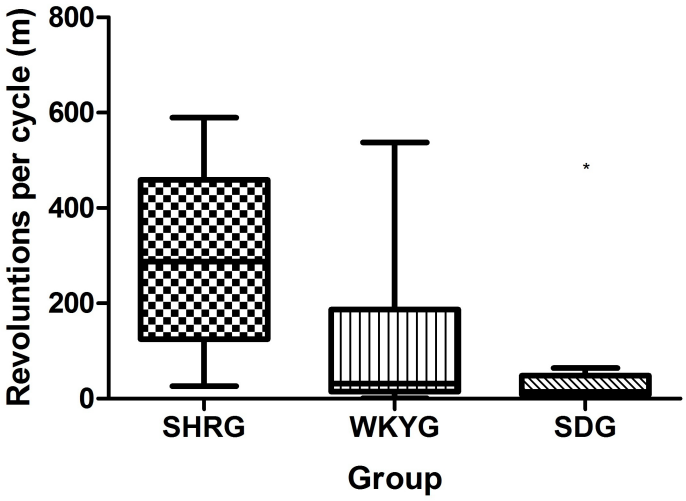

Figure 5.4 – Average number of revolutions of running wheels during light cycles in treated groups; SHRG ran significantly more than SDG (p=0.0095) but not WKYG (p=0.2283); H(2, N=23)=9.1511 p=0.0103; SHRG n=10, WKYG n=7, SDG n=6

5.3.1.3 Effect of guanfacine on running levels within each strain

No significant effect of guanfacine treatment was found for any of the strains, during the dark or light cycles (Fig. 5.5 ∼ Fig. 5.10).

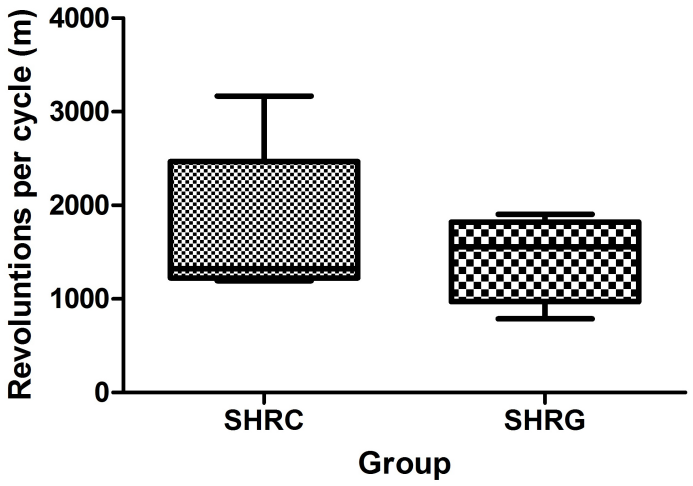

Figure 5.5 – Average number of revolutions of running wheels during dark cycles of SHRC and SHRG; p=0.5148; SHRC n=8; SHRG n=10

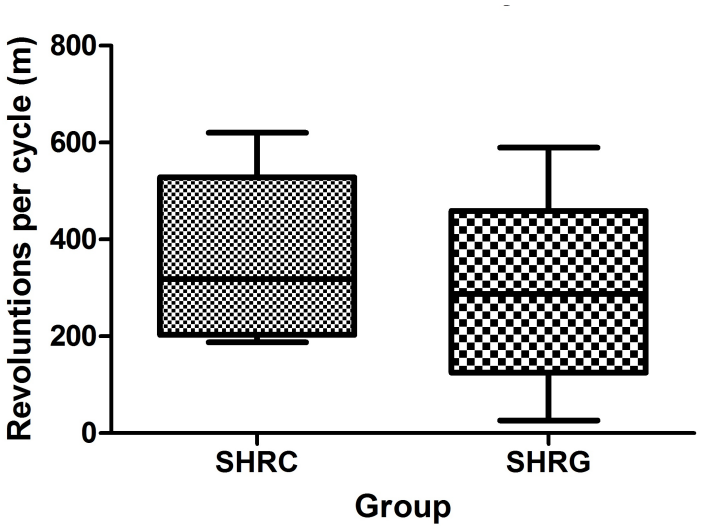

Figure 5.6 – Average number of revolutions of running wheels during light cycles of SHRC and SHRG; p=0.2743; SHRC n=8; SHRG n=10

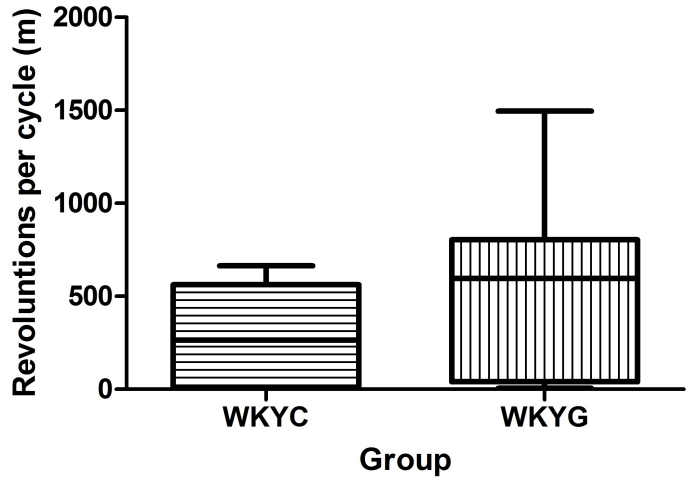

Figure 5.7 – Average number of revolutions of running wheels during dark cycles of WKYC and WKYG; p=0.3660; WKYC n=6; WKYG n=7

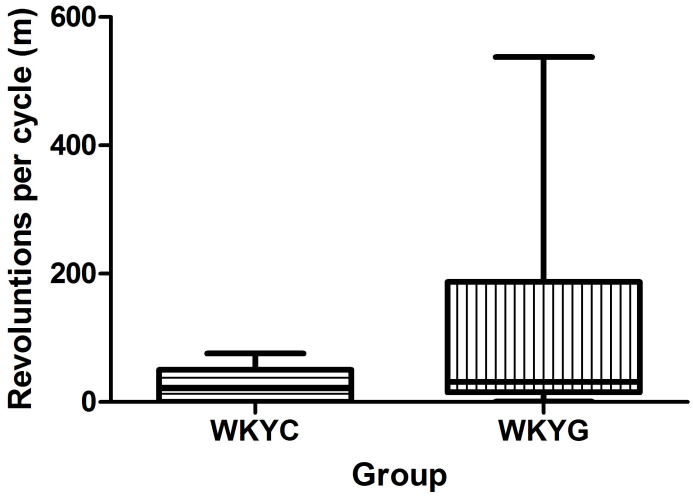

Figure 5.8 – Average number of revolutions of running wheels during light cycles of WKYC and WKYG; p=0.2343; WKYC n=6; WKYG n=7

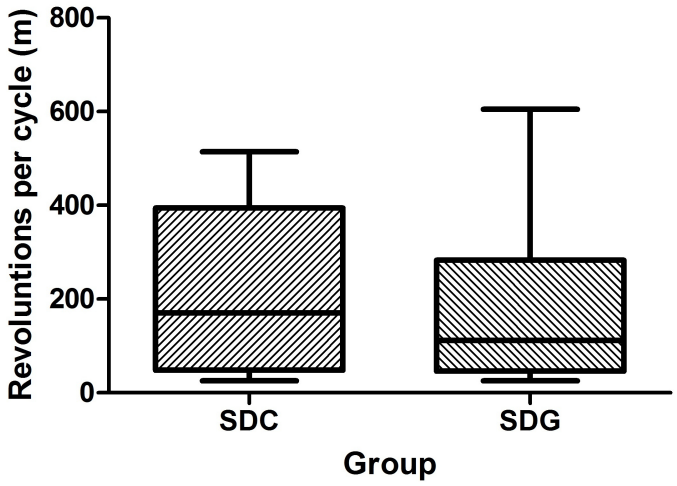

Figure 5.9 – Average number of revolutions of running wheels during dark cycles of SDC and SDG; p=0.7922; SDC n=5; SDG n=6

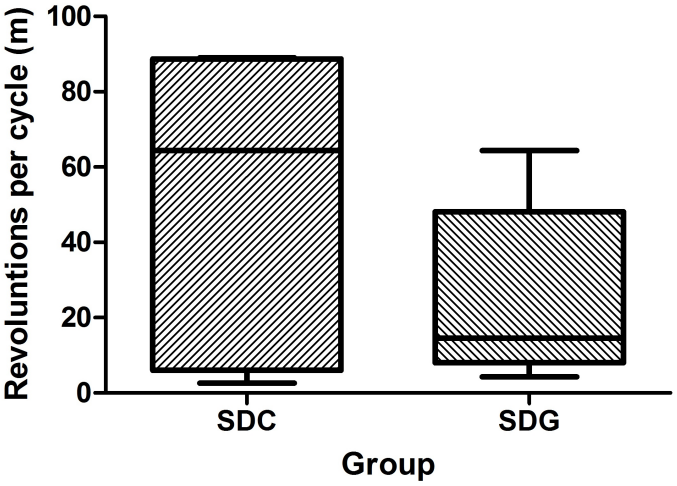

Figure 5.10 – Average number of revolutions of running wheels during light cycles of SDC and SDG; p=0.4286; SDC n=5; SDG n=6

5.3.2 Open Fields

5.3.2.1 Strain comparison of activities in the open field test

Prior to placement in cages with attached running wheels, the SHR and SD behaved similarly in the open field test, where both strains moved greater distances (Fig. 5.11), spent more time in the Inner Zone (Fig. 5.12) and had higher number of entries into the Inner zone (Fig. 5.13) than the WKY.

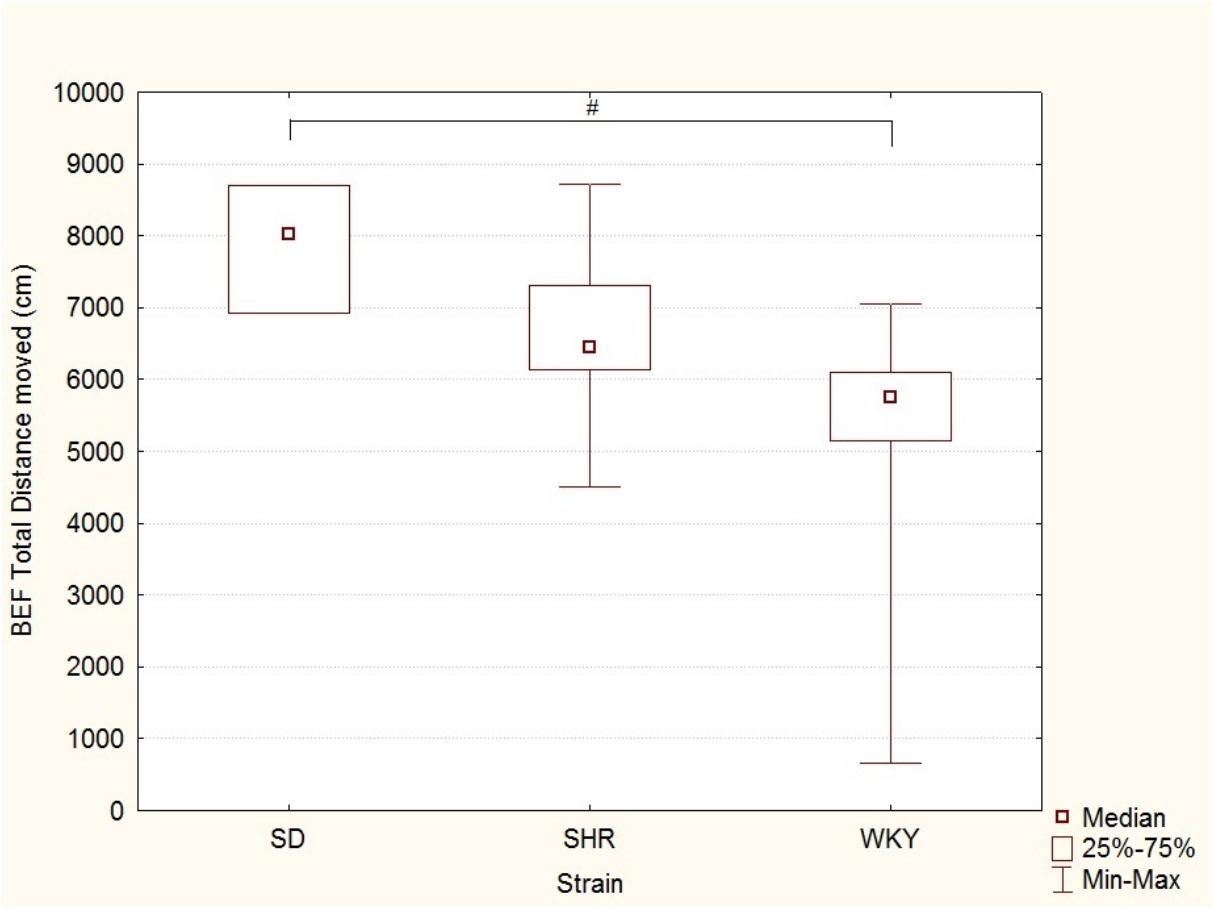

Figure 5.11 – Strain comparison of total distance moved in the open field before placement in running wheels; # p=0.0171; SHR n=18, WKY n=9, SD n=3

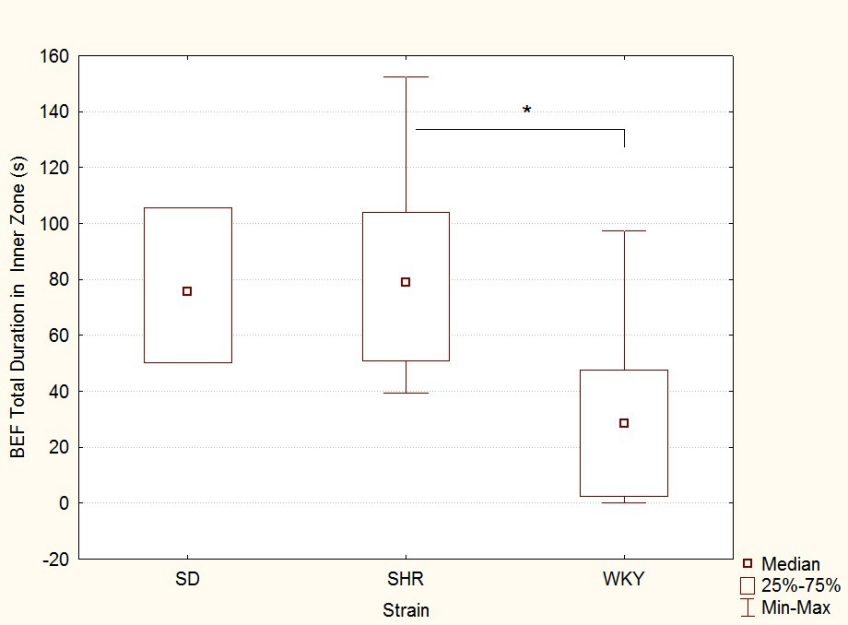

Figure 5.12 – Strain comparison of total duration in the Inner Zone of the open field before placement in running wheels; ∗p=0.0039; SHR n=18, WKY n=9, SD n=3

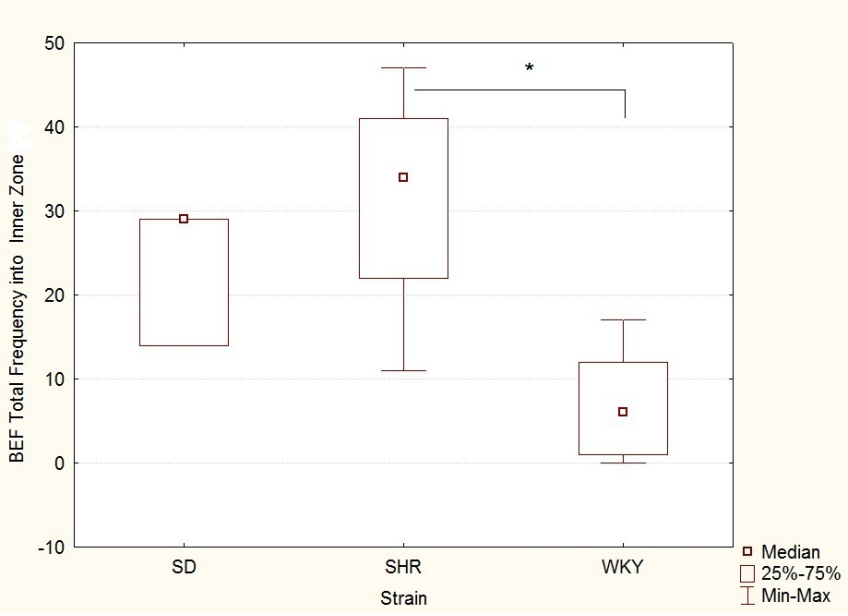

Figure 5.13 – Strain comparison of total number of entries into the Inner Zone of the open field before placement in running wheels; ∗p=0.0001; SHR n=18, WKY n=9, SD n=3

After the rats had been in the running wheels, the total distance travelled by the SHR decreased to levels that were similar to the WKY and significantly lower than the SD (Fig. 5.14), but the time spent in the Inner Zone and number of entries into the Inner Zone were similar in the SHR and SD, and both were significantly greater than for WKY (Fig. 5.15 and 5.16)

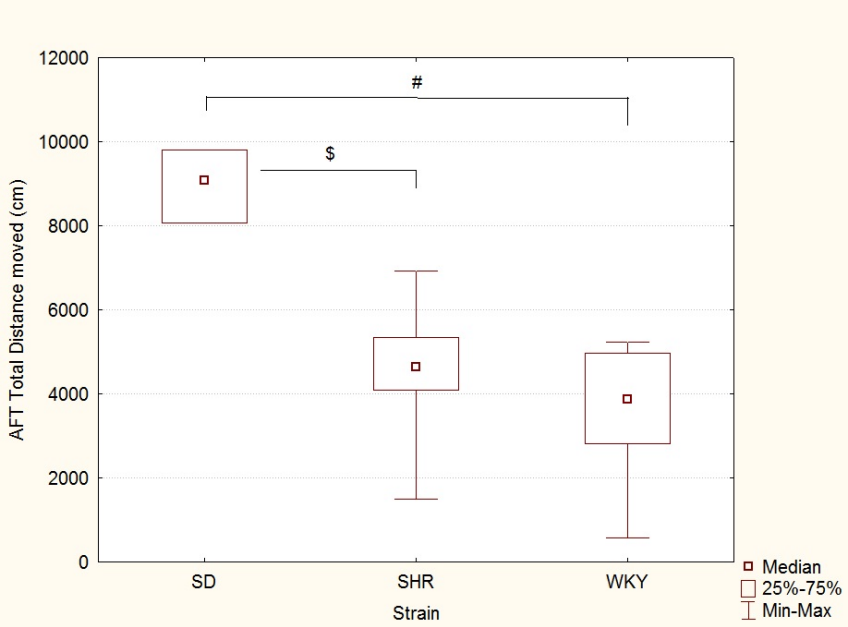

Figure 5.14 – Strain comparison of total distance moved in the open field after placement in running wheels; # p=0.0069, $ p=0.0406; SHR n=18, WKY n=9, SD n=3

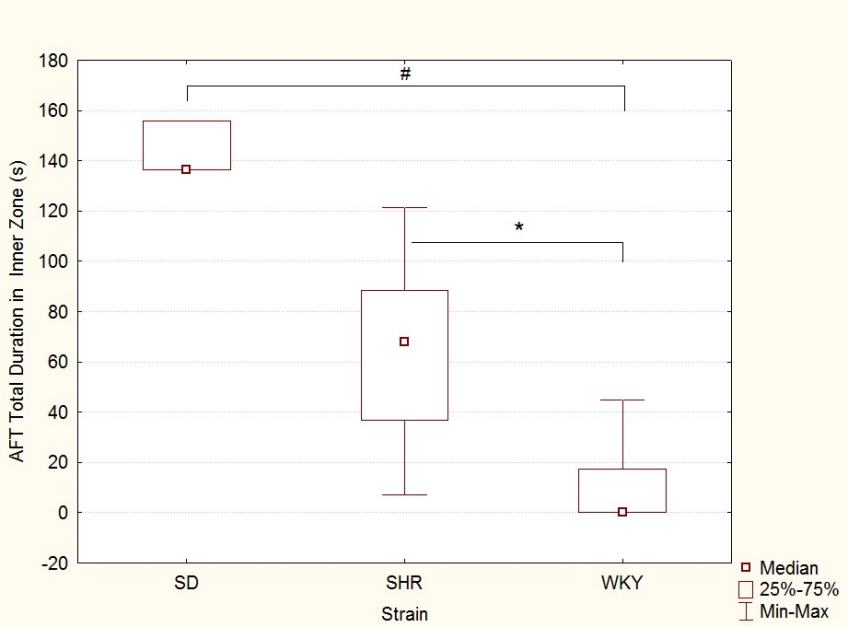

Figure 5.15 – Strain comparison of total duration in the Inner Zone of the open field after placement in running wheels; # p=0.0003, ∗p=0.0048; SHR n=18, WKY n=9, SD n=3

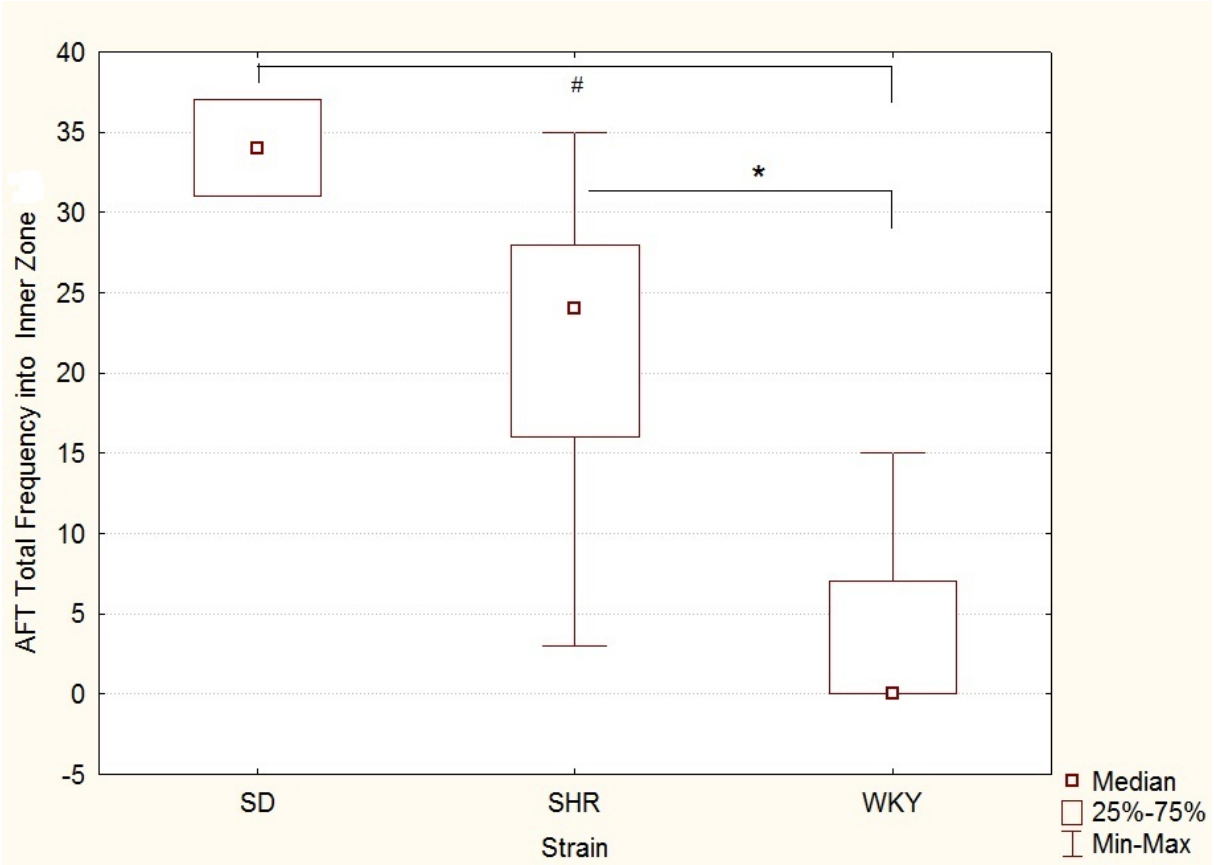

Figure 5.16 – Strain comparison of total number of entries into the Inner Zone of the open field after placement in running wheels; # p=0.000, ∗p=0.0014; SHR n=18, WKY n=9, SD n=3

Strain comparison of only the control groups revealed no differences between the strains for distances travelled or duration spent in the Inner Zone, but the SHR had significantly higher number of entries into the Inner Zone than the WKY (Fig. 5.17).

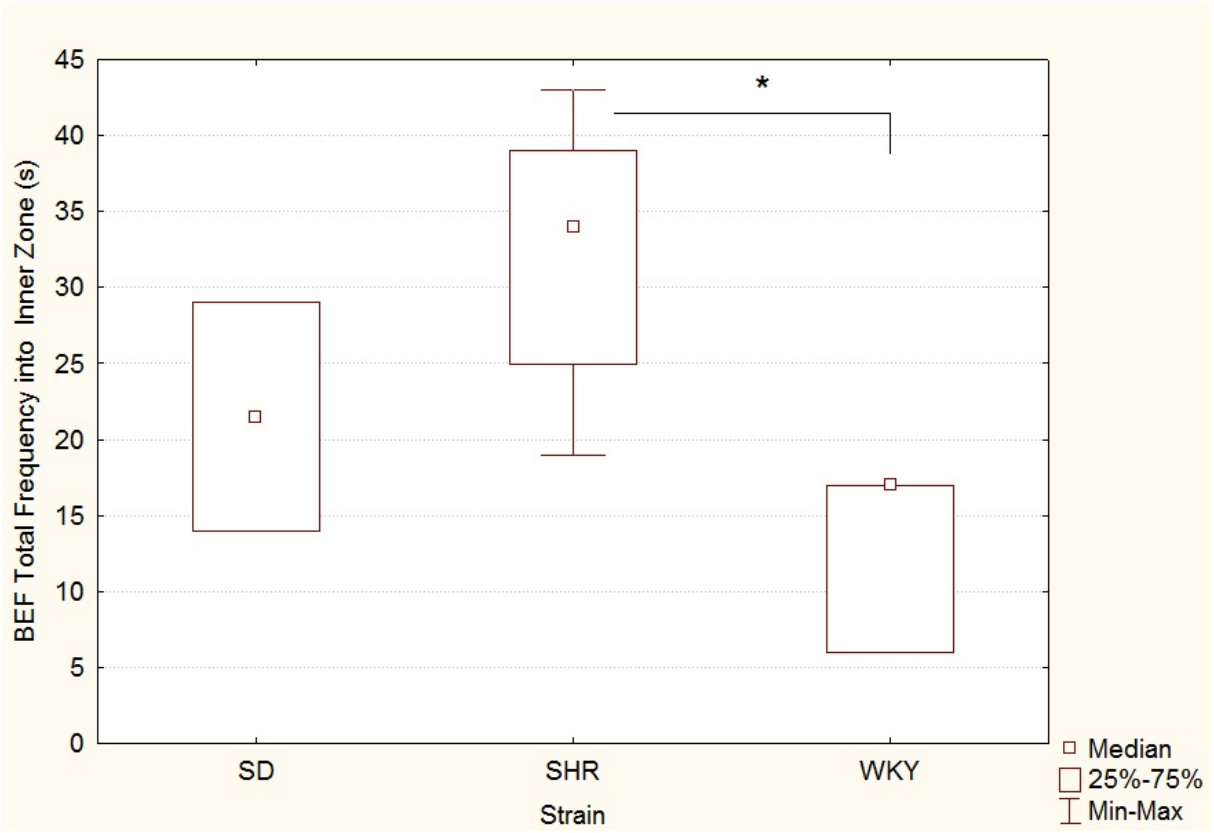

Figure 5.17 – Strain comparison of total number of entries into the Inner Zone of the open field after placement in running wheels for non treated groups only; ∗p=0.0429; SHR n=8, WKY n=3, SD n=2

5.3.2.2 Effect of guanfacine treatment in SHR and WKY

No effect of guanfacine treatment was detected in any open field parameter except for a tendency for the treated group to spend more time in the inner zone after placement in running wheels (p=0.083139) (Table. D.15). The WKYG spent significantly less time in the Inner Zone compared to the the WKYC before placement into the running wheels (p=0.0238), although this effect was not seen after the rats had been in the running wheels (Table. D.16).

5.3.2.3 Effects of SHR being placed in running wheels

5.3.2.3.1 Total distance covered in the open field The total distance covered in the open field by the both the control and treated SHR was significantly lower after they had been in the running wheels (Fig. 5.18 and Fig. 5.19).

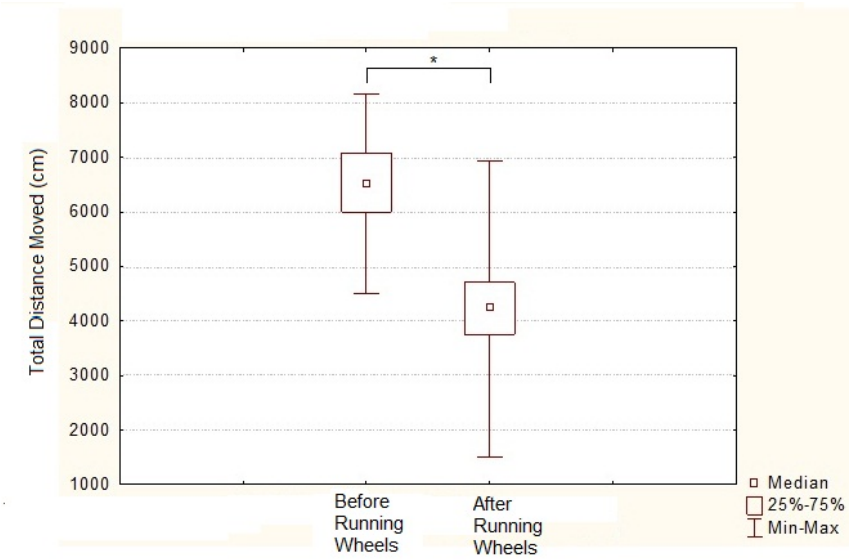

Figure 5.18 – Comparison of total distance covered before and after placement in running wheels in control SHR; n=8; ∗p=0.0117

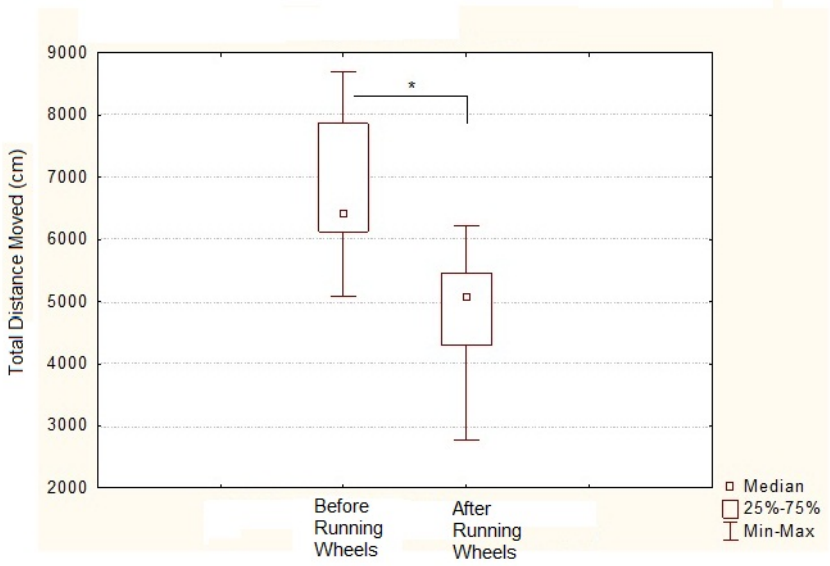

Figure 5.19 – Comparison of total distance covered before and after placement in running wheels in treated SHR; n=10; ∗p=0.0069

5.3.2.3.2 Total duration spent in Inner Zone The total duration of time spent in the Inner Zone of the open field was significantly lower after placement in the running wheels in the SHR control group, but not in the SHR treated group (Fig. 5.20 and Fig. 5.21).

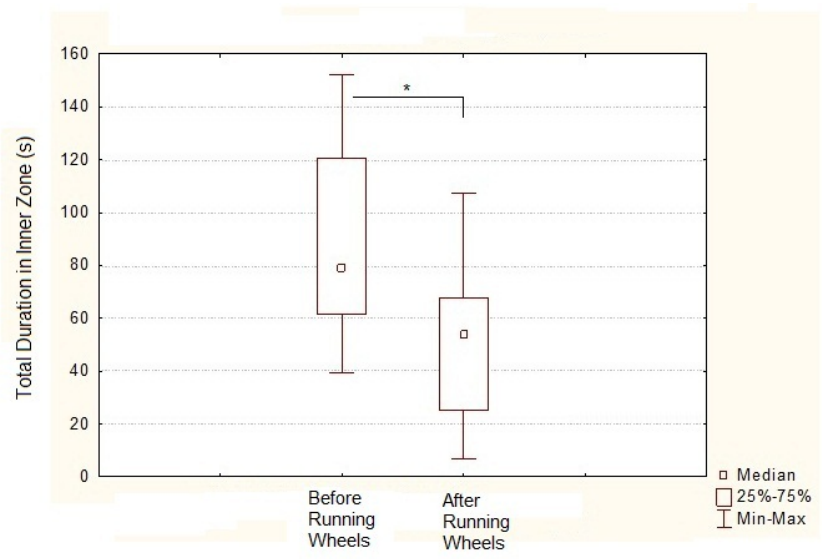

Figure 5.20 – Comparison of total duration in inner zone before and after placement in running wheels in control SHR; n=8; ∗p=0.0117

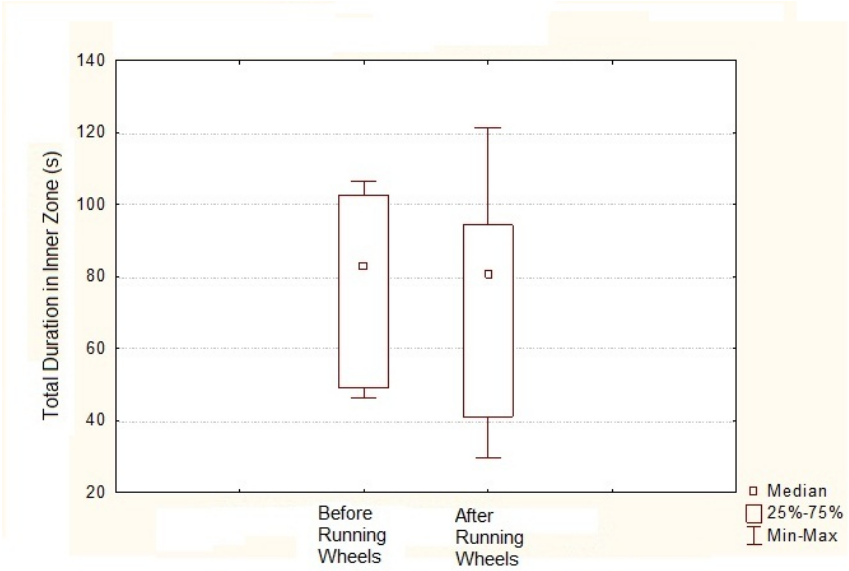

Figure 5.21 – Comparison of total duration in inner zone before and after placement in running wheels in treated SHR; n=10; p=0.7989

5.3.2.3.3 Total number of entries into Inner Zone The total number of entries into the Inner Zone was lower in both the control and treated SHR groups after placement in the running wheels (Fig. 5.22 and Fig. 5.23).

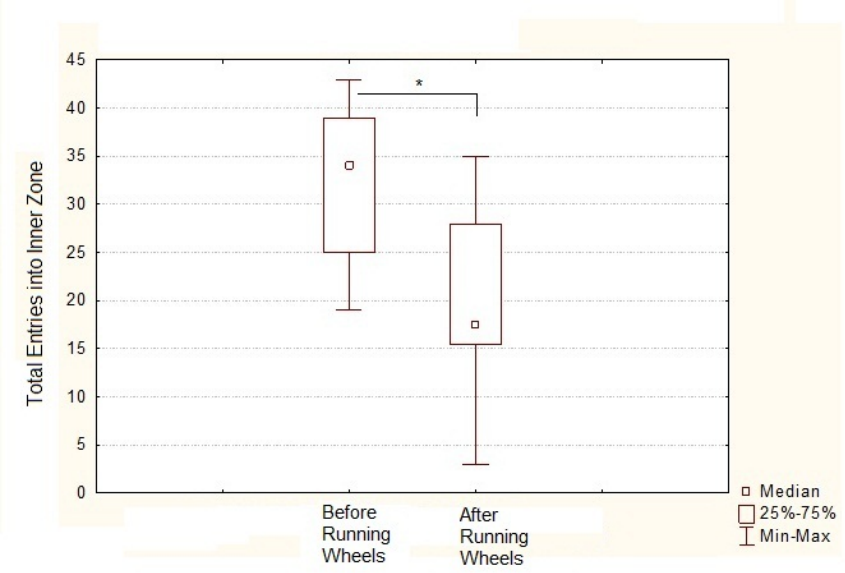

Figure 5.22 – Comparison of Frequency of entry into inner zone before and after placement in running wheels in control SHR; n=8; ∗p=0.0117

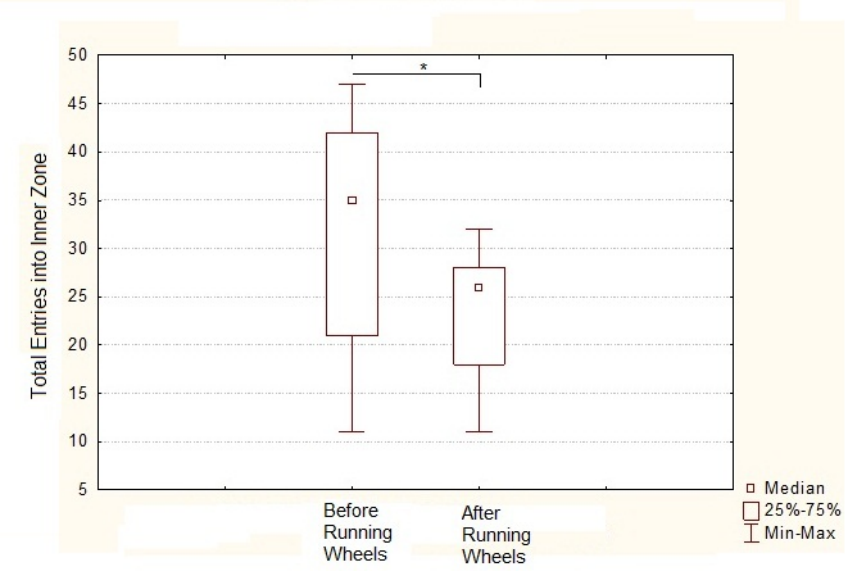

Figure 5.23 – Comparison of Frequency of entry into inner zone before and after placement in running wheels in treated SHR; n=10; ∗p=0.0593

5.4 Discussion

The non-treated SHR in this experiment ran significantly more than both the non-treated WKY and SD in the light and dark cycles. In the previous chapter, the vehicle treated SHR did not run significantly more than the WKY in the dark cycle, but only in the light cycle. Taken together with the previous results, it appears that the use of CM in the MPH experiments had increased the WKY activity levels in the dark cycle, since the jelly vehicle for the guanfacine experiments contains much lower levels of sucrose than the CM that was used in the MPH experiments. These results indicate that voluntary running activity could potentially be a measure with which to differentiate between strains if no substances or an appropriate vehicle is administered.

The treated SHR ran significantly more than the WKYG and SDG during the dark cycles, but no effects of guanfacine treatment on SHR voluntary activity levels were detected. These results contradict that of a previously published study that used the same dose of guanfacine via i.p. administration and showed that guanfacine decreased hyperactivity in SHR to levels that were similar to that of WKY (Sagvolden, 2006). The 'hyperactivity' in the previous study was measured by the total number of lever presses in a Random Interval/Extinction schedule experiment, where reinforcers were given within 180 s when the correct lever was pressed, while the incorrect lever resulted in an Extinction schedule (Sagvolden, 2006). The activity levels in the SHR in the lever pressing experiments were shaped by the presence of reinforcers and extinction schedules, much like that of children in social environments. Thus the hyperactivity as measured by the lever press experiment cannot be directly compared with the present activity in the voluntary running wheels, since there were no reinforcement or extiction schedules of any kind in this experiment. Thus according to Sagvolden's hypothesis of overactivity being due to a failure in the extinction of responses, without the application of extinction processes for decreasing activity, it appears that drugs alone cannot decrease the hyperactivity in the SHR.

In the light cycle, the WKYG ran similarly to that of SHRG, suggesting a possible increase in running activity, even though direct comparison of WKYC vs WKYG showed no significant difference. Whether the treated WKY group increased in locomotor activity could not be confirmed, however an increase in WKY as a result of guanfacine treatment would be interesting, since the WKY have been proposed to have low levels of arousal, on the left side of the upside-down U curve of arousal controlled by catecholamine imbalance (Arnsten, 2009), causing their typically low activity levels. Guanfacine administration could enhance the efficiency of NE transmission in the WKY PFC and generally increase arousal levels, and lead to higher voluntary activity levels.

Strain comparison of activities in the open field test showed that the WKY were the least mobile and the most anxious of the three strains, before or after their placement in the

running wheels. After their placement in running wheels, the SHR had spent less time in the Inner Zone than the SDs. These results agreed with previous forced exploration open field studies that found the SHR to be more active than the WKY (van den Buuse and de Jong, 1988), and that the SDs were more active than the SHR (Sagvolden et al., 1993b). Several files were corrupted during transfer and the sample size for the SD open fields were very low (n=3), however the patterns of activity suggest that the present results were similar to that of the previous study (Sagvolden et al., 1993b). Another study that measured open field activity of SHR, WKY and SD found that all 3 strains exhibited similar amounts of activity in the day time (Yang et al., 2006), which contradicts the present results that were also obtained during the light cycle, but showed marked lower activities in the WKY compared to the other strains, with or without guanfacine treatment.

Open field tests in the SHR before and after rats were in the running wheels showed a general pattern of decreased activity after being in the running wheels, and no difference between the treated and non-treated groups were observed. However, duration in the inner zone for the SHRG were not significantly different before and after placement in running wheels, and a direct comparison of the treated and non-treated SHR revealed a tendency for an increase in time spent in the Inner Zone after placement in the running wheels for the SHRG, suggesting that anxiety levels were lower in the treated group of SHR compared to the non-treated group. For the treated WKY, duration in the inner zone was significantly lower, and frequency to enter the inner zone had a tendency to be lower than the WKYC group before placement in the running wheels. The rats were treated for a week prior to the first open field measurement, and these results suggest that guanfacine could have induced higher levels of anxiety in the treated WKY compared with WKYC. However these parameters were not significantly different after the running wheels, suggesting that the experience of being in the running wheels could have reduced anxiety levels in the WKY. The apparent increases in time spent in the inner zone for both the SHR and WKY after placement in the running wheels suggests that exploratory behaviour increased, possibly as a result of isolation in the running wheels, but it could have also occured as a result of an increase in age. The first open field measurements were taken on P 28, and the second measurements was taken on P 35, which both fall within the prepubertal phase of rat development, however exploratory bahaviour differences between these two time points have never been compared. A separate experiment that does not involve the measurement of voluntary running would be required to determine whether the differences in behaviour after the placement in running wheels was an age effect or a running wheel effect. In fact, considering that the rats were re-habituated with their original littermates for 2 days after they were removed from the running wheels, the differences in open field activites between the 1st and 2nd measurements were most likely

due to age effects.

Activity in the open field test has been used to measure activity and anxiety (Goto et al., 1993). The use of this technique for the measurement of hyperactivity in the SHR has been criticized since increased activity could also be a reflection on decreased anxiety, which is not a behavioural characteristic of ADHD. In addition, other aspects of ADHD like impulsiveness and poor sustained attention are difficult to assess in the open field and are difficult to standardise for translational research in humans. Thus construct and predictive validities are impossible to obtain in open fields. Additionally, open field results were found to be sensitive to factors such as light conditions, size of the open field and duration of testing, which make the data difficult to interpret (Sagvolden et al., 1992a) and could explain the discrepancy between the present results and the Yang *et. al* study.

Similar to the results of the previous chapter, where the rats showed no effect of MPH treatment on distances covered in the running wheels, guanfacine produced no pronounced effects on the behavioural parameters used in the running wheel and open field tests in the present chapter. In retrospect, this result should have been expected, since MPH has been shown to inhibit both DAT and NET (Markowitz et al., 2006) and the increase in NE levels in the PFC (Kuczenski and Segal, 1997, 2001) was also shown to increase the intrinsic excitability of PFC neurons, that was mediated through the α_2-adrenoreceptor (Andrews and Lavin, 2006; Arnsten and Dudley, 2005). Thus the effects of both MPH and guanfacine on behaviour occurs through reinforcement-dependent, top-down regulation by the PFC, which cannot be measured by use of either the running wheel, or the open field tests.

Nevertheless, even though the motivation for activities in the open field, like that of voluntary running, are unknown, the patterns of activies of unknown motivation between the strains were similar across the tests, where the WKY showed consistently lower activity levels than the SHR and SDs. This suggests that voluntary running behaviour, and behaviour in the open field test can be used to measure general and intrinsic activity patterns within strains that are unaltered by any training protocols.

6 General Conclusions

The main aim of the present project was to set-up the *in vivo* chronoamperometry technique in South Africa, and compare the dopamine release and uptake responses between the SHR, WKY and SD rat strains. The technique was successfully set-up and results showed that the SHR released the most amount of DA from controlled potassium stimulation, the WKY released the least from similar stimulations, while the SDs release moderate amounts of DA in comparison. Since the SHR had the shortest T_{rise} times and required the least amount of applied DA to obtain similar amplitudes as the other rat strains, the results support that the SHR have decreased DAT activity in the rising phase of the DA peaks in comparison to the WKY and SD. The WKY showed decreased DAT activity in the falling phase of the DA peaks in comparison to the SHR and SD. The WKY also showed lower DAT activity in the rising phase of the DA peak when compared with the SDs. When DA amplitudes were low, all three strains clear DA at similar rates, however at higher amplitudes of DA, the defect in DAT function in the falling phase of the response becomes more evident in the WKY. These dopaminergic responses suggest that DAT activity in the WKY is most impaired compared to the SHR and SD, and that vesicular DA release in the SHR is not impaired, as suggested by previous *in vitro* studies.

The SHR were significantly more active than WKY in terms of voluntary behaviour in cages with attached running wheels and in the open field. These results showed that strain differences in voluntary behaviour could be measured with the cages with attached running wheels and the open field test.

Neither guanfacine nor methylphenidate produced significant changes in activity in voluntary running in cages with attached running wheels or in the open field. The results suggest that drug administration alone does not decrease voluntary activity, and has no effect on behaviours that are not controlled by reinforcer and extinction protocols.

The DNA screening results indicated a possible cross between the SHR and SD strains, although additional genetic screening is required to confirm this suggestion, since the results of this project found significant differences between the SHR and SD, as well as significant differences between the SD and WKY, indicating that the neurochemical and behavioural differences between the strains were not completely abolished. However the present results suggest that new animal stocks should be purchased for future studies.

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Appendices

A DNA screening at 3 SNP loci

A.1 Primer Sequences

The 3 pairs of primers defined SNP products of different sequences and expected size shifts of approximately 10 bp between the SHR and WKY.

The following primer pairs were manufactured by Whitehead Scientific:
D1RAT47 Forward: 5' - GCC TTG GGA ACC ACA TA - 3'
D1RAT47 Reverse: 5' - AGG AGT TTG GGA GAC ACC CT - 3'
Expected band size, approximately 162 bp
D2RAT62 Forward: 5' - CAA GGC CCT TAG CTT CCT G - 3'
D2RAT62 Reverse: 5' - CCC CCT GTC CTT TAT CTT GC - 3'
Expected band size, approximately 166 bp
D3RAT24 Forward: 5' - GCC ATG ATA TTG CAC CAT GA - 3'
D3RAT24 Reverse: 5' - GAC AAG GTC TTT GAT AGA TCC TCA - 3'
Expected band size, approximately 151 bp

Primer pellets were reconstituted in TE buffer in 100 μM stock solutions.

B *In vivo* Chronoamperometry

B.1 Histology

Table B.1 – Table detailing calculations for scaling 120 g fresh rat brain to the Rat Brain Atlas (Paxinos and Watson, 2005) at various AP loactions

Figure in Atlas	Atlas Total DV (mm)	Figure in Atlas	Atlas Total DV (mm)	Figure in Atlas	Atlas Total DV (mm)	Average Atlas Total DV (mm)	Ratio Atlas/Actual
10.00	109.90	11.00	109.90			109.90	13.24
11.00	109.90	12.00	112.34	13.00	118.10	113.45	13.67
12.00	112.34	13.00	118.10			115.22	13.65
						112.86	13.52
14.00	120.82	15.00	122.26	16.00	124.16	122.41	14.71
17.00	126.14	18.00	125.18	19.00	124.40	125.24	14.84
19.00	124.40	20.00	123.66	21.00	126.82	124.96	13.27
22.00	128.30	23.00	127.20	24.00	127.20	127.57	13.15
22.00	128.30	23.00	127.20	24.00	127.20	127.57	13.48
						126.33	13.69
29.00	130.04	30.00	130.00	31.00	129.90	129.98	13.68
28.00	131.48	29.00	130.04	30.00	130.00	130.51	13.45
33.00	131.28	34.00	132.66			131.97	13.20
						130.82	13.44

Table B.2 – Table detailing exact distances of DV points at 7 AP locations in the Rat Brain Atlas in relation to the 120 g rats

Rat	Figure in Atlas		3.5 * Ratio	4* Ratio	4.5* Ratio	5* Ratio	5.5* Ratio	6* Ratio	6.5* Ratio	7* Ratio	7.5* Ratio
SHR 4			46.34	52.96	59.58	66.20	72.83	79.45	86.07	92.69	99.31
SHR 3	13.00		47.84	54.67	61.51	68.34	75.18	82.01	88.84	95.68	102.51
SHR 4			47.78	54.61	61.43	68.26	75.08	81.91	88.74	95.56	102.39
		AVE	47.32	54.08	60.84	67.60	74.36	81.12	87.88	94.64	101.40
		0.5 mm= (cm)	6.76	6.76	6.76	6.76	6.76	6.76	6.76	6.76	
SHR 3	16.00		51.50	58.85	66.21	73.57	80.92	88.28	95.64	102.99	110.35
SHR 4	19.00		51.94	59.36	66.77	74.19	81.61	89.03	96.45	103.87	111.29
		0.5 mm= (cm)	7.42	7.42	7.42	7.42	7.42	7.42	7.42	7.42	
SHR 4	21.00		46.43	53.06	59.69	66.33	72.96	79.59	86.23	92.86	99.49
SHR 4	24.00		46.03	52.60	59.18	65.76	72.33	78.91	85.48	92.06	98.63
SHR 3	24.00		47.20	53.94	60.68	67.42	74.17	80.91	87.65	94.39	101.14
		AVE	39.80	45.28	50.75	56.22	61.70	67.17	72.65	78.12	102.64
		0.5 mm= (cm)	6.65	6.65	6.65	6.65	6.65	6.65	6.65	6.65	
SHR 3	31.00		47.89	54.73	61.57	68.41	75.25	82.09	88.93	95.77	102.62
SHR 4	30.00		47.09	53.82	60.54	67.27	74.00	80.73	87.45	94.18	100.91
SHR 4			46.19	52.79	59.39	65.99	72.58	79.18	85.78	92.38	98.98
		AVE	47.06	53.78	60.50	67.22	73.94	80.67	87.39	94.11	100.83
		0.5 mm= (cm)	6.72	6.72	6.72	6.72	6.72	6.72	6.72	6.72	

Table B.3 – The choice of each data point was determined according to histology

Rec	L/R	KCl/DA	Corresponding Atlas Figs	Recording Location Fig	3.5	4	4.5	5	5.5	6	6.5	7	7.5
41	L	DA	16	18	ST	ST	ST	ST	ST	ST	ST	Out	Out
	R	KCl	15	17	ST	ST	ST	ST	ST	ST	ST	Out	Out
40	L	KCl	16	18	ST	ST	ST	x	x	x	x	x	x
	R	DA	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
39	L	DA	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
38	L	DA	17	19	ST	ST	ST	x	x	x	x	x	x
	R	KCl	17	19	ST	ST	ST	ST	ST	ST	Out	Out	Out
37	L	DA	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	19	21	ST	ST	ST	ST	x	x	x	x	x
35	L	DA	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
34	L	DA	22	24	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	13	15	ST	ST	ST	ST	ST	ST	ST	OUT	OUT
32	L	DA	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
31	L	DA	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	20	22	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
30	L	DA	20	22	ST	ST	ST	ST	ST	ST	ST	ST	ST
	R	KCl	21	23	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
29	L	DA	21	23	ST	ST	ST	ST	x	x	x	x	x
	R	KCl	21	23	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
28	R	KCl	13	15	ST	ST	ST	ST	ST	ST	Nac	Out	Out
27	L	DA	22	24	ST	ST	ST	ST	x	x	x	x	x
	R	KCl	19	21	ST	ST	ST	ST	Nac	Out	Out	Out	Out
26	L	DA	21	23	x	x	x	x	x	x	x	x	x
	R	DA	21	23	x	x	x	ST	x	x	x	x	x
25	R	DA	21	23	x	x	x	ST	x	x	x	x	x
24	R	DA	13	15	x	x	x	ST	x	x	x	x	x
23	L	DA	17	19	ST	ST	ST	ST	ST	ST	Nac	Nac	out
	R	KCl	14	16	ST	ST	ST	ST	ST	ST	Nac	Nac	out
22	L	DA	13	15	ST	ST	ST	ST	ST	ST	Nac	Nac	out
	R	KCl	13	15	ST	ST	ST	ST	ST	ST	Nac	Nac	out
21	L	DA	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	19	21	ST	ST	ST	ST	ST	ST	ST	Nac	Nac

Table B.4 – Continued. The choice of each data point was determined according to histology

Rec	L/R	KCl/DA	Corresponding Atlas Figs	Recording Location Fig	3.5	4	4.5	5	5.5	6	6.5	7	7.5
20	L	DA	23	25	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	22	24	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
19	L	DA	21	23	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	21	23	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
18	L	DA	18	20	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	17	19	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
17	L	DA	18	20	ST	ST	ST	ST	ST	ST	ST	ST	ST
	R	KCl	14	16	ST	ST	ST	ST	ST	ST	OUT	OUT	OUT
15	L	DA	23	25	ST	ST	ST	ST	ST	ST	ST	ST	OUT
	R	KCl	22	24	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
14	L	DA	15	17	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
	R	KCl	14	16	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
13	R	KCl	22	24	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
12	L	DA	19	21	x	x	x	ST	ST	ST	ST	Nac	Nac
	R	KCl	7	9	x	x	x	x	x	x	x	x	x
11	R	KCl	13	15	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
10	R	KCl	10	12	CC	ST	OUT	OUT	OUT	OUT	OUT	OUT	OUT
9	L	DA	17	19	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
8	L	DA	12	14	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
	R	KCl	12	14	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
7	L	DA	21	23	ST	ST	ST	ST	ST	ST	ST	Nac	Nac
	R	KCl	19	21	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
6	R	KCl	12	14	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
5	R	KCl	12	14	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
4	R	KCl	11	13	CC	ST	ST	CC	CC	CC	OUT	OUT	OUT
3	L	DA	13	15	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT
	R	KCl	14	16	ST	ST	ST	ST	ST	ST	Nac	Nac	OUT

B.2 KCl Stimulated DA Release

B.2.1 KCl descriptive statistics

Table B.5 – Descriptive Statistics for KCl stimulation of DA release experiments, no strain breakdown

Descriptive Statistics (KCl 100~250 in Rec 9~41 Analysis 3 with k-1)

	Valid N	Mean	Confidence - - 95.000%	Confidence - 95.000	Median	Minimum	Maximum	Lower - Quartile	Upper - Quartile	Std.Dev.
Baseline	215	10.2531	8.7644	11.7417	4.3616	2.11621	38.354	3.32992	15.8498	11.0738
Amp	215	4.0007	3.0186	4.9828	1.7346	0.02098	53.692	0.40587	4.0511	7.3057
1st_K-1	215	0.0015	0.0010	0.0020	0.0004	0.00002	0.025	0.00018	0.0009	0.0037
1st_R^2	215	0.4947	0.4658	0.5236	0.4567	0.00575	0.959	0.33333	0.6510	0.2150
1st_Rate	215	0.0223	0.0062	0.0385	0.0008	0.00000	1.334	0.00008	0.0025	0.1202
Peak Area	215	151.9784	125.0591	178.8978	79.6068	2.73623	1130.476	28.49537	188.2939	200.2501
S/N	215	178.1003	150.4990	205.7016	103.9539	3.13700	1195.532	35.30035	273.4404	205.3232
Trise	215	31.6605	21.4652	41.8558	16.0000	3.00000	826.000	11.00000	26.0000	75.8416
T100	215	272.0047	246.2538	297.7555	255.0000	4.00000	837.000	85.00000	407.0000	191.5581
T80	215	67.9349	54.5230	81.3467	30.0000	3.00000	590.000	19.00000	63.0000	99.7692
T50	215	23.1674	20.2893	26.0456	17.0000	2.00000	134.000	11.00000	26.0000	21.4103
Tc	215	-0.2261	-0.2983	-0.1539	-0.0640	-4.18075	0.053	-0.17840	-0.0086	0.5371
Redox	215	-0.5604	-0.5794	-0.5414	-0.5637	-0.88351	-0.211	-0.66646	-0.4494	0.1413

Table B.6 – Descriptive Statistics for KCl stimulation of DA release experiments, with strain breakdown

	Mass	Mass	Mass	Mass	Mass	Mass	Mass	Mass
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	110.61	64.00	33.19	74.50	160.90	80.45	109.10	140.60
WKY	78.99	81.00	2.75	75.80	85.50	77.40	78.50	79.20
SHR	79.98	70.00	5.88	74.70	92.10	75.50	78.60	79.60
All Grps	88.73	215.00	23.29	74.50	160.90			
	Volume	Volume	Volume	Volume	Volume	Volume	Volume	Volume
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	177.34	64.00	57.17	100.00	250.00	125.00	150.00	250.00
WKY	201.23	81.00	42.00	100.00	250.00	175.00	200.00	225.00
SHR	212.86	70.00	38.01	150.00	250.00	200.00	225.00	250.00
All Grps	197.91	215.00	47.87	100.00	250.00			
	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	15.74	64.00	16.11	2.21	38.35	2.88	4.21	35.80
WKY	6.46	81.00	7.30	2.12	26.54	3.32	3.72	4.25
SHR	9.63	70.00	6.03	2.47	20.74	5.70	6.44	16.00
All Grps	10.25	215.00	11.07	2.12	38.35			
	Amp	Amp	Amp	Amp	Amp	Amp	Amp	Amp
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	6.80	64.00	11.93	0.05	53.69	0.89	1.78	5.45
WKY	2.02	81.00	2.73	0.02	12.79	0.17	0.90	2.93
SHR	3.73	70.00	3.86	0.03	18.03	1.06	2.59	4.76
All Grps	4.00	215.00	7.31	0.02	53.69			
	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	0.00	64.00	0.00	0.00	0.02	0.00	0.00	0.00
WKY	0.00	81.00	0.00	0.00	0.01	0.00	0.00	0.00
SHR	0.00	70.00	0.00	0.00	0.02	0.00	0.00	0.00
All Grps	0.00	215.00	0.00	0.00	0.02			
	Trise	Trise	Trise	Trise	Trise	Trise	Trise	Trise
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	24.36	64.00	39.67	5.00	325.00	12.00	17.50	25.00
WKY	40.54	81.00	107.89	3.00	826.00	10.00	17.00	29.00
SHR	28.06	70.00	52.45	4.00	402.00	10.00	13.50	23.00
All Grps	31.66	215.00	75.84	3.00	826.00			

Table B.7 – Continued. Descriptive Statistics for KCl stimulation of DA release experiments, with strain breakdown

	T100	T100	T100	T100	T100	T100	T100	T100
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	226.89	64.00	218.24	6.00	837.00	53.50	144.50	399.50
WKY	291.00	81.00	192.33	4.00	828.00	97.00	323.00	410.00
SHR	291.27	70.00	157.30	13.00	647.00	172.00	274.50	402.00
All Grps	272.00	215.00	191.56	4.00	837.00			
	T80	T80	T80	T80	T80	T80	T80	T80
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	67.27	64.00	127.08	4.00	590.00	15.00	21.50	45.00
WKY	74.74	81.00	91.65	3.00	451.00	21.00	38.00	82.00
SHR	60.67	70.00	79.26	8.00	567.00	25.00	36.50	68.00
All Grps	67.93	215.00	99.77	3.00	590.00			
	T50	T50	T50	T50	T50	T50	T50	T50
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	18.88	64.00	21.18	2.00	134.00	8.00	13.00	18.50
WKY	26.31	81.00	24.81	2.00	122.00	11.00	18.00	30.00
SHR	23.46	70.00	16.42	2.00	108.00	14.00	18.00	30.00
All Grps	23.17	215.00	21.41	2.00	134.00			
	Tc	Tc	Tc	Tc	Tc	Tc	Tc	Tc
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	-0.49	64.00	0.89	-4.18	0.05	-0.47	-0.13	-0.01
WKY	-0.08	81.00	0.12	-0.56	0.02	-0.09	-0.03	0.00
SHR	-0.16	70.00	0.23	-1.28	0.00	-0.17	-0.09	-0.03
All Grps	-0.23	215.00	0.54	-4.18	0.05			
	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	183.53	64.00	267.64	3.95	1130.48	34.33	64.52	226.90
WKY	112.92	81.00	167.26	2.74	833.68	9.95	47.83	116.58
SHR	168.33	70.00	153.43	3.66	698.97	64.70	121.20	232.48
All Grps	151.98	215.00	200.25	2.74	1130.48			

B.2.2 KCl Shapiro-Wilks' Normality tests

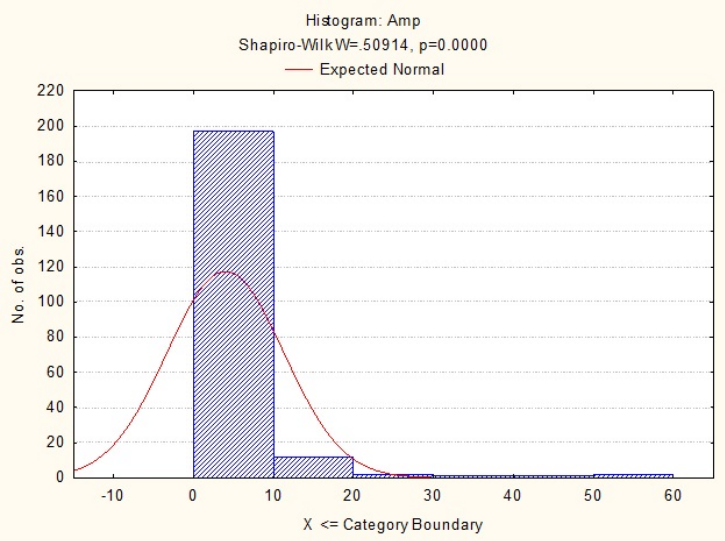


Figure B.1 – Shapiro-Wilks' normality histogram of amplitudes obtained from KCl stimulation

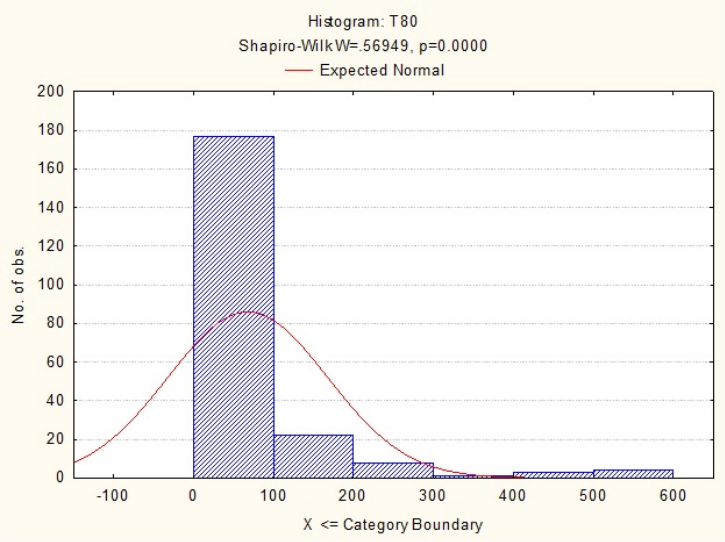


Figure B.2 – Shapiro-Wilks' normality histogram of T_{80} from KCl stimulation

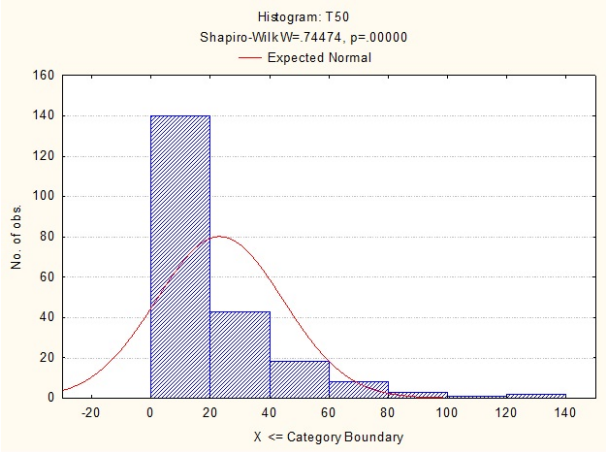


Figure B.3 – Shapiro-Wilks' normality histogram of T_{50} from KCl stimulation

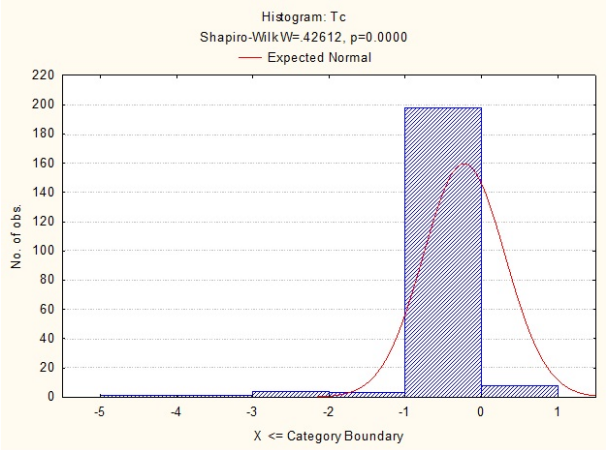


Figure B.4 – Shapiro-Wilks' normality histogram of T_c from KCl stimulation

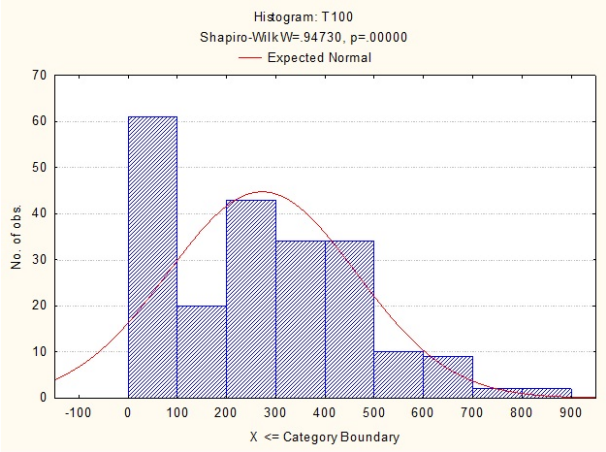


Figure B.5 – Shapiro-Wilks' normality histogram of T_{100} from KCl stimulation

B.2.3 Calibration slopes

Table B.8 – Descriptive statistics of calibration slopes

2-Way Tables of Descriptive Statistics (KCl one slope per rat in Final KCl and DA datasets) N=23 (No missing data in dep. var. list)

	Slope - Means	Slope - N	Slope - Std.Dev.	Slope - Std.Err.	Slope - Minimum	Slope - Maximum
SD	-0.435895	7	0.330701	0.124993	-0.839163	-0.042610
WKY	-0.451596	8	0.185461	0.065570	-0.683731	-0.123411
SHR	-0.379626	8	0.206069	0.072856	-0.709652	-0.115312
All Grps	-0.421815	23	0.235192	0.049041	-0.839163	-0.042610

Table B.9 – 1-way ANOVA strain comparison of calibration slopes in the KCl experiments

Analysis of Variance (KCl one slope per rat in Final KCl and DA datasets) Marked effects are significant at p < .05000

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
Slope	0.022742	2	0.011371	1.194199	20	0.059710	0.190439	0.828078

B.2.4 Analysis of rat mass

Table B.10 – Strain comparison of rat mass with REC 13

Multiple Comparisons p values (2-tailed); Mass (KCl 100~250 in Rec3~41 Analysis 2 KCl results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 215) =36.25178 p =.0000

	SD - R:146.88	WKY - R:95.019	SHR - R:87.479
SD		0.000002	0.000000
WKY	0.000002		1.000000
SHR	0.000000	1.000000	

Table B.11 – Strain comparison of rat mass without REC 13

Multiple Comparisons p values (2-tailed); Mass (KCl 100~250 in Rec3~41 Analysis 2 KCl results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 199) =14.25611 p =.0008 Exclude condition: Rec=13

	SD - R:126.67	WKY - R:95.019	SHR - R:87.479
SD		0.007660	0.000847
WKY	0.007660		1.000000
SHR	0.000847	1.000000	

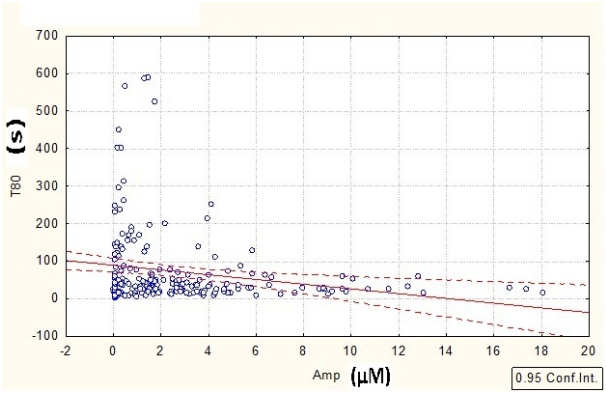


Figure B.6 – Scatterplot of T_{80} vs amplitudes with the exclusion of Rec 13, r=-0.2055

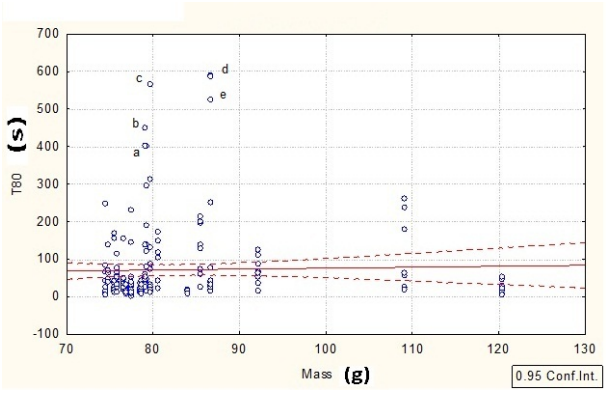


Figure B.7 – Scatterplot of T_{80} vs rat mass with the exclusion of Rec 13, r=0.0282

B.2.5 Analysis of KCl ejection volumes

Table B.12 – Strain comparison of KCl ejection volumes with REC 13

Multiple Comparisons p values (2-tailed); Volume (KCl 100~250 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 215) =13.64053 p =.0011			
	SD - R: 86.898	**WKY - R: 109.41**	**SHR - R: 125.66**
SD		0.091381	0.000946
WKY	0.091381		0.328774
SHR	0.000946	0.328774	

Table B.13 – Strain comparison of KCl ejection volumes without REC 13

Multiple Comparisons p values (2-tailed); Volume (KCl 100~250 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 199) =5.589900 p =.0611 Exclude condition: Rec=13			
	SD - R:88.302	**WKY - R: 96.556**	**SHR - R: 112.01**
SD		1.000000	0.084182
WKY	1.000000		0.300474
SHR	0.084182	0.300474	

B.2.6 Strain difference in Amplitude

The data quality verification suggested it prudent to perform strain comparisons of amplitudes both with and without the inclusion of the outlier Rec 13 to ensure that the results obtained were similar. Analysis including Rec 13 was presented in the results. Analysis excluding Rec 13 is included below.

Analysis of strain difference in amplitudes with the exclusion of Rec 13 showed the SHR released significantly more DA than the WKY, with the SDs releasing moderate amounts that were not significantly different from that of the other two strains (Fig. B.9). This difference occured in the ST ($H(2, N=144)=13.0919$ $p=0.0014$) but not the NAc ($H(2, N=27)=3.304246$ $p=0.1916$). Results excluding Rec 13 were similar to those that included Rec 13. Although the significant difference between the SD and WKY was lost, the pattern of the data was identical.

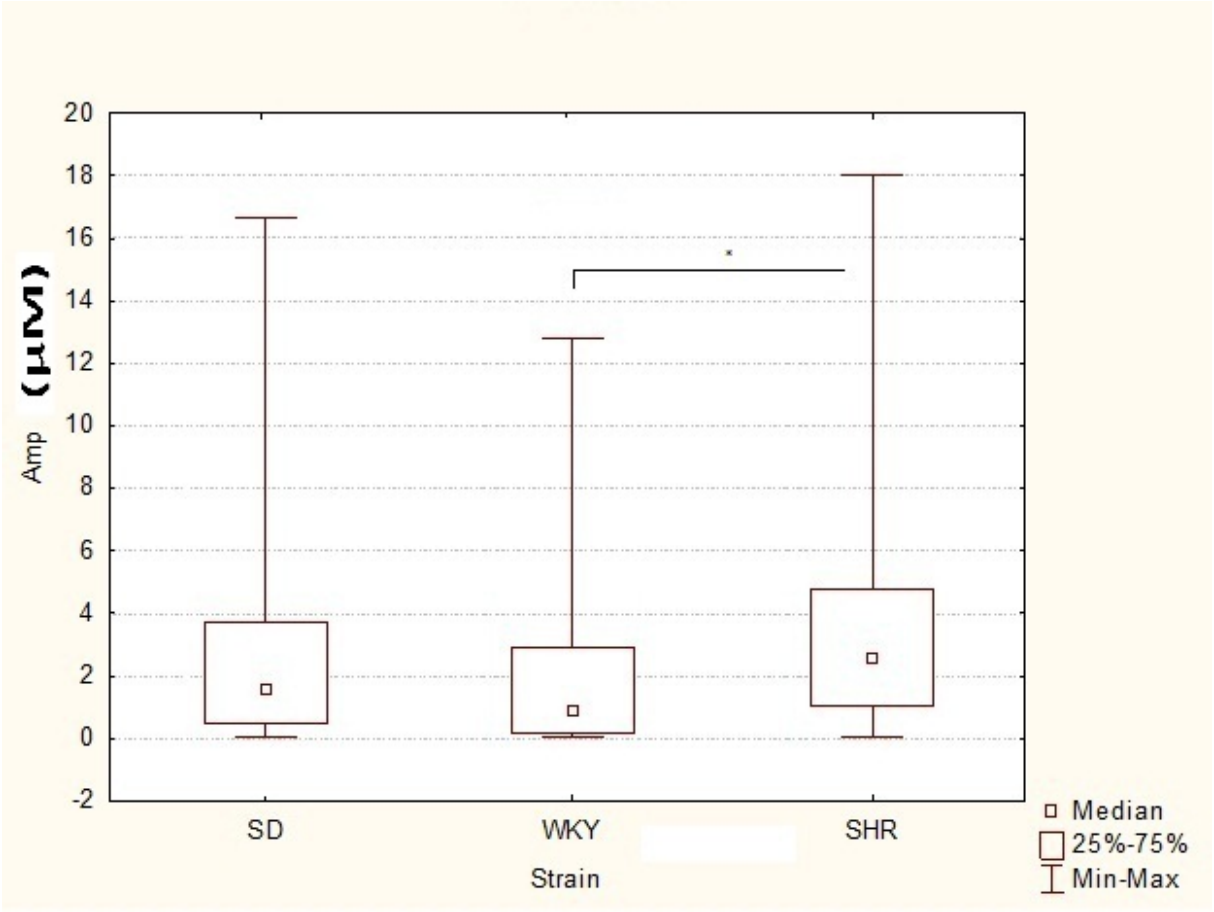


Figure B.8 – Strain difference in amplitudes where the SHR released significantly larger amounts of DA compared to WKY, $*p=0.0002$; Overall: $H(2, N=199)=15.5725$ $p=0.0004$

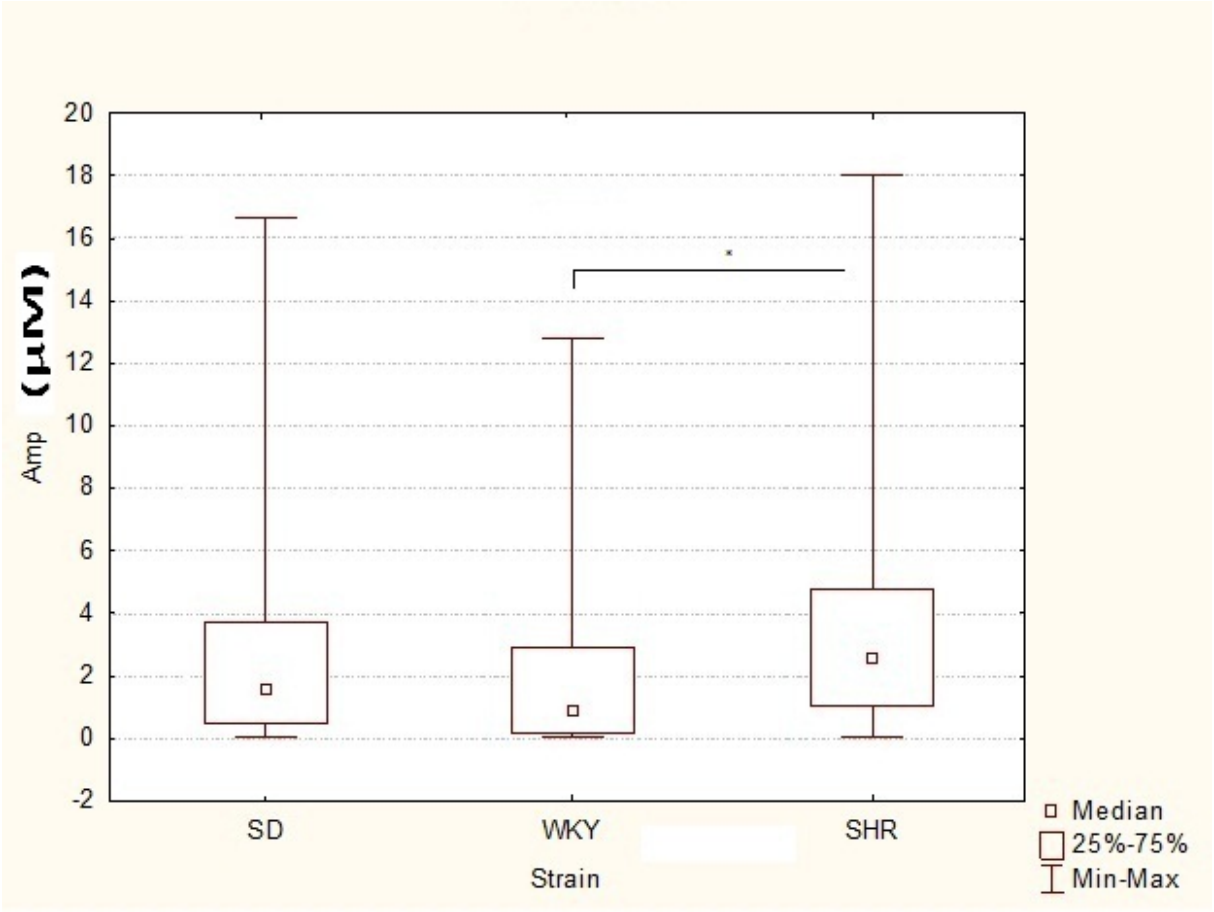


Figure B.9 – Strain comparison of amplitude without REC 13 Kruskal-Wallis test; H(2, N=199)=15.5725 p=0.0004

Table B.14 – Strain comparison of amplitude without REC 13 Kruskal-Wallis test

Multiple Comparisons p values (2-tailed); Amp (KCl-Rec13 in Rec 9~41 Analysis 3 with k-1) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 199) =15.57254 p =.0004			
	WKY - R:83.185	**SD - R:98.854**	**SHR - R:120.24**
WKY		0.405776	0.000241
SD	0.405776		0.142502
SHR	0.000241	0.142502	

Table B.15 – Strain comparison of amplitude without REC 13 in ST only Kruskal-Wallis test

Multiple Comparisons p values (2-tailed); Amp (KCl-Rec13 in Rec 9~41 Analysis 3 with k-1) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 144) =13.09194 p =.0014 Include condition: "Area 2"="ST"			
	WKY - R:58.203	**SD - R:75.656**	**SHR - R:86.509**
WKY		0.170037	0.001009
SD	0.170037		0.735446
SHR	0.001009	0.735446	

Table B.16 – Strain comparison of amplitude without REC 13 in NAc only Kruskal-Wallis test

Multiple Comparisons p values (2-tailed); Amp (KCl-Rec13 in Rec 9~41 Analysis 3 with k-1) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 27) =3.304246 p =.1916 Include condition: "Area 2"="NAC"			
	WKY - R:11.385	**SD - R:18.600**	**SHR - R:15.222**
WKY		0.252252	0.794561
SD	0.252252		1.000000
SHR	0.794561	1.000000	

B.2.7 Strain difference in T_{80} and T_{rise}

Table B.17 – Strain comparison of T_{80} as from KCl stimulations

Multiple Comparisons p values (2-tailed); T80 (KCl 100~250 in Rec 3~41 Analysis 2 KCl results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 215) =11.73721 p =.0028			
	SD - R: 85.680	**WKY - R: 117.60**	**SHR - R: 117.29**
SD		0.006454	0.009900
WKY	0.006454		1.000000
SHR	0.009900	1.000000	

Table B.18 – Strain comparison of T_{rise} as from KCl stimulations

Multiple Comparisons p values (2-tailed); Trise (KCl 100~250 in Rec 9~41 Analysis 3 with k-1) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 215) =1.643204 p =.4397			
	SD - R: 112.95	**WKY - R: 110.75**	**SHR - R: 100.29**
SD		1.000000	0.717124
WKY	1.000000		0.907534
SHR	0.717124	0.907534	

B.2.8 Strain comparison of parameters when results were averaged per rat

B.2.8.1 Averaged results in the ST

Table B.19 – Strain comparison of averaged amplitudes in the ST with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Amp (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =1.921584 p =.3826 Include condition: "Area 2"="ST"			
	SD - R:12.857	WKY - R:9.3750	SHR - R:13.875
SD		0.963578	1.000000
WKY	0.963578		0.553548
SHR	1.000000	0.553548	

Table B.20 – Strain comparison of averaged T_{80} in the ST with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); T80 (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =3.114907 p =.2107 Include condition: "Area 2"="ST"			
	SD - R:8.5714	WKY - R:14.750	SHR - R:12.250
SD		0.235131	0.883958
WKY	0.235131		1.000000
SHR	0.883958	1.000000	

Table B.21 – Strain comparison of averaged baselines in the ST with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Baseline (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =1.921584 p =.3826 Include condition: "Area 2"="ST"			
	SD - R:11.143	WKY - R:10.125	SHR - R:14.625
SD		1.000000	0.963578
WKY	1.000000		0.553548
SHR	0.963578	0.553548	

Table B.22 – Strain comparison of averaged ejection KCl volumes in the ST with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Volume (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =1.606580 p =.4479 Include condition: "Area 2"="ST"			
	SD - R:10.286	WKY - R:11.125	SHR - R:14.375
SD		1.000000	0.732086
WKY	1.000000		1.000000
SHR	0.732086	1.000000	

B.2.8.2 Averaged results in the NAc

Table B.23 – Strain comparison of averaged amplitudes in the NAc with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Amp (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 15) =3.260000 p =.1959 Include condition: "Area 2"="NAC"			
	SD - R:10.800	**WKY - R:5.8000**	**SHR - R:7.4000**
SD		0.231300	0.687996
WKY	0.231300		1.000000
SHR	0.687996	1.000000	

Table B.24 – Strain comparison of averaged T_{80} in the NAc with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); T80 (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 15) =.7200000 p =.6977 Include condition: "Area 2"="NAC"			
	SD - R:6.8000	**WKY - R:9.2000**	**SHR - R:8.0000**
SD		1.000000	1.000000
WKY	1.000000		1.000000
SHR	1.000000	1.000000	

Table B.25 – Strain comparison of averaged baselines in the NAc with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Baseline (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 15) =3.380000 p =.1845 Include condition: "Area 2"="NAC"			
	SD - R:9.6000	**WKY - R:5.0000**	**SHR - R:9.4000**
SD		0.311628	1.000000
WKY	0.311628		0.359385
SHR	1.000000	0.359385	

Table B.26 – Strain comparison of averaged KCl ejection volumes in the NAc with Kruskal-Wallis

Multiple Comparisons p values (2-tailed); Volume (KCl AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 15) =.9915596 p =.6091 Include condition: "Area 2"="NAC"			
	SD - R:7.1000	**WKY - R:7.3000**	**SHR - R:9.6000**
SD		1.000000	1.000000
WKY	1.000000		1.000000
SHR	1.000000	1.000000	

B.2.9 Analysis of calibrations

Table B.27 – Calibration parameters prior to Rec 39

LOD	0.009166
Selectivity (Ox)	1748.583344
Selectivity (Re)	-26276.787754
Slope (Ox)	-0.130292
Slope (Re)	0.017238
Baseline SD (Ox)	0.000398
Baseline SD (Re)	0.000780
Y Intercept (Ox)	-0.972187
Y Intercept (Re)	0.610404
R Squared (Ox)	0.999688
R Squared (Re)	0.998704
Impedance	0.000000

Table B.28 – Calibration parameters after Rec 39 recording

LOD	0.040693
Selectivity (Ox)	334.710003
Selectivity (Re)	2595.407935
Slope (Ox)	-0.032602
Slope (Re)	0.011026
Baseline SD (Ox)	0.000442
Baseline SD (Re)	0.000829
Y Intercept (Ox)	-1.734420
Y Intercept (Re)	0.822115
R Squared (Ox)	0.998284
R Squared (Re)	0.995083
Impedance	0.000000

Table B.29 – Calibration parameters prior to Rec 40

LOD	0.003714
Selectivity (Ox)	2122.126804
Selectivity (Re)	-2026.382336
Slope (Ox)	-0.393112
Slope (Re)	0.055586
Baseline SD (Ox)	0.000487
Baseline SD (Re)	0.000867
Y Intercept (Ox)	-1.415295
Y Intercept (Re)	1.014784
R Squared (Ox)	0.999702
R Squared (Re)	0.998586
Impedance	0.000000

Table B.30 – Calibration parameters after Rec 40 recording

LOD	0.011057
Selectivity (Ox)	238.656313
Selectivity (Re)	-5251.662650
Slope (Ox)	-0.101441
Slope (Re)	0.068037
Baseline SD (Ox)	0.000374
Baseline SD (Re)	0.000495
Y Intercept (Ox)	-1.183944
Y Intercept (Re)	0.759231
R Squared (Ox)	0.999927
R Squared (Re)	0.999810
Impedance	0.000000

B.3 Time of clearance of DA Uptake

B.3.1 DA descriptive statistics

Table B.31 – Descriptive Statistics for exogenously applied DA experiments, no strain break down

Descriptive Statistics (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July)							
	Valid N	Confidence - -95.000%	Confidence - 95.000	Median	Minimum	Maximum	Std.Dev.
Baseline	306	5.0154	6.1986	3.7671	2.006876	24.741	5.2591
Amp	306	1.2789	1.3499	1.2900	0.808560	1.999	0.3153
1st_K-1	306	0.0038	0.0067	0.0010	0.000018	0.070	0.0132
Peak Area	306	47.0695	56.7882	41.9635	6.186224	259.346	43.1982
Trise	306	9.5693	12.9274	8.0000	2.000000	215.000	14.9260
T100	306	177.8403	214.4604	172.5000	6.000000	1031.000	162.7705
T80	306	38.6834	49.9963	30.5000	4.000000	463.000	50.2841
T50	306	17.6987	21.7588	15.0000	3.000000	126.000	18.0464
Tc	306	-0.1027	-0.0808	-0.0464	-0.407539	-0.002	0.0972

Table B.32 – Descriptive Statistics for exogenously applied DA experiments, with strain break down

	Mass	Mass	Mass	Mass	Mass	Mass	Mass	Mass
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	90.00	95.00	17.32	74.50	120.30	74.50	84.00	109.10
WKY	79.34	116.00	2.62	76.50	85.50	77.40	78.50	80.30
SHR	83.79	95.00	10.33	75.00	105.90	75.50	79.60	92.10
All Grps	84.03	306.00	12.14	74.50	120.30			
	Volume	Volume	Volume	Volume	Volume	Volume	Volume	Volume
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	343.72	95.00	591.18	3.50	4000.00	25.00	150.00	375.00
WKY	258.38	116.00	375.26	5.00	2000.00	25.00	75.00	362.50
SHR	88.51	95.00	288.55	3.00	2500.00	5.00	20.00	50.00
All Grps	232.14	306.00	443.89	3.00	4000.00			
	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	3.17	95.00	0.86	2.01	4.59	2.19	3.39	3.93
WKY	6.55	116.00	7.20	2.15	24.74	2.99	3.50	4.31
SHR	6.90	95.00	4.09	3.28	16.43	4.89	5.58	6.55
All Grps	5.61	306.00	5.26	2.01	24.74			
	Amp	Amp	Amp	Amp	Amp	Amp	Amp	Amp
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	1.33	95.00	0.31	0.81	2.00	1.04	1.33	1.55
WKY	1.29	116.00	0.33	0.81	2.00	1.01	1.23	1.53
SHR	1.34	95.00	0.31	0.83	1.96	1.11	1.33	1.58
All Grps	1.31	306.00	0.32	0.81	2.00			
	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	46.26	95.00	37.19	6.19	219.35	10.53	37.29	73.24
WKY	59.94	116.00	50.41	6.54	259.35	25.37	46.40	75.23
SHR	47.82	95.00	37.87	7.42	217.05	22.82	37.32	58.03
All Grps	51.93	306.00	43.20	6.19	259.35			
	Trise	Trise	Trise	Trise	Trise	Trise	Trise	Trise
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	9.80	95.00	8.06	2.00	35.00	3.00	8.00	14.00
WKY	12.68	116.00	10.96	2.00	62.00	6.00	9.00	16.00
SHR	10.95	95.00	22.50	2.00	215.00	3.00	5.00	13.00
All Grps	11.25	306.00	14.93	2.00	215.00			

Table B.33 – Continued. Descriptive Statistics for exogenously applied DA experiments, with strain break down

	T100	T100	T100	T100	T100	T100	T100	T100
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	170.75	95.00	140.67	6.00	707.00	23.00	205.00	268.00
WKY	231.56	116.00	188.25	14.00	1031.00	102.50	195.00	282.50
SHR	178.32	95.00	142.48	10.00	700.00	80.00	139.00	233.00
All Grps	196.15	306.00	162.77	6.00	1031.00			
	T80	T80	T80	T80	T80	T80	T80	T80
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	40.43	95.00	33.34	4.00	158.00	10.00	37.00	62.00
WKY	50.92	116.00	55.91	5.00	361.00	15.00	33.00	65.00
SHR	40.21	95.00	56.41	4.00	463.00	11.00	23.00	52.00
All Grps	44.34	306.00	50.28	4.00	463.00			
	T50	T50	T50	T50	T50	T50	T50	T50
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	19.01	95.00	14.17	3.00	65.00	5.00	17.00	30.00
WKY	22.19	116.00	20.82	3.00	118.00	8.00	16.00	27.50
SHR	17.44	95.00	17.67	3.00	126.00	5.00	10.00	26.00
All Grps	19.73	306.00	18.05	3.00	126.00			
	Tc	Tc	Tc	Tc	Tc	Tc	Tc	Tc
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	-0.10	95.00	0.11	-0.41	-0.01	-0.15	-0.04	-0.02
WKY	-0.07	116.00	0.08	-0.41	0.00	-0.10	-0.04	-0.02
SHR	-0.10	95.00	0.10	-0.39	0.00	-0.17	-0.07	-0.03
All Grps	-0.09	306.00	0.10	-0.41	0.00			
	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	0.01	95.00	0.02	0.00	0.07	0.00	0.00	0.01
WKY	0.00	116.00	0.00	0.00	0.02	0.00	0.00	0.00
SHR	0.00	95.00	0.00	0.00	0.03	0.00	0.00	0.00
All Grps	0.01	306.00	0.01	0.00	0.07			

Table B.34 – Descriptive Statistics for exogenously applied DA experiments, with strain break down, 'Large' amp only

	Mass	Mass	Mass	Mass	Mass	Mass	Mass	Mass
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	85.73	15.00	12.41	76.90	120.30	76.90	84.00	84.00
WKY	78.80	23.00	2.39	76.50	85.50	77.40	78.50	79.20
SHR	84.02	23.00	9.43	75.00	105.90	75.80	80.70	92.10
All Grps	82.47	61.00	8.91	75.00	120.30			
	Volume	Volume	Volume	Volume	Volume	Volume	Volume	Volume
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	904.33	15.00	1179.45	5.00	4000.00	100.00	275.00	1325.00
WKY	175.22	23.00	173.76	5.00	675.00	50.00	100.00	300.00
SHR	35.70	23.00	45.84	3.00	200.00	5.00	25.00	50.00
All Grps	301.90	61.00	678.59	3.00	4000.00			
	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	3.16	15.00	0.62	2.46	4.20	2.53	2.85	3.70
WKY	6.07	23.00	6.62	2.19	24.74	2.53	3.20	3.90
SHR	7.28	23.00	4.33	3.31	16.43	5.07	5.64	7.00
All Grps	5.81	61.00	5.06	2.19	24.74			
	Amp	Amp	Amp	Amp	Amp	Amp	Amp	Amp
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	1.80	15.00	0.12	1.60	2.00	1.72	1.82	1.90
WKY	1.79	23.00	0.11	1.63	2.00	1.71	1.78	1.86
SHR	1.75	23.00	0.10	1.62	1.96	1.66	1.72	1.82
All Grps	1.78	61.00	0.11	1.60	2.00			
	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1	1st_K-1
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	0.01	15.00	0.01	0.00	0.05	0.00	0.00	0.01
WKY	0.00	23.00	0.00	0.00	0.02	0.00	0.00	0.00
SHR	0.00	23.00	0.01	0.00	0.03	0.00	0.00	0.00
All Grps	0.00	61.00	0.01	0.00	0.05			
	Trise	Trise	Trise	Trise	Trise	Trise	Trise	Trise
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	10.00	15.00	8.08	2.00	35.00	4.00	8.00	14.00
WKY	12.04	23.00	9.22	2.00	43.00	8.00	9.00	12.00
SHR	6.61	23.00	6.13	3.00	27.00	3.00	4.00	6.00
All Grps	9.49	61.00	8.12	2.00	43.00			

Table B.35 – Continued. Descriptive Statistics for exogenously applied DA experiments, with strain break down, 'Large' amp only

	T100	T100	T100	T100	T100	T100	T100	T100
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	231.40	15.00	204.37	8.00	707.00	41.00	251.00	279.00
WKY	268.13	23.00	189.57	26.00	696.00	121.00	205.00	383.00
SHR	171.35	23.00	160.73	10.00	665.00	73.00	119.00	225.00
All Grps	222.61	61.00	184.98	8.00	707.00			
	T80	T80	T80	T80	T80	T80	T80	T80
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	40.73	15.00	35.91	6.00	140.00	16.00	23.00	64.00
WKY	50.13	23.00	43.74	6.00	181.00	21.00	40.00	55.00
SHR	28.43	23.00	31.67	6.00	142.00	8.00	18.00	30.00
All Grps	39.64	61.00	38.22	6.00	181.00			
	T50	T50	T50	T50	T50	T50	T50	T50
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	19.07	15.00	14.02	4.00	55.00	9.00	12.00	29.00
WKY	22.70	23.00	17.63	4.00	76.00	10.00	20.00	23.00
SHR	13.26	23.00	11.30	4.00	43.00	5.00	10.00	16.00
All Grps	18.25	61.00	14.96	4.00	76.00			
	Tc	Tc	Tc	Tc	Tc	Tc	Tc	Tc
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	-0.10	15.00	0.08	-0.30	-0.02	-0.12	-0.09	-0.03
WKY	-0.07	23.00	0.08	-0.39	-0.01	-0.08	-0.05	-0.04
SHR	-0.15	23.00	0.11	-0.39	-0.02	-0.24	-0.11	-0.07
All Grps	-0.11	61.00	0.10	-0.39	-0.01			
	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area	Peak Area
	Means	N	StdDev	Min	Max	Q25	Median	Q75
SD	65.50	15.00	55.95	10.50	219.35	23.86	42.43	98.29
WKY	83.14	23.00	58.67	16.57	259.35	47.04	72.11	91.80
SHR	52.67	23.00	45.37	14.09	217.05	27.16	36.76	57.88
All Grps	67.31	61.00	54.10	10.50	259.35			

B.3.2 Application of exogenous DA Shapiro-Wilks' Normality tests

B.4 Analysis of amplitudes

Figure B.10 – Shapiro-Wilks' Normality tests of controlled amplitudes from application of exogenous DA

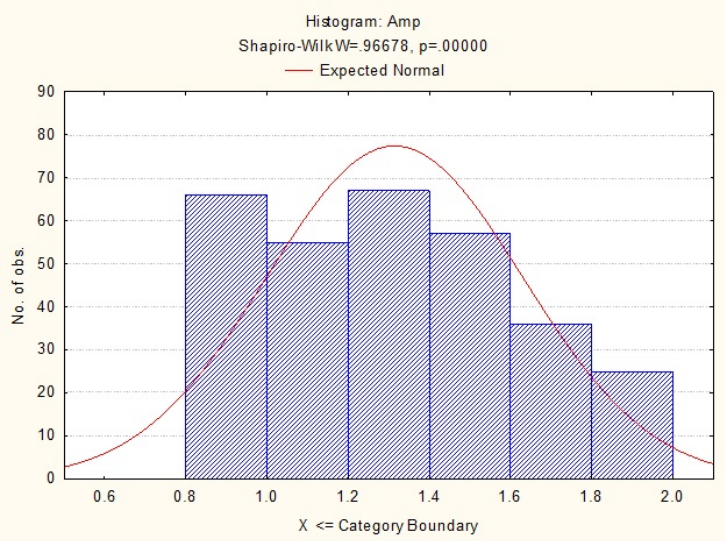


Figure B.11 – Shapiro-Wilks' Normality tests of T_{80} from application of exogenous DA

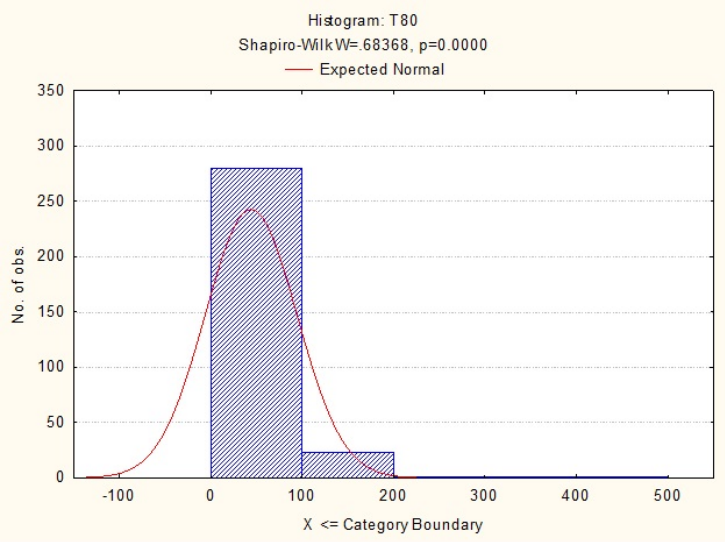


Figure B.12 – Shapiro-Wilks' Normality tests of T_{50} from application of exogenous DA

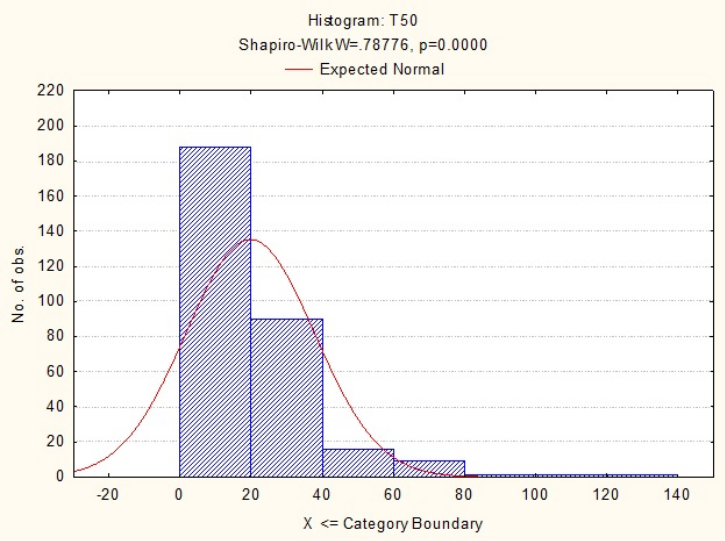


Figure B.13 – Shapiro-Wilks' Normality tests of T_c from application of exogenous DA

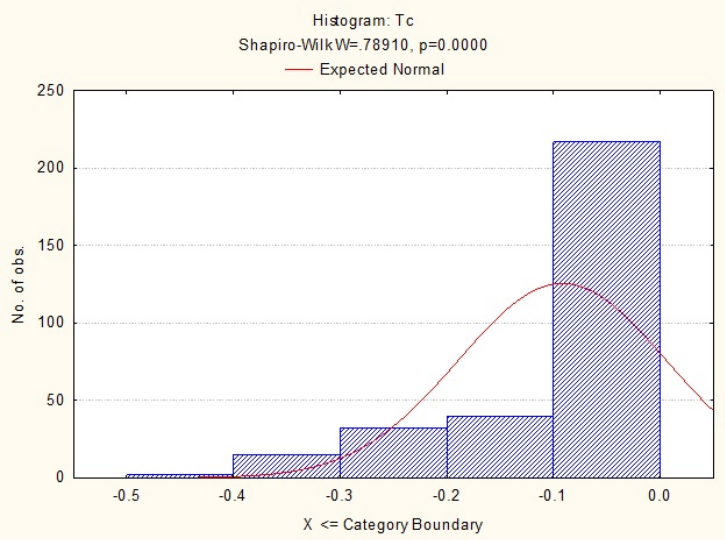


Figure B.14 – Shapiro-Wilks' Normality tests of T_{rise} from application of exogenous DA

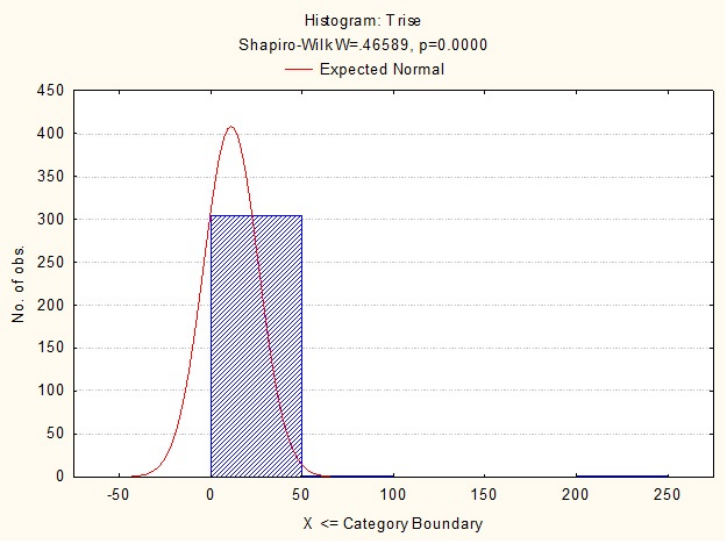


Figure B.15 – Shapiro-Wilks' Normality tests of T_{100} from application of exogenous DA

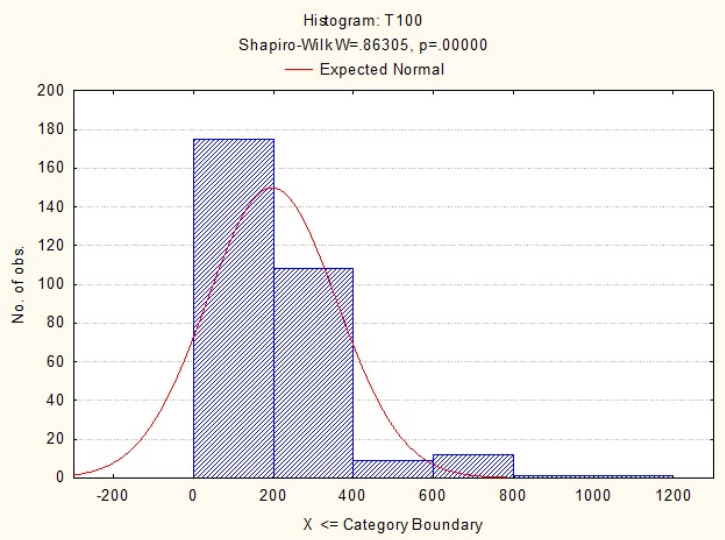


Figure B.16 – Shapiro-Wilks' Normality tests of baseline from application of exogenous DA

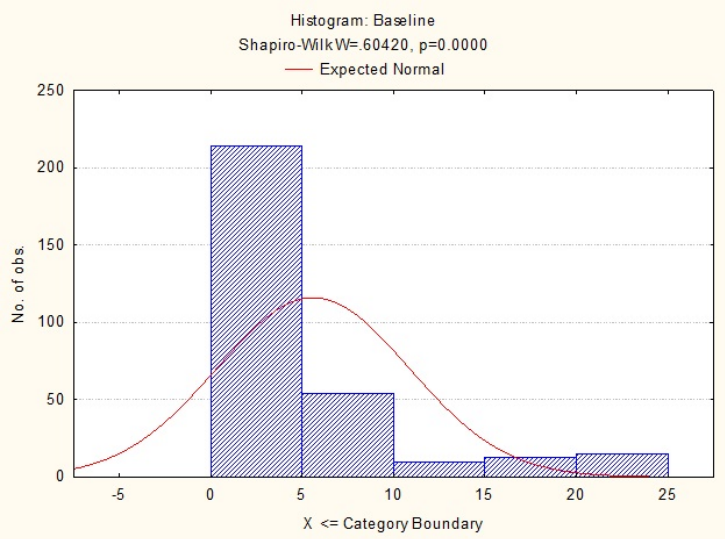


Figure B.17 – Shapiro-Wilks' Normality tests of K^{-1} from application of exogenous DA

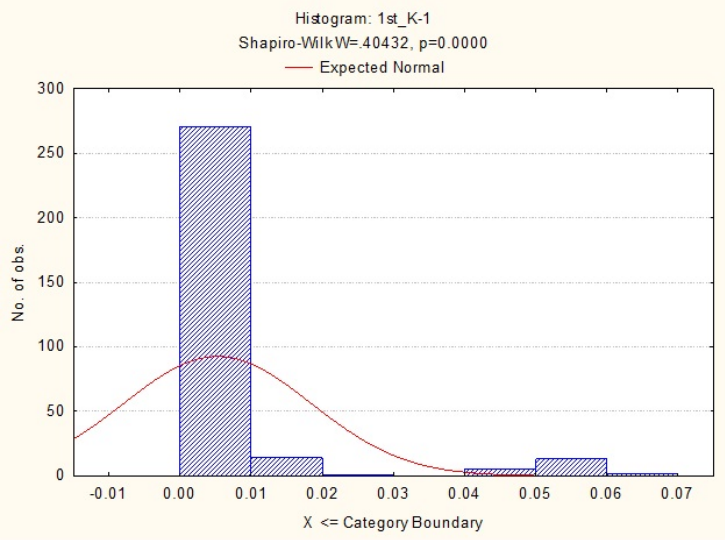


Figure B.18 – Shapiro-Wilks'

B.4.1 Analysis of working electrode calibration slopes

Table B.36 – Descriptive statistics of calibrations slopes of electrodes used in the application of exogenous DA experiments

2-Way Tables of Descriptive Statistics (DA one slope per rat in Final KCl and DA datasets) N=23 (No missing data in dep. var. list)

	Slope - Means	Slope - N	Slope - Std.Dev.	Slope - Std.Err.	Slope - Minimum	Slope - Maximum
SHR	-0.335517	9	0.177547	0.059182	-0.709652	-0.115312
WKY	-0.402847	9	0.223584	0.074528	-0.683731	-0.041401
SD	-0.593122	5	0.236707	0.105859	-0.839163	-0.317145
All Grps	-0.417866	23	0.222880	0.046474	-0.839163	-0.041401

Table B.37 – Strain comparison of calibrations slopes of electrodes used in the application of exogenous DA experiments

Analysis of Variance (DA one slope per rat in Final KCl and DA datasets) Marked effects are significant at p < .05000

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
Slope	0.216636	2	0.108318	0.876223	20	0.043811	2.472380	0.109776

B.4.2 Strain comparison of of rat mass and amplitudes

Table B.38 – Strain comparison of rat mass in the application of exogenous DA experiments

Multiple Comparisons p values (2-tailed); Mass (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =1.500934 p =.4721			
	SD - R: 162.72	**WKY - R: 149.18**	**SHR - R: 149.56**
SD		0.806823	0.916190
WKY	0.806823		1.000000
SHR	0.916190	1.000000	

Table B.39 – Strain comparison of amplitudes in the application of exogenous DA experiments

Multiple Comparisons p values (2-tailed); Amp (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =2.086749 p =.3523			
	SD - R: 157.99	**WKY - R: 144.22**	**SHR - R: 160.34**
SD		0.782608	1.000000
WKY	0.782608		0.564452
SHR	1.000000	0.564452	

Table B.40 – Kruskal-Wallis

B.4.3 Strain comparison of T_{80} in small and medium amplitude ranges

Table B.41 – Strain comparison of T_{80} in the small amplitude range

	Multiple Comparisons p values (2-tailed); T80 (DA vol toc Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 66) =1.899932 p =.3868 Include condition: "Amp Size"="Small"		
Depend.: T80	SD R:28.395	WKY R:35.328	SHR R:35.944
SD		0.663282	0.695437
WKY	0.663282		1.000000
SHR	0.695437	1.000000	

Table B.42 – Strain comparison of T_{80} in the medium amplitude range

	Multiple Comparisons p values (2-tailed); T80 (DA vol t Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 179) =1.199606 p =.548 Include condition: "Amp Size"="Medium"		
Depend.: T80	SD R:88.984	WKY R:95.250	SHR R:84.926
SD		1.00000C	1.00000C
WKY	1.00000C		0.84274
SHR	1.00000C	0.84274	

B.4.4 Strain comparison of T_{50} across all amplitude ranges

Table B.43 – Strain comparison of T_{50} in across all amplitude ranges

Multiple Comparisons p values (2-tailed); T50 (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =3.813889 p =.1485			
	SD - R:153.78	**WKY - R:164.12**	**SHR - R:140.25**
SD		1.000000	0.875597
WKY	1.000000		0.153827
SHR	0.875597	0.153827	

Table B.44 – Strain comparison of T_{50} in the small amplitude range

Multiple Comparisons p values (2-tailed); T50 (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 66) =1.838147 p =.3989 Include condition: "Amp Size"="Small"			
	SD - R:28.474	**WKY - R:35.397**	**SHR - R:35.750**
SD		0.665279	0.747465
WKY	0.665279		1.000000
SHR	0.747465	1.000000	

Table B.45 – Strain comparison of T_{50} in the medium amplitude range

Multiple Comparisons p values (2-tailed); T50 (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 179) =1.310280 p =.5194 Include condition: "Amp Size"="Medium"			
	SD - R:92.049	**WKY - R:93.656**	**SHR - R:83.352**
SD		1.000000	1.000000
WKY	1.000000		0.845502
SHR	1.000000	0.845502	

Table B.46 – Strain comparison of T_{50} in the large amplitude range

Multiple Comparisons p values (2-tailed); T50 (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 61) =6.457083 p =.0396 Include condition: "Amp Size"="Large"			
	SD - R:33.100	**WKY - R:36.804**	**SHR - R:23.826**
SD		1.000000	0.346448
WKY	1.000000		0.039514
SHR	0.346448	0.039514	

B.4.5 Strain comparison of T_c

Table B.47 – Strain comparison of T_c across all amplitude ranges

Multiple Comparisons p values (2-tailed); Tc (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =4.475753 p =.1067			
	SD - R:151.17	**WKY - R:166.09**	**SHR - R:140.46**
SD		0.669137	1.000000
WKY	0.669137		0.109084
SHR	1.000000	0.109084	

Table B.48 – Strain comparison of T_c in the small amplitude range

Multiple Comparisons p values (2-tailed); Tc (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 66) =1.210709 p =.5459 Include condition: "Amp Size"="Small"			
	SD - R:29.474	**WKY - R:35.552**	**SHR - R:34.444**
SD		0.850145	1.000000
WKY	0.850145		1.000000
SHR	1.000000	1.000000	

Table B.49 – Strain comparison of T_c in the medium amplitude range

Multiple Comparisons p values (2-tailed); Tc (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 179) =1.194159 p =.5504 Include condition: "Amp Size"="Medium"			
	SD - R:88.787	**WKY - R:95.313**	**SHR - R:85.074**
SD		1.000000	1.000000
WKY	1.000000		0.854779
SHR	1.000000	0.854779	

Table B.50 – Strain comparison of T_c in the large amplitude range

Multiple Comparisons p values (2-tailed); Tc (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 61) =6.530504 p =.0382 Include condition: "Amp Size"="Large"			
	SD - R:32.067	**WKY - R:37.304**	**SHR - R:24.000**
SD		1.000000	0.512885
WKY	1.000000		0.033123
SHR	0.512885	0.033123	

B.4.6 Strain comparison of T_{rise}

Table B.51 – Strain comparison of T_{rise} across all amplitude ranges

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 306) =9.853931 p =.0072

	SD - R:144.22	WKY - R:173.54	SHR - R:138.31
SD		0.049803	1.000000
WKY	0.049803		0.012015
SHR	1.000000	0.012015	

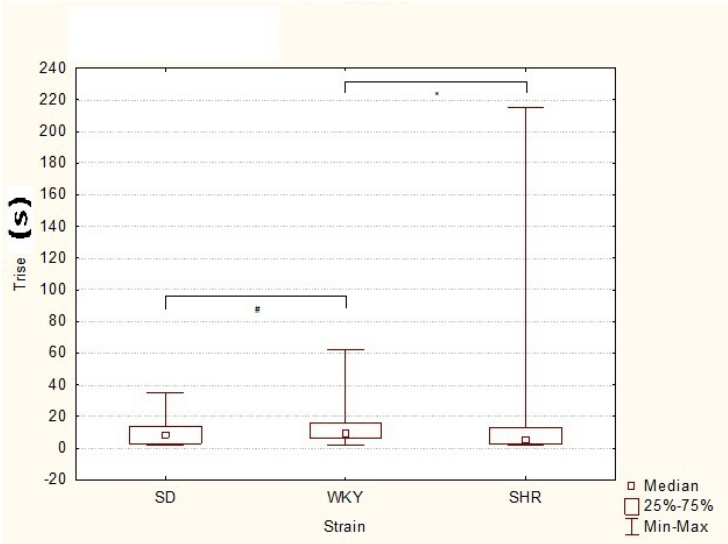


Figure B.19 – Overall strain difference in T_{rise} of peaks from exogenously applied DA; H(2, N=306)=9.853931 p=0.0072

Table B.52 – Strain comparison of T_{rise} in the ST only

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain
Kruskal-Wallis test: H (2, N= 254) =7.166937 p =.0278 Include condition: Area2="ST"

	SD - R:124.83	WKY - R:142.88	SHR - R:112.77
SD		0.306449	0.878848
WKY	0.306449		0.025372
SHR	0.878848	0.025372	

Table B.53 – Strain comparison of T_{rise} in the NAc only

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 34) =.8531081 p =.6528 Include condition: Area2="NAC"			
	SD - R:21.000	**WKY - R:18.395**	**SHR - R:15.654**
SD		1.000000	1.000000
WKY	1.000000		1.000000
SHR	1.000000	1.000000	

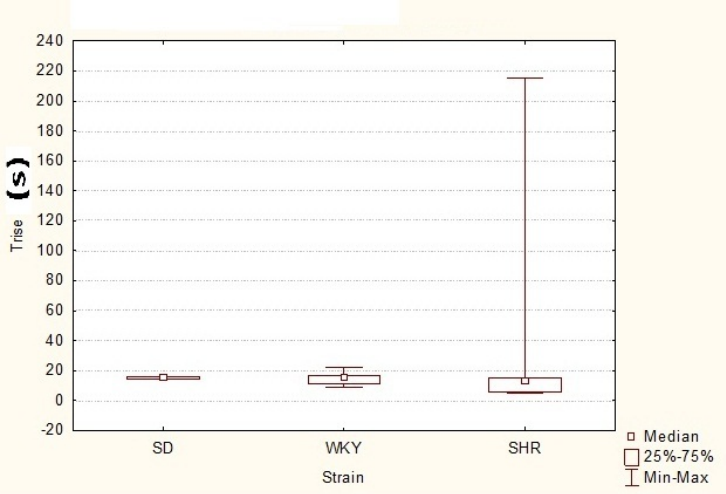


Figure B.20 – Strain comparison of T_{rise} in the NAc; H(2, N=34) =0.8531081 p=0.6628

Table B.54 – Strain comparison of T_{rise} in small amplitude range

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 66) =3.672752 p =.1594 Include condition: "Amp Size"="Small"

	SD - R:26.474	WKY - R:36.948	SHR - R:35.361
SD		0.193490	0.477777
WKY	0.193490		1.000000
SHR	0.477777	1.000000	

Table B.55 – Strain comparison of T_{rise} in medium amplitude range

Multiple Comparisons z' values; Trise (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 179) =4.007922 p =.1348 Include condition: "Amp Size"="Medium"

	SD - R:86.361	WKY - R:100.11	SHR - R:82.130
SD		1.482825	0.437005
WKY	1.482825		1.877834
SHR	0.437005	1.877834	

Table B.56 – Strain comparison of T_{rise} in large amplitude range

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 61) =11.71361 p =.0029 Include condition: "Amp Size"="Large"			
	SD - R:33.400	**WKY - R:39.000**	**SHR - R:21.435**
SD		1.000000	0.126824
WKY	1.000000		0.002378
SHR	0.126824	0.002378	

Table B.57 – Strain comparison of T_{rise} in large amplitude range in the ST only

Multiple Comparisons p values (2-tailed); Trise (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 53) =10.38336 p =.0056 Include condition: "Amp Size"="Large" Exclude condition: Area2="NAC"			
	SD - R:29.714	**WKY - R:33.921**	**SHR - R:18.525**
SD		1.000000	0.112795
WKY	1.000000		0.005577
SHR	0.112795	0.005577	

B.4.7 Strain comparison for T_{100}

Table B.58 – Strain comparison of T_{100} across all amplitude ranges

Multiple Comparisons p values (2-tailed); T100 (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =6.665384 p =.0357			
	SD - R:142.89	**WKY - R:170.21**	**SHR - R:143.71**
SD		0.077077	1.000000
WKY	0.077077		0.091249
SHR	1.000000	0.091249	

B.4.8 Strain comparison of DA volume required to reach amplitude range

Table B.59 – Strain comparison of DA volume required to reach small amplitude range

Multiple Comparisons p values (2-tailed); Volume (DA vol too in Rec 3~41 Analysis 2 DA results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 66) =14.09062 p =.0009 Include condition: "Amp Size"="Small"			
	SD - R:42.921	**WKY - R:35.759**	**SHR - R:19.917**
SD		0.618534	0.000807
WKY	0.618534		0.017864
SHR	0.000807	0.017864	

Table B.60 – Strain comparison of DA volume required to reach medium amplitude range

Multiple Comparisons p values (2-tailed); Volume (DA vol too in Rec 3~41 Analysis 2 DA results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 179) =20.38795 p =.0000 Include condition: "Amp Size"="Medium"			
	SD - R:93.279	**WKY - R:107.77**	**SHR - R:65.231**
SD		0.353956	0.011307
WKY	0.353956		0.000027
SHR	0.011307	0.000027	

Table B.61 – Strain comparison of DA volume required to reach large amplitude range

Multiple Comparisons p values (2-tailed); Volume (DA vol too in Rec 3~41 Analysis 2 DA results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 61) =22.86445 p =.0000 Include condition: "Amp Size"="Large"			
	SD - R:44.167	**WKY - R:35.674**	**SHR - R:17.739**
SD		0.448382	0.000022
WKY	0.448382		0.001838
SHR	0.000022	0.001838	

Table B.62 – Strain comparison of DA volume required to reach required amplitude range in ST only

Multiple Comparisons p values (2-tailed); Volume (DA vol too in Rec 3~41 Analysis 2 DA results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 254) =37.83900 p =.0000 Include condition: Area2="ST"			
	SD - R:144.71	**WKY - R:147.45**	**SHR - R:84.773**
SD		1.000000	0.000001
WKY	1.000000		0.000000
SHR	0.000001	0.000000	

Table B.63 – Strain comparison of DA volume required to reach required amplitude range in NAc only

Multiple Comparisons p values (2-tailed); Volume (DA vol too in Rec 3~41 Analysis 2 DA results) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 34) =8.946923 p =.0114 Include condition: Area2="NAC"			
	SD - R:25.500	**WKY - R:20.974**	**SHR - R:11.192**
SD		1.000000	0.175636
WKY	1.000000		0.019063
SHR	0.175636	0.019063	

B.4.9 Strain comparison of Baseline levels

Table B.64 – Strain comparison of baseline levels in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Baseline (DA 0.8~1.99 in Rec 9~41 Analysis 3 with k-1 24July) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 306) =83.12241 p =.0000			
	SD - R:103.42	**WKY - R:141.63**	**SHR - R:218.07**
SD		0.005411	0.000000
WKY	0.005411		0.000000
SHR	0.000000	0.000000	

B.4.10 Strain comparison of parameters when results were averaged per rat

B.4.10.1 Averaged results in the ST

Table B.65 – Strain comparison of averaged T_{80} in the ST in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); T80 (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =.0772947 p =.9621 Include condition: Area2="ST"			
	SHR - R:11.556	WKY - R:12.444	SD - R:12.000
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

Table B.66 – Strain comparison of averaged T_{50} in the ST in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); T50 (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =.0772947 p =.9621 Include condition: Area2="ST"			
	SHR - R:11.556	WKY - R:12.444	SD - R:12.000
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

Table B.67 – Strain comparison of averaged T_c in the ST in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Tc (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =.5381643 p =.7641 Include condition: Area2="ST"			
	SHR - R:10.778	WKY - R:13.111	SD - R:12.200
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

Table B.68 – Strain comparison of averaged baseline in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Baseline (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =4.969082 p =.0834 Include condition: Area2="ST"			
	SHR - R:15.556	WKY - R:11.000	SD - R:7.4000
SHR		0.462603	0.093288
WKY	0.462603		1.000000
SD	0.093288	1.000000	

Table B.69 – Strain comparison of averaged T_{rise} in the ST in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Trise (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 23) =1.485024 p =.4759 Include condition: Area2="ST"

	SHR - R:10.333	WKY - R: 14.111	SD - R: 11.200
SHR		0.712115	1.000000
WKY	0.712115		1.000000
SD	1.000000	1.000000	

B.4.10.2 Averaged results in the NAc

Table B.70 – Strain comparison of averaged T_{80} in the NAc in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); T80 (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 14) =.7020408 p =.7040 Include condition: Area2="NAC"

	SHR - R:6.5714	WKY - R:8.5000	SD - R:8.0000
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

Table B.71 – Strain comparison of averaged T_{50} in the NAc in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); T50 (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 14) =1.289796 p =.5247 Include condition: Area2="NAC"

	SHR - R:6.2857	WKY - R:8.5000	SD - R:10.000
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

Table B.72 – Strain comparison of averaged T_c in the NAc in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Tc (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 14) =1.474830 p =.4783 Include condition: Area2="NAC"

	SHR - R:6.1429	WKY - R:8.8333	SD - R:9.0000
SHR		0.743026	1.000000
WKY	0.743026		1.000000
SD	1.000000	1.000000	

Table B.73 – Strain comparison of averaged baselines in the exogenously applied DA experiments

Multiple Comparisons p values (2-tailed); Baseline (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 14) =4.446259 p =.1083 Include condition: Area2="NAC"

	SHR - R:9.8571	WKY - R:5.1667	SD - R:5.0000
SHR		0.131603	0.832317
WKY	0.131603		1.000000
SD	0.832317	1.000000	

Table B.74 – Strain comparison of averaged T_{rise} in the NAc in the exogenously applied DA experiments

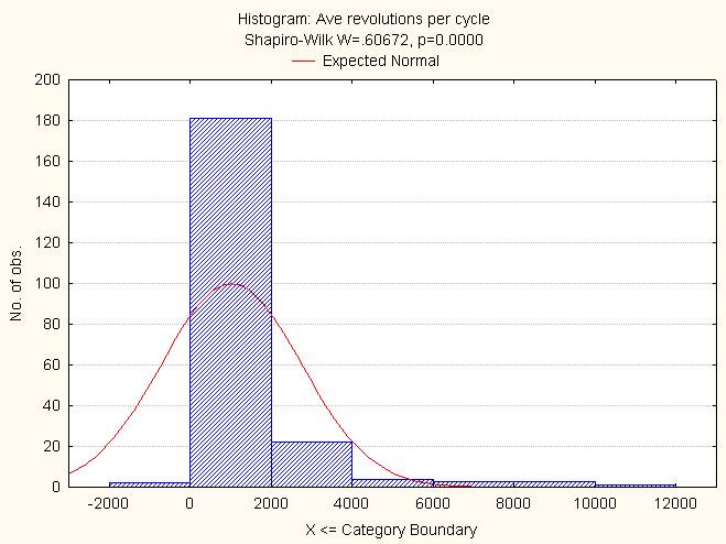
Multiple Comparisons p values (2-tailed); Trise (DA AVE in One rat one point Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 14) =.0807898 p =.9604 Include condition: Area2="NAC"

	SHR - R:7.5714	WKY - R:7.2500	SD - R:8.5000
SHR		1.000000	1.000000
WKY	1.000000		1.000000
SD	1.000000	1.000000	

C Behavioural Response to Methylphenidate

C.1 Running Wheels

C.1.1 Normality histogram of averaged running wheel revolutions



C.1.2 Strain comparison of MPH effect during each dosage period

Table C.1 – Strain comparison of MPH effect in dosage period 1, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =5.913617 p =.0520 Include condition: Dosage="CM" Exclude condition: DL="L"

	SHR - R:11.333	SHRM - R:12.600	WKY - R:5.7143
SHR		1.000000	0.175521
SHRM	1.000000		0.082832
WKY	0.175521	0.082832	

Table C.2 – Strain comparison of MPH effect in dosage period 1, light cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =9.225898 p =.0099 Include condition: Dosage="CM" Exclude condition: DL="D"

	SHR - R:12.333	SHRM - R:12.800	WKY - R:4.7143
SHR		1.000000	0.030930
SHRM	1.000000		0.029073
WKY	0.030930	0.029073	

Table C.3 – Strain comparison of MPH effect in dosage period 2, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =7.260986 p =.0265 Include condition: Dosage=0.5 Exclude condition: DL="L"

	SHR - R:11.667	SHRM - R:12.800	WKY - R:5.2857
SHR		1.000000	0.095045
SHRM	1.000000		0.048669
WKY	0.095045	0.048669	

Table C.4 – Strain comparison of MPH effect in dosage period 2, lightcycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =12.49242 p =.0019 Include condition: Dosage=0.5 Exclude condition: DL="D"

	SHR - R:12.167	SHRM - R:14.000	WKY - R:4.0000
SHR		1.000000	0.017898
SHRM	1.000000		0.004136
WKY	0.017898	0.004136	

Table C.5 – Strain comparison of MPH effect in dosage period 3, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =10.56274 p =.0051 Include condition: Dosage=1 Exclude condition: DL="L"

	SHR - R:10.833	SHRM - R:14.600	WKY - R:4.7143
SHR		0.731819	0.118131
SHRM	0.731819		0.004693
WKY	0.118131	0.004693	

Table C.6 – Strain comparison of MPH effect in dosage period 3, light cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =12.26211 p =.0022 Include condition: Dosage=1 Exclude condition: DL="D"

	SHR - R:11.667	SHRM - R:14.400	WKY - R:4.1429
SHR		1.000000	0.033909
SHRM	1.000000		0.003100
WKY	0.033909	0.003100	

Table C.7 – Strain comparison of MPH effect in dosage period 4, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =11.00301 p =.0041 Include condition: Dosage=2 Exclude condition: DL="L"

	SHR - R:11.000	SHRM - R:14.600	WKY - R:4.5714
SHR		0.796303	0.091292
SHRM	0.796303		0.004007
WKY	0.091292	0.004007	

Table C.8 – Strain comparison of MPH effect in dosage period 4, light cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =12.27368 p =.0022 Include condition: Dosage=2 Exclude condition: DL="D"

	SHR - R:12.500	SHRM - R:13.600	WKY - R:4.0000
SHR		1.000000	0.012635
SHRM	1.000000		0.006398
WKY	0.012635	0.006398	

Table C.9 – Strain comparison of MPH effect in dosage period 5, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =7.800334 p =.0202 Include condition: Dosage=5 Exclude condition: DL="L"

	SHR - R:11.667	SHRM - R:13.000	WKY - R:5.1429
SHR		1.000000	0.084168
SHRM	1.000000		0.035858
WKY	0.084168	0.035858	

Table C.10 – Strain comparison of MPH effect in dosage period 5, light cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =12.15789 p =.0023 Include condition: Dosage=5 Exclude condition: DL="D"

	SHR - R:13.000	SHRM - R:13.000	WKY - R:4.0000
SHR		1.000000	0.007331
SHRM	1.000000		0.011963
WKY	0.007331	0.011963	

Table C.11 – Strain comparison of MPH effect in dosage period 6, dark cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =5.348872 p =.0689 Include condition: Dosage=0 Exclude condition: DL="L"

	SHR - R:12.000	SHRM - R:11.600	WKY - R:5.8571
SHR		1.000000	0.115852
SHRM	1.000000		0.198555
WKY	0.115852	0.198555	

Table C.12 – Strain comparison of MPH effect in dosage period 6, light cycle; Kruskal-Wallis and Dunn's Multiple comparisons tests

Multiple Comparisons p values (2-tailed); Ave revolutions per cycle (MPH Statistica in MPH combine May July Data) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 18) =8.139181 p =.0171 Include condition: Dosage=0 Exclude condition: DL="D"

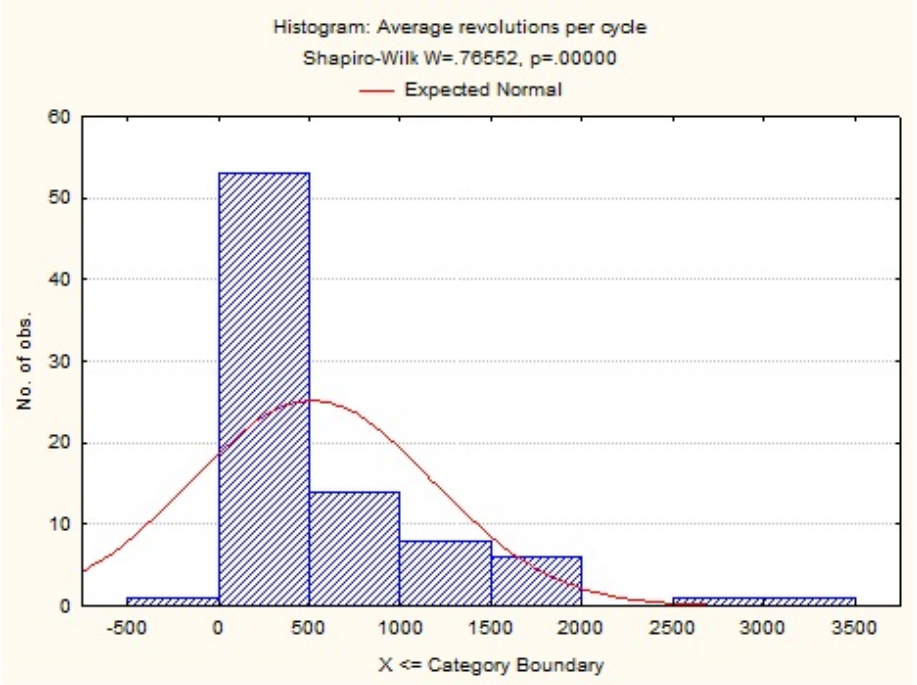
	SHR - R:12.333	SHRM - R:12.400	WKY - R:5.0000
SHR		1.000000	0.040640
SHRM	1.000000		0.053756
WKY	0.040640	0.053756	

D Behavioural Response to Guanfacine

D.1 Running Wheels

D.1.1 Normality histogram of averaged running wheel revolutions

Figure D.1 – Shapiro-Wilks' Normality test of averaged running wheel revolutions



D.1.2 Kruskal-Wallis and Dunn's Multiple comparison test statistics

Table D.1 – Running wheel revolutions in the dark cycle for control groups

Multiple Comparisons p values (2-tailed); Average revolutions per cycle (controls in GUA combine May July Data averaged) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 19) =13.21158 p =.0014 Include condition: DIcycle="D"			
	SHRC - R:15.500	**WKYC - R:6.1667**	**SDC - R:5.8000**
SHRC		0.006398	0.007493
WKYC	0.006398		1.000000
SDC	0.007493	1.000000	

Table D.2 – Running wheel revolutions in the light cycle for control groups

Multiple Comparisons p values (2-tailed); Average revolutions per cycle (controls in GUA combine May July Data averaged) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 19) =13.62880 p =.0011 Include condition: DIcycle="L"			
	SHRC - R:15.500	**WKYC - R:5.0000**	**SDC - R:7.2000**
SHRC		0.001651	0.029025
WKYC	0.001651		1.000000
SDC	0.029025	1.000000	

Table D.3 – Running wheel revolutions in the dark cycle for treated groups

Multiple Comparisons p values (2-tailed); Average revolutions per cycle (treated in GUA combine May July Data averaged) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 23) =13.79462 p =.0010 Include condition: DIcycle="D"			
	SHRG - R:17.900	**WKYG - R:8.5714**	**SDG - R:6.1667**
SHRG		0.015763	0.002423
WKYG	0.015763		1.000000
SDG	0.002423	1.000000	

Table D.4 – Running wheel revolutions in the light cycle for treated groups

Multiple Comparisons p values (2-tailed); Average revolutions per cycle (treated in GUA combine May July Data averaged) Independent (grouping) variable: Group Kruskal-Wallis test: H (2, N= 23) =9.151139 p =.0103 Include condition: DIcycle="L"			
	SHRG - R:16.500	**WKYG - R:10.571**	**SDG - R:6.1667**
SHRG		0.228307	0.009522
WKYG	0.228307		0.729222
SDG	0.009522	0.729222	

D.1.3 Mann-Whitney U test statistics

Table D.5 – SHR running wheel revolutions in the dark cycle

Mann-Whitney U Test (GUA SHR in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - SHRC	Rank Sum - SHRG	U	Z	p-value	Z - adjusted	p-value	Valid N - SHRC	Valid N - SHRG	2*1sided - exact p
Average revolutions per cycle	84.00000	87.00000	32.00000	0.666392	0.505161	0.666392	0.505161	8	10	0.514786

Table D.6 – SHR running wheel revolutions in the light cycle

Mann-Whitney U Test (GUA SHR in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - SHRC	Rank Sum - SHRG	U	Z	p-value	Z - adjusted	p-value	Valid N - SHRC	Valid N - SHRG	2*1sided - exact p
Average revolutions per cycle	89.00000	82.00000	27.00000	1.110654	0.266718	1.110654	0.266718	8	10	0.274281

Table D.7 – WKY running wheel revolutions in the dark cycle

Mann-Whitney U Test (GUA WKY in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - WKYC	Rank Sum - WKYG	U	Z	p-value	Z - adjusted	p-value	Valid N - WKYC	Valid N - WKYG	2*1sided - exact p
Average revolutions per cycle	35.00000	56.00000	14.00000	-0.928571	0.353112	-0.928571	0.353112	6	7	0.366967

Table D.8 – WKY dark cycle

Table D.9 – WKY running wheel revolutions in the light cycle

Mann-Whitney U Test (GUA WKY in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - WKYC	Rank Sum - WKYG	U	Z	p-value	Z - adjusted	p-value	Valid N - WKYC	Valid N - WKYG	2*1sided - exact p
Average revolutions per cycle	33.00000	58.00000	12.00000	-1.21429	0.224640	-1.21596	0.224002	6	7	0.234266

Table D.10 – SD running wheel revolutions in the dark cycle

Mann-Whitney U Test (GUA SD in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - SDC	Rank Sum - SDG	U	Z	p-value	Z - adjusted	p-value	Valid N - SDC	Valid N - SDG	2*1sided - exact p
Average revolutions per cycle	32.00000	34.00000	13.00000	0.273861	0.784191	0.273861	0.784191	5	6	0.792208

Table D.11 – SD running wheel revolutions in the light cycle

Mann-Whitney U Test (GUA SD in GUA combine May July Data averaged) By variable Group Marked tests are significant at p										
	Rank Sum - SDC	Rank Sum - SDG	U	Z	p-value	Z - adjusted	p-value	Valid N - SDC	Valid N - SDG	2*1sided - exact p
Average revolutions per cycle	34.50000	31.50000	10.50000	0.730297	0.465209	0.731962	0.464192	5	6	0.428571

D.2 Open Field

D.2.1 Descriptive Statistics

Table D.12 – Descriptive statistics of Non treated rat groups in the Open Field

Strain	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	7478.27	2.00	780.77	6926.18	8030.36	6926.18	7478.27	8030.36
SHR	6476.89	8.00	1071.67	4501.09	8150.00	5997.04	6510.17	7074.80
WKY	6368.61	3.00	649.41	5750.81	7045.58	5750.81	6309.43	7045.58
All Grps	6605.96	13.00	971.11	4501.09	8150.00	6154.31	6570.30	7045.58

Strain	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	8580.58	2.00	716.59	8073.87	9087.29	8073.87	8580.58	9087.29
SHR	4233.40	8.00	1510.30	1502.64	6932.99	3752.84	4248.73	4714.23
WKY	3739.57	3.00	2366.77	1011.16	5239.50	1011.16	4968.04	5239.50
All Grps	4788.24	13.00	2276.86	1011.16	9087.29	4086.32	4581.61	5239.50

Strain	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	63.00	2.00	17.82	50.40	75.60	50.40	63.00	75.60
SHR	89.23	8.00	39.26	39.20	152.40	61.50	78.80	120.80
WKY	67.73	3.00	26.23	47.60	97.40	47.60	58.20	97.40
All Grps	80.23	13.00	34.39	39.20	152.40	50.80	75.60	97.40

Strain	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	136.50	2.00	0.14	136.40	136.60	136.40	136.50	136.60
SHR	51.13	8.00	31.82	7.00	107.60	25.30	54.10	67.80
WKY	27.67	3.00	24.21	0.00	45.00	0.00	38.00	45.00
All Grps	58.85	13.00	44.46	0.00	136.60	26.80	50.40	70.40

Strain	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	21.50	2.00	10.61	14.00	29.00	14.00	21.50	29.00
SHR	32.25	8.00	8.53	19.00	43.00	25.00	34.00	39.00
WKY	13.33	3.00	6.35	6.00	17.00	6.00	17.00	17.00
All Grps	26.23	13.00	11.31	6.00	43.00	17.00	27.00	34.00

Strain	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	33.00	2.00	1.41	32.00	34.00	32.00	33.00	34.00
SHR	22.00	8.00	9.64	6.00	36.00	17.00	20.00	30.00
WKY	11.33	3.00	7.64	3.00	18.00	3.00	13.00	18.00
All Grps	21.23	13.00	10.58	3.00	36.00	16.00	19.00	32.00

Strain	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	21.50	2.00	10.61	14.00	29.00	14.00	21.50	29.00
SHR	32.25	8.00	8.53	19.00	43.00	25.00	34.00	39.00
WKY	13.00	3.00	6.08	6.00	17.00	6.00	16.00	17.00
All Grps	26.15	13.00	11.39	6.00	43.00	17.00	27.00	34.00

Strain	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	31.50	2.00	2.12	30.00	33.00	30.00	31.50	33.00
SHR	19.50	8.00	9.80	3.00	34.00	15.00	17.00	27.50
WKY	8.33	3.00	7.64	0.00	15.00	0.00	10.00	15.00
All Grps	18.77	13.00	10.99	0.00	34.00	14.00	16.00	29.00

Table D.13 – Descriptive statistics of Treated rat groups in the Open Field

Strain	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)	BEF Total Distance moved (cm)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	8693.87	1.00	0.00	8693.87	8693.87	8693.87	8693.87	8693.87
SHR	6707.49	10.00	1193.69	5085.02	8709.46	6127.85	6422.26	7870.40
WKY	4615.84	6.00	2073.28	652.34	6100.45	4105.37	5443.37	5950.12
Grps	6086.11	17.00	1903.09	652.34	8709.46	5250.81	6127.85	6969.91

Strain	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)	AFT Total Distance moved (cm)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	9802.95	1.00	0.00	9802.95	9802.95	9802.95	9802.95	9802.95
SHR	4833.58	10.00	1011.24	2772.14	6226.47	4298.02	5064.21	5462.44
WKY	3477.16	6.00	1636.49	564.43	5220.34	2822.09	3851.61	4552.89
Grps	4647.16	17.00	1899.62	564.43	9802.95	3829.17	4682.26	5333.23

Strain	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)	BEF Total Duration in Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	105.60	1.00	0.00	105.60	105.60	105.60	105.60	105.60
SHR	78.18	10.00	26.37	46.40	106.60	49.20	83.30	102.80
WKY	11.30	6.00	15.55	0.00	33.80	0.00	2.70	28.60
Grps	56.19	17.00	40.95	0.00	106.60	28.60	49.20	100.80

Strain	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)	AFT Total Duration in Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	156.00	1.00	0.00	156.00	156.00	156.00	156.00	156.00
SHR	75.50	10.00	31.09	29.60	121.40	41.20	80.50	94.40
WKY	4.17	6.00	7.11	0.00	17.20	0.00	0.00	7.80
Grps	55.06	17.00	49.28	0.00	156.00	7.80	41.20	88.60

Strain	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)	BEF Total Frequency into Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	29.00	1.00	0.00	29.00	29.00	29.00	29.00	29.00
SHR	31.40	10.00	12.03	11.00	47.00	21.00	35.00	42.00
WKY	4.67	6.00	5.50	0.00	12.00	0.00	2.50	11.00
Grps	21.82	17.00	16.18	0.00	47.00	11.00	21.00	37.00

Strain	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)	AFT Total Frequency into Inner Zone (s)
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	40.00	1.00	0.00	40.00	40.00	40.00	40.00	40.00
SHR	26.10	10.00	6.97	14.00	35.00	21.00	29.00	31.00
WKY	4.83	6.00	2.99	3.00	10.00	3.00	3.00	7.00
Grps	19.41	17.00	12.82	3.00	40.00	7.00	21.00	30.00

Strain	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions	BEF Total Frequency of zone transitions
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	29.00	1.00	0.00	29.00	29.00	29.00	29.00	29.00
SHR	31.10	10.00	11.72	11.00	46.00	21.00	35.00	41.00
WKY	4.67	6.00	5.50	0.00	12.00	0.00	2.50	11.00
Grps	21.65	17.00	15.94	0.00	46.00	11.00	21.00	37.00

Strain	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions	AFT Total Frequency of zone transitions
	Mean	N	StedDev	Min	Max	Q25	Median	Q75
SD	37.00	1.00	0.00	37.00	37.00	37.00	37.00	37.00
SHR	23.30	10.00	7.02	11.00	32.00	18.00	26.00	28.00
WKY	1.83	6.00	2.99	0.00	7.00	0.00	0.00	4.00
Grps	16.53	17.00	12.90	0.00	37.00	4.00	18.00	28.00

D.2.2 Effect of guanfacine treatment on open field parameters

Table D.14 – Mann-Whiteny U tests of guanfacine effect

Mann-Whitney U Test (GUA bef-aft in GUA OF 2010 Analysis2) By variable G/C Marked tests are significant at p <.05000

	Rank Sum - C	Rank Sum - G	U	Z	p-value	Z - adjusted	p-value	Valid N - C	Valid N - G	2*1sided - exact p
BEF Total Distance moved (cm)	227.0000	238.0000	85.0000	1.046294	0.295426	1.046294	0.295426	13	17	0.300326
BEF Total Duration in Inner Zone (s)	239.0000	226.0000	73.0000	1.548515	0.121499	1.548687	0.121458	13	17	0.122716
BEF Total Frequency into Inner Zone (s)	220.0000	245.0000	92.0000	0.753332	0.451251	0.753919	0.450899	13	17	0.457046
BEF Frequency of zone transitions in bin 3	238.5000	226.5000	73.5000	1.527589	0.126616	1.535638	0.124628	13	17	0.122716
AFT Total Distance moved (cm)	201.0000	264.0000	110.0000	0.000000	1.000000	0.000000	1.000000	13	17	1.000000
AFT Total Duration in Inner Zone (s)	208.0000	257.0000	104.0000	0.251111	0.801729	0.251671	0.801296	13	17	0.804693
AFT Total Frequency into Inner Zone (s)	216.5000	248.5000	95.5000	0.606850	0.543951	0.608545	0.542826	13	17	0.535769
AFT Total Frequency of zone transitions	216.5000	248.5000	95.5000	0.606850	0.543951	0.608613	0.542781	13	17	0.535769

Table D.15 – Mann-Whiteny U tests of guanfacine effect in SHR

Mann-Whitney U Test (GUA bef-aft in GUA OF 2010 Analysis2) By variable G/C Marked tests are significant at p

	Rank Sum - C	Rank Sum - G	U	Z	p-value	Z - adjusted	p-value	Valid N - C	Valid N - G	2*1sided - exact p
BEF Total Distance moved (cm)	75.00000	96.0000	39.00000	-0.04443	0.964565	-0.04443	0.964565	8	10	0.965401
BEF Total Duration in Inner Zone (s)	84.00000	87.0000	32.00000	0.66639	0.505161	0.66639	0.505161	8	10	0.514786
BEF Total Frequency into Inner Zone (s)	76.50000	94.5000	39.50000	0.00000	1.000000	0.00000	1.000000	8	10	0.965401
BEF Frequency of zone transitions in bin 3	89.50000	81.5000	26.50000	1.15508	0.248058	1.16535	0.243879	8	10	0.236985
AFT Total Distance moved (cm)	61.00000	110.0000	25.00000	-1.28836	0.197622	-1.28836	0.197622	8	10	0.203071
AFT Total Duration in Inner Zone (s)	56.00000	115.0000	20.00000	-1.73262	0.083164	-1.73262	0.083164	8	10	0.083139
AFT Total Frequency into Inner Zone (s)	68.50000	102.5000	32.50000	-0.62197	0.533964	-0.62325	0.533118	8	10	0.514786
AFT Total Frequency of zone transitions	68.50000	102.5000	32.50000	-0.62197	0.533964	-0.62390	0.532693	8	10	0.514786

Table D.16 – Mann-Whiteny U tests of guanfacine effect in WKY

Mann-Whitney U Test (GUA bef-aft in GUA OF 2010 Analysis2) By variable G/C Marked tests are significant at p

	Rank Sum - C	Rank Sum - G	U	Z	p-value	Z - adjusted	p-value	Valid N - C	Valid N - G	2*1sided - exact p
BEF Total Distance moved (cm)	22.00000	23.00000	2.000000	1.678293	0.093291	1.678293	0.093291	3	6	0.095238
BEF Total Duration in Inner Zone (s)	24.00000	21.00000	0.000000	2.194691	0.028187	2.203893	0.027533	3	6	0.023810
BEF Total Frequency into Inner Zone (s)	22.00000	23.00000	2.000000	1.678293	0.093291	1.692456	0.090560	3	6	0.095238
BEF Frequency of zone transitions in bin 3	18.00000	27.00000	6.000000	0.645497	0.518605	0.653720	0.513292	3	6	0.547619
AFT Total Distance moved (cm)	18.00000	27.00000	6.000000	0.645497	0.518605	0.645497	0.518605	3	6	0.547619
AFT Total Duration in Inner Zone (s)	20.00000	25.00000	4.000000	1.161895	0.245279	1.272792	0.203093	3	6	0.261905
AFT Total Frequency into Inner Zone (s)	20.00000	25.00000	4.000000	1.161895	0.245279	1.272792	0.203093	3	6	0.261905
AFT Total Frequency of zone transitions	20.00000	25.00000	4.000000	1.161895	0.245279	1.272792	0.203093	3	6	0.261905

D.2.3 Strain comparison of open field test activities

Table D.17 – Strain comparison of open field test activities before running wheel placement

Multiple Comparisons p values (2-tailed); BEF Total Distance moved (cm) (GUA bef-aft in GUA OF 2010 Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =9.045161 p =.0109

	SD - R:25.333	SHR - R:17.056	WKY - R:9.1111
SD		0.394800	0.017125
SHR	0.394800		0.081214
WKY	0.017125	0.081214	

Multiple Comparisons p values (2-tailed); BEF Total Duration in Inner Zone (s) (GUA bef-aft in GUA OF 2010 Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =10.77157 p =.0046

	SD - R:18.667	SHR - R:19.000	WKY - R:7.4444
SD		1.000000	0.167575
SHR	1.000000		0.003910
WKY	0.167575	0.003910	

Multiple Comparisons p values (2-tailed); BEF Total Frequency into Inner Zone (s) (GUA bef-aft in GUA OF 2010 Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =17.12613 p =.0002

	SD - R:15.333	SHR - R:20.472	WKY - R:5.6111
SD		1.000000	0.292828
SHR	1.000000		0.000106
WKY	0.292828	0.000106	

Multiple Comparisons p values (2-tailed); BEF Total Frequency of zone transitions (GUA bef-aft in GUA OF 2010 Analysis) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =17.12232 p =.0002

	SD - R:15.333	SHR - R:20.472	WKY - R:5.6111
SD		1.000000	0.292828
SHR	1.000000		0.000106
WKY	0.292828	0.000106	

Table D.18 – Strain comparison of open field test activities after running wheel placement

Multiple Comparisons p values (2-tailed); AFT Total Distance moved (cm) (GUA bef-aft in GUA OF 2010 Analysis2)
Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =9.292473 p =.0096

	SD - R:29.000	SHR - R:15.444	WKY - R:11.111
SD		0.040626	0.006910
SHR	0.040626		0.683775
WKY	0.006910	0.683775	

Multiple Comparisons p values (2-tailed); AFT Total Duration in Inner Zone (s) (GUA bef-aft in GUA OF 2010 Analysis2)
Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =17.86227 p =.0001

	SD - R:29.000	SHR - R:17.778	WKY - R:6.4444
SD		0.122813	0.000364
SHR	0.122813		0.004841
WKY	0.000364	0.004841	

Multiple Comparisons p values (2-tailed); AFT Total Frequency into Inner Zone (s) (GUA bef-aft in GUA OF 2010 Analysis2) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =19.08518 p =.0001

	SD - R:28.000	SHR - R:18.306	WKY - R:5.7222
SD		0.232252	0.000441
SHR	0.232252		0.001389
WKY	0.000441	0.001389	

Multiple Comparisons p values (2-tailed); AFT Total Frequency of zone transitions (GUA bef-aft in GUA OF 2010 Analysis2) Independent (grouping) variable: Strain Kruskal-Wallis test: H (2, N= 30) =18.92668 p =.0001

	SD - R:28.000	SHR - R:18.278	WKY - R:5.7778
SD		0.229716	0.000459
SHR	0.229716		0.001515
WKY	0.000459	0.001515	

D.2.3.1 Effects of running wheel placement in SHR

Table D.19 – SHRC Total Distance covered in open field

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Distance moved (cm) & AFT Total Distance moved (cm)	8	0.00	2.520504	0.011719

Table D.20 – SHRG Total Distance covered in open field

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Distance moved (cm) & AFT Total Distance moved (cm)	10	1.000000	2.701130	0.006911

D.2.3.1.1 Total distance covered in the open field

Table D.21 – SHRC Total duration in Inner Zone

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Duration in Inner Zone (s) & AFT Total Duration in Inner Zone (s)	8	0.00	2.520504	0.011719

Table D.22 – SHRG Total duration in Inner Zone

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Duration in Inner Zone (s) & AFT Total Duration in Inner Zone (s)	10	25.00000	0.254824	0.798860

D.2.3.1.2 Total duration spent in Inner Zone

Table D.23 – SHRC Total frequency into Inner Zone

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Frequency into Inner Zone (s) & AFT Total Frequency into Inner Zone (s)	8	0.00	2.520504	0.011719

Table D.24 – SHRG Total frequency into Inner Zone

Wilcoxon Matched Pairs Test (GUA bef-aft in GUA OF 2010 Analysis2) Marked tests are significant at p				
	Valid - N	T	Z	p-value
BEF Total Frequency into Inner Zone (s) & AFT Total Frequency into Inner Zone (s)	10	9.000000	1.885695	0.059337

D.2.3.1.3 Total frequency of entry into Inner Zone