ORAL AMPHETAMINE ADMINISTRATION'S EFFECTS ON PREFRONTAL MONOAMINES AND THE BEHAVIOURAL COMORBIDITIES OF TEMPORAL LOBE EPILEPSY

A POTENTIAL NEW MODEL FOR ATTENTION-DEFICIT HYPERACTIVITY DISORDER

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Abstract

One of the most common syndromes involving attention disruption is Attention-Deficit Hyperactivity Disorder (ADHD), which is characterized by symptoms of inattention and/or hyperactivity with impulsivity. The prevalence of ADHD in Temporal Lobe Epilepsy (TLE) patients is approximately 5-times the general population. Interestingly, one TLE-prone rat strain (called 'Fast' rats) has been observed to have ADHD behaviours but further tests were needed to specifically asses Fast rats differences in impulsivity and attention compared to the control strain, the TLE-resistant 'Slow' rats.

Microdialysis was used to study the *in vivo* basal and changes in ADHD-implicated neurotransmitters in the right prefrontal cortex before and after oral amphetamine (AMPH) administration. At baseline, the Slow strain had more norepinephrine and dopamine than the Fast strain. Oral 5.0 mg/kg AMPH did not affect the neurotransmitters, nor the metabolites, which contrasts with published articles using injected administration. While this could indicate that the dose was too low, evidence suggests that non-PFC neurochemistry was affected.

Impulsivity was investigated with the Delay of Reward and Differential Reinforcement of Low rate responding procedures, which compared the strains' delay preference depending on the reward size and ability to withhold a response, respectively. The attentional test, the Lat Maze, looked at non-selective attention; this has been shown to be altered in other rat ADHD models. Based on these experiments and published studies, the male Fast strain showed signs consistent with ADHD models.

Oral AMPH had both positive and negative effects on behaviour, typically in a strain-independent manner. Primarily, if self-control was not necessary, attention and

impulsivity were negatively affected by the drug. Otherwise, AMPH could have a positive impact on impulsivity. Thus, the administration of the drug should be tailored to the task, but is not expected to have a global improvement in all ADHD behaviours and can be addictive.

In conclusion, the Fast strain appears to be a good ADHD model but more measures are needed to clarify which aspects of ADHD are best modelled. Additionally, the therapeutic use of ADHD drugs, like amphetamine, should be investigated in the laboratory with the oral administration method to better mimic the human condition.

I dedicate this thesis to my family, especially Mom and Dad.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

5HIAA: 5-hydroxyindoleacetic acid
5HT: Serotonin
6-OHDA: 6-Hydroxydopamine
ACSF: Artificial cerebral spinal fluid
ACTH: Adrenocorticotropic hormone
ADHD: Attention-Deficit Hyperactivity Disorder
ADHD-C: Combined ADHD subtype
ADHD-HI: Predominantly Hyperactive/Impulsive ADHD subtype
ADHD-I: Predominantly Inattentive ADHD subtype
AMPH: Amphetamine
ANOVA: Analysis of variance
AP: Anterior-posterior
CNS: Central nervous system
DA: Dopamine
DAT: Dopamine Transporter
DOPAC: 3,4-dihydroxyphenylacetic acid
DOR: Delay of reward
DRL: Differential reinforcement of low rate responding
DSM-III: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition
DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DV: Dorsal-ventral
FCN: Fixed consecutive number
GAB A: gamma-aminobutyric acid
ITI: Intertrial interval
LEH: Long Evan Hooded
MCL: Mesocorticolimbic
MHPG: 3 -Methoxy-4-hydroxyphenylglycol
ML: Medial-lateral
MPH: Methylphenidate
MRI: Magnetic Resonance Imaging
NA: Nucleus accumbens
NE: Norepinephrine
NHE: Naples High Excitability
NLE: Naples Low Excitability
NS: Nigrostriatal
NSA: Non-selective attention
PET: Positron Emission Tomography
PFC: Prefrontal cortex
SEM: Standard error of mean
SHR: Spontaneously Hypertensive Rat
TLE: Temporal lobe epilepsy
VTA: Ventral tegmental area
WKY: Wistar Kyoto

Introduction

Attention is a very complicated behaviour; it is controlled by a variety of central nervous system structures and neurotransmitters. Since many factors work together to produce attention, it is not surprising that there are many causes of poor attention. Some of the more common syndromes related to attention are Attention Deficit Hyperactivity Disorder (ADHD), primary disorder of vigilance, narcolepsy, affective disorders like depression, learning disabilities, hormonal disorders (Weinberg et al., 1997), metabolic disorders (Norris, 2000; Weinberg et al., 1997), inadequate sleep (Kohrman & Carnery, 2000; Norris, 2000; Weinberg et al., 1997), and epilepsy (Carlson, 1995; Norris, 2000; Powell et al., 1997; Semrud-Clikeman & Wical, 1999; Weinberg et al., 1997). Poor attention can be caused by other means, too: for example, medications, toxins, and neurological insults, especially in the midbrain and right cerebral hemisphere (Weinberg etal., 1997). This range of medical conditions can make diagnosis difficult. However, proper diagnosis is imperative because the ADHD treatment protocol could actually worsen the true underlying disorder, like depression (Weinberg et al., 1997).

Prevalence

Despite these many causes of attention problems, ADHD appears to be one of the more prevalent syndromes (Mannuzza & Klein, 2000; Papa et al., 2002). Of all the children referred for mental health testing, approximately half are diagnosed with ADHD (Mannuzza & Klein, 2000), and ADHD is now the most commonly diagnosed childhood disorder in North America (Norris, 2000), with a prevalence of 3 to 5% (American Psychiatric Association, 1994) but other authors indicate that it can range from 1 to 8% (Faraone & Doyle, 2001; Hess et al., 1995; Jensen et al, 1997; Kohlert & Block, 1993; Madras et al., 2002; Paule et al., 2000; Puumala et al., 1996; Rhee et al., 2001; Seeman &

Madras, 1998; Viggiano & Sadile, 2000). Some studies, though, state that the prevalence could be as low as 0.1% (Puumala et al., 1996) to as high as 10% (Faraone & Doyle, 2001),15% (Paule et al, 2000) or 20% (Barr et al., 2001a; Kohlert & Block, 1993). The high variability in the prevalence could be due to inconsistent diagnostic criteria, resulting in under- or over-diagnosis of the disorder.

Remarkably, many more boys are diagnosed with ADHD than girls, somewhere in the range of 2 to 9 times more (Andersen & Teicher, 2000; Aspide et al., 1998; Biederman et al., 1994; Bull et al., 2000; Mannuzza & Klein, 2000; Rhee et al., 2001; Silverthorn et al., 1996; Viggiano & Sadile, 2000) even though the symptoms tend to be the same (Biederman et al., 1994; Silverthorn et al., 1996) and the adult ratio may be the same (Biederman et al., 1994; Mannuzza & Klein, 2004). Reasons have been proposed but none can explain why males greatly outnumber females. As a result, the significance of this sex difference has been the subject of debate (Andersen & Teicher, 2000; Baird et al., 2000; Biederman et al., 1994; Faraone & Doyle, 2001; Hess et al., 1995; Mannuzza & Klein, 20004; Rhee et al., 2001; Sawada & Shimohama, 2000; Silverthorn et al., 1996).

ADHD

DSM-IV

Various definitions and labels have been used to describe ADHD. These include a 'deficit in moral control' (seen in 1902), Minimal Brain Dysfunction (following the pandemic of encephalitis lethargica in the mid-1920s; Shaywitz et al., 1976), problems with hyperactivity and impulsivity (since the 1950s), and the more "modern" focus on attentional problems (starting in the 1970s). This latter definition was then accepted into the DSM-III (Diagnostic and Statistical Manual, third edition) as ADD with and without Hyperactivity. The classification then went through some modifications in the next two

editions, DSM-III-R and DSM-IV (Levy, 2001). In the DSM-IV, ADHD is characterized by symptoms of inattention and/or hyperactivity with impulsivity, which cause impairments in at least 2 settings, such as school, work, home, sports, etc., that are evident before the individual is 7 years old (American Psychiatric Association, 1994).

Rather than a global attention deficit, ADHD is associated with problems in focusing on a subset of sensory information (i.e., selective attention; Carli et al., 1983; Hawk et al., 2003; Kemner et al., 2004; Nieoullon, 2002; Puumala & Sirvio, 1998; Robbins, 2002; Russell, 2002) and/or remaining vigilant in order to detect a rare event (i.e., sustained attention; Dalley et al., 2001; Nieoullon, 2002; Puumala & Sirvio, 1998; Puumala et al., 1996; Robbins, 2002; Russell, 2002). The two types of attention problems form the basis of two of the three subtypes (more below). Interestingly, genetic research supports these symptom-based subdivisions (Barr et al., 2001a; Levy et al., 2001; Todd, 2000), but is subject to debate (Adriani et al, 2003; Barkley, 1997; Hay et al., 2001b; Levy, 2001). There are also perception problems (Russell, 2002) like time awareness, and colour and position discrimination (Paule et al., 2000).

ADHD Subtypes

For the Predominantly Inattentive (ADHD-I) subtype, the deficit appears to reside in selective attention (Barkley, 1997). ADHD-I typically emerges around 5-7 years of age, which is later than the Predominantly Hyperactive/Impulsive subtype (ADHD-HI), and persists much longer (Hay et al., 2001b; Levy, 2001). This subtype is associated with school performance problems and reading disabilities (Levy, 2001). Fortunately, treatment appears to decrease the severity of inattention in this group (Hay et al., 2001b).

Hyperactivity and impulsivity are generally diagnosed together, as demonstrated by the DSM-IV subtype, Predominantly Hyperactive/Impulsive (ADHD-HI). Indeed, some researchers have argued that hyperactivity is simply impulsive motor behaviour and should not be treated separately (Adriani et al., 2003). The word 'impulsivity' encompasses a range of behaviours. In general, an impulsive person will not consider alternatives and/or consequences (Adriani et al., 2003), will tend to prefer immediate and smaller rewards (Bull et al., 2000; Monterosso & Ainslie, 1999; Sonuga-Barke, 2003), have poor inhibition control (Monterosso & Ainslie, 1999) and be less responsive to feedback (Russell, 2002). These changes are suggestive of altered reinforcement processes (Bull et al., 2000; Russell, 2002; Sonuga-Barke, 2003). Their impulsive nature can also manifest as an exaggerated response when compared to other people (Adriani et al., 2003) and cognitive disorganization (Nieoullon, 2002). The inability to control their responses can be seen in tests of executive functions (Narbona-Garcia & Sanchez-Carpintero, 1999, Sonuga-Barke, 2003), including planning and preparing (e.g., Tower of Hanoi), executing and then inhibiting a response (e.g., Go/No-Go; Wisconsin Card Sorting Task, WCST), and working memory (e.g., backwards digit span) (Paule et al., 2000).

ADHD-HI symptoms start around 3-4 years of age (Hay et al., 2001b). In general, ADHD-HI, which does not have symptoms of inattention, tends to evolve into the Combined subtype (ADHD-C), which does have inattention as a symptom (Barkley, 1997; Levy, 2001). Given time, the hyperactivity/impulsivity will decrease (Barkley, 1997; Faraone & Doyle, 2001; Hay et al., 2001b; Levy, 2001). Interestingly, the reduction in hyperactivity appears to be independent of treatment. Thus, this decline

could be related to developmental changes (Hay et al., 2001b). The net result, then, is that the combined subtype has become an inattentive subtype (Barkley, 1997; Biederman et al., 1994; Faraone & Doyle, 2001). How does the "left-over" inattention compare to the ADHD-I inattention? Essentially, the attention problems for this evolving subtype seem to be with sustained attention, contrasting with selective attention problems in ADHD-I (Barkley, 1997).

Outcome

Children diagnosed with ADHD can have a range of outcomes (Hechtman, 1991; Ingram et al., 1999). As adults, they will typically fall into one of three categories: (1) 30 to 60% are symptom-free (Andersen & Teicher, 2000; Ernst et al., 1998; Hechtman, 1991; Mannuzza & Klein, 2000), (2) as many as 60% have ongoing ADHD (Biederman et al, 1994; Ernst et al., 1998; Faraone & Doyle, 2001; Ingram et al., 1999; Madras et al., 2002), including the negative social and emotional implications (Barr et al., 2001a; Faraone & Doyle, 2001; Gentschel & McLaughlin, 2000; Hechtman, 1991; International Consensus Statement on ADHD, 2002; Mannuzza & Klein, 2000), or (3) psychiatric and/or criminal behaviour (Ernst et al., 1998; Gentschel & McLaughlin, 2000; Hechtman, 1991; Mannuzza & Klein, 2000). Somewhat predictably, ADHD is considered to be the greatest risk factor for these negative outcomes (Mannuzza & Klein, 200). Furthermore, ADHD individuals are more likely to drop out of school, become pregnant as teenagers, contract a sexually transmitted disease, get into car accidents and under-perform at work (International Consensus Statement on ADHD, 2002). As a result, this disorder can be costly not only to the individual but to the general public (Barr et al., 2001a; Ernst et al., 1998; Faraone & Doyle, 2001; Mannuzza & Klein, 2000), especially since a large proportion of the population may have this disorder.

Treatment

There are two forms of treatment available to ADHD patients: medication and behavioural therapy.

Medication

The most effective ADHD drugs are those that change catecholamine (DA & NE) levels, for example, stimulants (Puumala & Sirvio, 1998; Solanto, 2000). Other drugs that target just NE or DA or neither (i.e., 5HT) have either controversial effects or are less effective; some may even have detrimental effects on cognitive functioning (Solanto, 1998). Stimulant medications produce their effects by increasing catecholamine release (for example, amphetamine [Seeman & Madras, 1998]), blocking catecholamine reuptake (for example, methylphenidate [Aspide et al., 1998; Seeman & Madras, 1998; Sergeant et a1, 2003; Swanson & Volkow, 2003] and amphetamine [Seeman & Madras, 1998]) or interfering with monoamine oxidase degradation of catecholamines (Solanto, 2000). Thus, stimulant medications like methylphenidate (MPH) and amphetamine (AMPH) are typically used to treat ADHD symptoms. The more consistent drug-induced changes include improved attention, response organization (as opposed to stimulus-evaluation), retention (as opposed to acquisition), and executive functions, as well as decreased impulsivity (Malone & Swanson, 1993; Solanto, 2000) and hyperactivity. The latter result is also seen during sleep, suggesting that this is a primary effect (Seeman & Madras, 1998; Solanto, 1998, 2000). Interestingly, non-clinical populations can also show improvements with stimulants (Weinberg et al., 1997).

Behavioural

Non-drug treatments include individual and family therapy (Hay & Levy, 2001; Norris, 2000). Individual therapy is geared to teaching the ADHD patients social skills

and developing better coping strategies (Gentschel & McLaughlin, 2000; Miller, 2002; Norris, 2000). Increasing working memory through training improved not only prefrontal cortical function in ADHD and control subjects, but it also reduced the hyperactivity seen in ADHD children (Russell, 2003). Family therapy, in turn, tries to encourage a positive environment for all involved (Norris, 2000). Despite these encouraging drug-free effects, the best combination is psychosocial and pharmacological interventions (Paule et al., 2000).

Etiology

Studying the etiology of ADHD is very complicated. While genetic and phenotype studies are pointing to a genetic origin (Faraone & Doyle, 2001), these genes are not a requirement for ADHD (Swanson et al., 2000a). As a result, environmental contributions cannot be ignored (Hay et al., 2001a; Swanson et al., 2000a). These etiologies could also combine to create a neurodevelopment dysfunction (Sadile, 2000). With all of these different causes, it is not surprising that the array of different animal models seems to parallel this heterogeneity. A brief summary of these more common models is provided for two reasons: (1) they have provided most of the data for the theories, and (2) to indicate their limitations.

Genetics & the Environment

Genes have a strong influence on the development of attention problems (Gjone et al., 1996). ADHD is no exception; this disorder is highly heritable, with 30 to 95% of the cases existing within families (Aspide et al., 1998; Barr et al., 2001a; Hay et al., 2001b; Viggiano et al., 2003; Winsberg & Comings, 1999). Genes can also determine the subtype (Todd, 2000) and outcome (Andersen & Teicher, 2000; Ernst et al., 1998; Faraone, 2000). Two of the most investigated genes for DA function, DA receptor D4

(DRD4) (Baird et al., 2000; Barr, 2001; Biederman & Spencer, 2000; Ernst et al., 1998; Faraone & Doyle, 2001; Paterson et al, 1999; Seeman & Madras, 1998; Swanson et al., 1998, 2000b; Viggiano et al., 2003a; Winsberg & Comings, 1999) and DA transporter (DAT) (Baird et al, 2000; Cheon et al., 2005; Faraone & Doyle, 2001; Gainetdinov & Caron, 2001; Kirley et al., 2003; Leo et al., 2003; Marx, 1999; Swanson et al., 1998; Winsberg & Comings, 1999), have been linked to ADHD. Despite this link, their individual effects maybe small (Barr et al., 2001b; Faraone & Doyle, 2001; Marx, 1999); any complex syndrome could very likely have many different causes (Barr, 2001; Faraone, 2000; Faraone & Doyle, 2001; Krause et al., 2003; Leo et al, 2003; Swanson et al., 2000a; Winsberg & Comings, 1999; Xu et al., 2001).

There are some ADHD subjects without a genetic predisposition, thus, the environment must be contributing substantially to these cases (Faraone & Doyle, 2001). Many different prenatal, perinatal and postnatal environmental factors have been linked to an increased risk of ADHD, including toxins, stress, diet, injury, hypoxia, very low birth weight, neglect, institutional rearing and other social adversity measures (Sullivan & Brake, 2003). The timing of these insults could affect different brain structures and result in a heterogeneous phenotype (Faraone & Doyle, 2001; Jensen et al., 1997). Interestingly, non-genetic origins could result in both behavioural and cognitive symptoms (Paule et al., 2000). Conversely, a positive environment could be protective because not all subjects with the 'ADHD gene(s)' actually display the disorder. A similar result has been demonstrated in a genetic ADHD model, the SHR strain: an enriched environment prevented or minimized the neurological differences (Sadile, 2000).

Models

In theory, an animal model of ADHD should have differences within the DA and/or NE systems, little to no learning deficits, sustained hyperactivity with attention deficits and/or poor inhibitory control, response to a comparable dose of stimulant medications that is the same in control animals, and no signs of tolerance to, sensitization to or long-term effects of the drug (Sagvolden, 2000). While none of the common ADHD models appear to meet all of these qualifications, they can provide useful insights (Aspide et al., 1998).

The genetic models include the Spontaneously Hypertensive Rat (SHR) and Naples High Excitability (NHE) strains. By far the most studied genetic model is the SHR. Each has varying degrees of hyperactivity, with only the SHR strain showing consistent sustained attention impairment (Leo et al., 2003). These types of models have the advantage of studying the genetic causes, but tend to be an extreme situation. The environmental models, on the other hand, have the advantage of no genetic anomalies, but do not give any information about genetic predisposition. This other type of model is a broader category; it includes any ADHD models that do not have a concrete genetic link.

Genetic Models

SHR. The SHR strain, which first emerged in 1963 (Bull et al, 2000), was bred from Wistar Kyoto (WKY) (Sagvolden, 2000) rats selected for their high blood pressure (Bull et al., 2000; Kohlert & Block, 1993) and has become the most widely used model of hypertension (Adriani et al., 2003; Hendley, 2000). This strain, though, has also been accepted as a validated genetic animal model of ADHD (Adriani et al., 2003; Papa et al.,

2000, 2002; Russell, 2002; Sadile, 2000; Sagvolden, 2000) and it has become the most-studied strain for ADHD (Papa et al, 2000, 2002).

The biggest advantage for the SHR is that it is the only strain of rat that models all of the primary ADHD characteristics: impulsivity (Bull et al., 2000; Russell, 2000,2002,2003; Russell et al., 2000; Sagvolden, 2000), both behavioural (e.g., hyperactivity) (Adriani et al., 2003; Aspide et al., 1998; Bull et al., 2000; Hendley, 2000; Papa et al., 2002; Paule et al., 2000; Russell, 2000,2002,2003; Sagvolden, 2000) and cognitive (Sagvolden, 2000); deficits in attention (Adriani et al., 2003; Aspide et al., 1998; Bull et al., 2000; Papa et al., 2002; Russell, 2000, 2003; Russell et al., 2000; Sagvolden, 2000); a reduction in hyperactivity, following low doses of MPH and AMPH (Russell, 2003); and changes in the dopaminergic system (see below).

Aside from the fact that these rats are hypertensive, the main disadvantage of this model is that both hypertension and these ADHD behaviours are based in the brain. Hence, separating out the effects of either disorder is very difficult or not possible (Bull et al., 2000; Hendley, 2000). Another disadvantage to the SHR strain is that they have an attenuated response to MPH and AMPH compared to the WKY strain (Hendley, 2000; Russell, 2003; Russell et al, 1998, 2000; Yang et al., 2003). This could limit its application to human ADHD research because both normal and ADHD children respond similarly to MPH (Russell et al., 2000).

NHE. Starting in 1976 (Lipp et al., 1987; Viggiano et al., 2002), Sprague-Dawley rats were selectively bred together, based on their extreme rearing and corner-crossing scores in a spatial novelty test, the Lat maze (Lipp et al., 1987; Sadile et al., 1996; Viggiano et al., 2002). Eventually, the Naples high-excitability (NHE) and low-

excitability (NLE) strains emerged (Sadile et al., 1996). The NHE strain is considered to be another model of ADHD, because it is hyperactive (Aspide et al., 1998; Gonzalez-Lima & Sadile, 2000; Russell, 2003; Viggiano et al., 2003b), impulsive (Gonzalez-Lima & Sadile, 2000; Viggiano et al., 2003b), and shows attentional problems (Gonzalez-Lima & Sadile, 2000; Viggiano et al., 2003b). The biggest advantage to this strain over SHR is a normal blood pressure (Sadile et al., 1996). Unfortunately, their 24-hour activity level does not differ from control (Solanto, 1998; Viggiano & Sadile, 2000), plus there is a high degree of inbreeding and impaired reference memory (Viggiano et al., 2002).

Environment Models

ADHD-like symptoms have been induced with various lesion techniques. Some of the more common techniques are 6-OHDA (Paule et al., 2000; Russell, 2003; Sagvolden, 2000; Viggiano et al., 2003b), hippocampal X-irradiation (Gonzalez-Lima & Sadile, 2000; Sagvolden, 2000; Viggiano et al., 2003b), methylazoxymethanol acetate, glucocorticoid dexamethasone, cerebellar irradiation (Paule et al., 2000; Viggiano et al., 2003b) and anoxia (Sagvolden, 2000; Sullivan & Brake, 2003; Viggiano et al., 2003b). The first two techniques, however, result in impaired cognitive functioning (Paule et al., 2000; Russell, 2003), which limits their validity. The other models do not appear to have the same complications (Paule et al., 2000; Sullivan & Brake, 2003). Regardless, various laboratory experiments have shown that the catecholamine systems (Cirulli & Laviola, 2000), particularly the PFC DA system (Sullivan & Brake, 2003), appear to be especially sensitive to environmental manipulations (Sullivan & Brake, 2003) and could be a risk factor for ADHD (Cirulli & Laviola, 2000).

Anatomy of ADHD

A variety of human studies have been done using magnetic resonance imaging (MRI), positron emission tomography (PET) and blood oxygenation levels. These studies have shown differences between the ADHD and non-ADHD brains in terms of size, blood and nutrient supply, and activity; changes that are highly inter-correlated. Interestingly, differences were found in structures, neurotransmitters and receptors involved with executive functions, attention and reinforcement processes. Furthermore, these structures are typically rich in the neurotransmitters and receptors that are targeted by the drugs used to treat this disorder. Important to note, though, is that some of these differences are not specific to the disorder, some of the results are not consistent, and most of the studies were done on boys.

Comparative neuroanatomy studies between ADHD subjects and controls have found differences. Unfortunately, the location and degree of differences vary greatly. However, one of the most consistent findings is a decreased overall brain volume (Castellanos et al., 1996; Solanto, 1998; Zametkin et al., 2001). ADHD patients have also presented with the opposite ventricular asymmetry (Castellanos et al., 1996; Solanto, 1998). Together, these variations suggest altered neurodevelopment and pruning on a large scale. Most of the physical abnormalities within ADHD subjects, though, have been found within the right hemisphere, along with correlated changes in neuropsychological tasks typically linked to this hemisphere (Sullivan & Brake, 2003). More specific differences have been found within the frontal cortex, basal ganglia and limbic structures.

Frontal cortex

The frontal cortex is involved with the planning and monitoring of movements, vital components to the executive function (Narbona-Garcia & Sanchez-Carpintero, 1999). The right frontal cortex may be predominantly involved in general attention and motivation while more specific aspects of attention (i.e., stimulus identification) are controlled by the left frontal cortex (Oades, 1998). Within each hemisphere, the attentional systems can be divided into anterior and posterior (Aspide et al., 2000; Oades, 1998; Papa et al., 2000). The anterior system, located in the frontal cortex (Aspide et al., 2000; Oades, 1998; Papa et al., 2000), is involved in non-selective attention (NSA), which encompasses the more basic attention behaviours of scanning the environment, detecting and orienting to stimuli (Aspide et al., 2000). As a consequence, pathology isolated to the frontal cortex could account for a variety of dysfunctions found in ADHD (Papa et al., 2002).

In general, ADHD children had frontal lobes that were approximately 10% smaller than non-ADHD children (Swanson et al., 1998). Of particular importance are the differences found within the PFC/anterior frontal cortex, an area that integrates working memory and attention in order to determine the relevance of the behaviours and develop strategies to maximize reward (Cohen et al., 2002; Kolb et al., 1994). Indeed, this subdivision (Cirulli & Laviola, 2000; Narbona-Garcia & Sanchez-Carpintero, 1999), especially in the right hemisphere (Arnsten, 2000; Castellanos et al., 1996; Solanto, 1998, 2000), typically had less volume than controls. Furthermore, the smaller the volume, the worse their performance is on inhibitory tasks (Solanto, 2000). Interestingly, this difference was found in both medicated and non-medicated patients (Krause et al., 2003). The PFC's contribution to the etiology of ADHD may be due to its unique connections

with subcortical monoaminergic structures. The PFC is the only cortical structure that connects with the monoaminergic nuclei (Robbins & Everitt, 1995) which, in turn, make reciprocal connections with the PFC. The monoamines produced by these nuclei, DA, NE and 5HT, have all been linked with cognitive processes and behavioural control (more below).

Additionally, ADHD has been linked with decreased metabolism (Paule et al., 2000), blood perfusion (Krause et al., 2003) and activity (Nieoullon, 2002; Rubia et al., 1999, 2000; Russell, 2003) in the frontal cortex, with some evidence isolating the (right) PFC (Paule et al., 2000; Solanto, 2000). Similar to the volume difference, decreased activity was correlated with poor performance on an inhibitory task (Paule et al., 2000; Solanto, 1998; especially in the right hemisphere [Sullivan & Brake, 2003]). Thus, an abnormality in this area, particularly the PFC, could explain the poor working memory, attention, strategy development and disinhibition typically seen in ADHD.

Basal Ganglia

The basal ganglia are involved with the coordination of movement (Purves et al., 1997; Solanto, 2000), maintaining an ability to respond to the environment (Oades, 1998), and relaying information between the executive functions and the other attentional networks (Berger & Posner, 2000). They consist of the striatum, globus pallidus, substantial nigra and subthalamic nucleus (Purves et al., 1997). In ADHD subjects, the basal ganglia may function differently (Cirulli & Laviola, 2000). This has been suggested by the fact that the basal ganglia were smaller in ADHD subjects (Paule et al., 2000), both in medicated and non-medicated individuals (Krause et al., 2003), although the side that was smaller varied (Paule et al., 2000). Additional research suggests that the

differences in this collection of nuclei may be due to the differences found in specific subnuclei: the striatum and globus pallidus. For example, ADHD subjects tend to have a smaller and under-perfused dorsal striatum (Paule et al., 2000) and globus pallidus (Narbona-Garcia & Sanchez-Carpintero, 1999), especially in the right hemisphere (Castellanos et al., 1996; Solanto, 1998, 2000; Swanson et al., 1998; Viggiano & Sadile, 2000). These alterations have been correlated with greater difficulty performing inhibitory tasks (Carey et al., 1998; Krause et al., 2003; Paule et al., 2000; Russell, 2003; Solanto, 1998, 2000)

Amygdala & the Extended Amygdala

The limbic system is composed of several key structures, one of which is the amygdala (Carlson, 1995). This structure is also anatomically- and functionally-related to a collection of nuclei called the 'extended amygdala' (Koob, 2003; Papa et al., 2000), which includes the nucleus accumbens (NA) (Koob, 2003; Mink, 2003). The amygdala sends projections to the thalamus, striatum and brainstem to develop a coordinated motor response (Kent et al., 2002). The amygdala also sends information to the ventral striatum, thereby bringing in emotion and contributing to reinforcement (Sonuga-Barke, 2003).

The NA is ideally suited for integrating information from a variety of structures: glutamate innervation comes from the PFC, thalamus, hippocampus and amygdala (Papa et al., 2002; Russell, 2000; Sadile, 2000); DA comes from the ventral tegmental area (VTA) (Mink, 2003; Papa et al., 2002; Sadile, 2000); the Raphe nucleus sends 5HT; and the hypothalamus afferents bring histamine (Papa et al., 2002; Sadile, 2000). The NA, in turn, communicates with the limbic and motor systems in order to influence a wide range

of behaviours, from integration of information, to initiating, planning and carrying out motor behaviour, to attention (Russell, 2000). This structure is typically associated with reward and addiction (Sonuga-Barke, 2003; Tanaka et al., 2004; Viggiano et al., 2003a). Indeed, in ADHD research, evidence is pointing to a dysfunction in the NA system that may be causing the differences in the reinforcement mechanisms between ADHD and non-ADHD subjects (de Villiers et al., 1995). Interestingly, the SHR strain may have a network dysfunction within the "extended amygdala." Thus, this collection of nuclei could account for a proportion of ADHD patients with emotional problems (Papa et al., 2000).

Neurochemical Theories of ADHD

Neurochemical dysfunctions have been proposed for ADHD. By far, the greatest amount of research has focused on DA. Neither NE nor 5HT should be ignored, though. The main reasons for this are that both can contribute to the pathologies and behaviours to some extent. However, the fact that these neurotransmitter systems interact indicates that the underlying problem(s) may have very dynamic mechanisms.

DA plays a significant role in memory processes (e.g., working memory, procedural learning, and conditioning), especially through the interconnection of two brain regions: the striatum and the PFC (Olvera-Cortes et al., 2008). NE is an inhibitory neurotransmitter (Carlson, 1995; Xu et al., 2001) typically found in structures controlling alertness levels (Carlson, 1995; Purves et al., 1997) and, with the PFC, mediates focusing attention, inhibiting behaviour and supporting short-term memory (Solanto, 2000). Serotonergic systems, including those in the PFC, have been strongly implicated in the regulation of impulsivity (Boulougouris & Tsaltas, 2008; Quist & Kennedy, 2001;

Rogers et al., 1999; Tanaka et al., 2004; Viggiano et al., 2003a) and learning (Olvera-Cortes et al., 2008).

Consequently, it may not be surprising to find that altered activity in any of these systems affects cognitive functions. Decreased activity of the mesocortical DA projection has been implicated in working memory deficits (Olvera-Cortes et al., 2008), ADHD (Boulougouris & Tsaltas, 2008) and schizophrenia (Boulougouris & Tsaltas, 2008; Jentsch et al., 1999), while too much DA could lead to perseveration (Cohen et al., 2002). Stimulation of the noradrenergic systems (Solanto, 1998; Viggiano et al., 2003a; Xu et al., 2001) or depleting NE in the PFC (Arnsten, 2000; Barr et al., 2001b) results in high distractibility, high exploration and poor habituation. Serotonin imbalance has been implicated in impulsivity, but the actual correlation (i.e., inverse or positive) is a matter of debate (Quist & Kennedy, 2001). The net implication of all these patterns is that a moderate amount of each monoamine is needed to function normally.

DA theory of ADHD

ADHD DA dysfunction theories tend to fall under two categories: hypofunction (Barr et al, 2001; Cirulli & Laviola, 2000; Ernst et al., 1998; Gainetdinov & Caron, 2001; Leo et al., 2003; Russell, 2000, 2002, 2003; Sagvolden, 2000; Viggiano et al., 2003a) and hyperfunction (Gainetdinov & Caron, 2001; Papa et al., 2002; Russell, 2003; Sadile, 2000; Solanto, 1998, 2000; Viggiano et al., 2002, 2003a,b). There are reasonable arguments for either side. A compromise, of sorts, has been proposed, apart from merely stating that ADHD is linked to altered DA function. With great overlap between dopaminergic systems (e.g., mesocorticolimbic [MCL], nigrostriatal pathways [NS]), it is

very likely that the imbalance is complicated and the direction of this alteration can be different between individuals (Vaidya et al., 1998).

Many studies have found altered NS in the SHR strain (Aspide et al., 1998; Bull et al., 2000; Papa et al., 2002; Sadile, 2000; Sullivan & Brake, 2003; Viggiano et al., 2003a), potentially hypofunctioning (Adriani et al., 2003; Aspide et al., 1998; de Villiers et al., 1995; Papa et al, 2000; Paule et al., 2000; Russell, 2003; Russell et al., 1998; Sagvolden, 2000; Sullivan & Brake, 2003; Viggiano et al., 2003a). Similarly, the SHR MCL DA system is affected (Aspide et al, 1998; Bull et al., 2000; Papa et al., 2002; Sadile, 2000; Sullivan & Brake, 2003; Viggiano et al, 2003a), potentially hypofunctioning (Adriani et al, 2003; Aspide et al., 1998; de Villiers et al., 1995; Papa et al., 2000; Paule et al., 2000; Russell, 2003; Russell et al., 1998; Sagvolden, 2000; Sullivan & Brake, 2003; Viggiano et al., 2003a). In contrast, the NHE NS system appears to be normal under basal conditions (Sadile et al., 1996; Viggiano & Sadile, 2000; Viggiano et al., 2003b), but not when activated (Viggiano et al., 2003b). Their MCL pathway, though, may be hyperfunctioning (Russell, 2003; Viggiano & Sadile, 2000). In fact, the PFC shows the greatest alterations during basal activity (Viggiano et al., 2003b).

Despite these model differences, the general theme is an altered NS and/or MCL pathway. The NS changes could lead to poor motor output integration (Aspide et al., 1998; Russell, 2003; Sagvolden, 2000). The MCL pathway changes, on the other hand, could lead to problems integrating attention and reward processes (Aspide et al., 1998; Papa et al., 2002; Russell, 2003; Sadile, 2000; Sagvolden, 2000). Interestingly, these are the very behaviours associated with ADHD.

NE theory of ADHD

Further paralleling DA function, debate surrounds the actual levels of NE in ADHD patients. Some evidence suggests that NE could be overactive (Arasten, 2000; Solanto, 1998; Xu et al., 2001). In support of this theory, some ADHD children have higher levels of the NE metabolite, MHPG (Barr et al., 2001b). Other studies, though, have found ADHD children with lower MHPG levels (Adriani et al., 2003; Barr et al., 2001b). In fact, low doses of stimulants will preferentially release NE (Arnsten, 2000; Franowicz et al., 2002; Groves et al., 1989; Rothman et al., 2001), indicating that NE changes could be more clinically relevant. Of the ADHD models, it appears as though the SHR strain also has a widespread noradrenergic dysfunction (Leo et al., 2003; Russell, 2002, 2003).

Serotonin theory of ADHD

The SHR model shows decreased 5HT utilization (Puumala & Sirvio, 1998). However, whether 5HT is under-active or up-regulated is a matter of debate (Quist & Kennedy, 2001). Indeed, the results are part of a controversial field investigating serotonin's role in response accuracy and impulsivity: agonists have been shown to decrease accuracy with no change in impulsivity, but antagonists have no effect on either behaviour (Puumala & Sirvio, 1998). Perhaps more selective ligands need to be tested (Barnes & Sharp, 1999; Etchepareborda, 2002; Millan et al., 2003).

Even non-drug experiments have produced conflicting results. For instance, impulsive rats had higher bilateral 5HT turnover in the frontal cortex (Koskinen et al., 2000; Puumala & Sirvio, 1998). In contrast, lesioning the ascending serotonergic pathway will shift a rat to prefer smaller immediate rewards over larger future rewards

(Tanaka et al., 2004) and increase premature responding (Koskinen et al., 2000; Muir et al., 1995; Robbins & Everitt, 1995).

*A Need **for** New **Models***

A review of ADHD research definitely has revealed a wide-range of (and often conflicting) theories, be they anatomy- or neurotransmitter-based. A number of models based on genes or environmental causes have been developed, each with advantages and disadvantages, in order to clarify one point of view or another. The main advantage for all these models is the ADHD-like behaviours, like hyperactivity, while the main disadvantage is the etiology of the attentional problems, like learning deficits, neuropathology, high blood pressure, or extreme genetic situations.

With the vast majority of ADHD cases potentially linked to genes, a genetic ADHD model could reveal more relevant information, particularly if the genetic source is not an extreme situation. Granted, even the two non-extreme genetic models, the SHR and NHE strains, indicate that there is room for improvement. Consequently, another genetic model with CNS etiology is needed. One potential model is based on epilepsy; not only are epilepsy and ADHD highly comorbid, but a genetic epilepsy model displays behaviours consistent with ADHD.

ADHD & Epilepsy

Epilepsy

Anyone can have a seizure, be it drug-, trauma- or electroshock-induced. When someone has spontaneously recurring seizures, these seizures are part of a common neurological syndrome called epilepsy. Some epilepsies, identified as symptomatic, have a known cause, such as trauma, stroke or tumour. Other epilepsies are idiopathic (i.e.,

without known causes). Despite the different causes, there are patterns that allow for the categorization of seizures based on their neurological and behavioural presentations. The first distinction is neurologically-based; a seizure episode that is isolated in one area of the brain is known as a partial seizure. On the other hand, a generalized seizure describes seizures that (a) involve the whole brain from the outset, or (b) evolve over time to engage the whole brain. The former is known as a primary generalized seizure, and the latter is a secondary generalized seizure. Whatever the neurological situation, consciousness is lost in all generalized seizures (Alloy et al., 1999).

Unlike generalized seizures, partial seizures do not involve the whole brain and consciousness is not always lost. If the person's consciousness is not affected by the seizure, it is called a simple partial seizure; otherwise, it is a complex seizure (Alloy et al., 1999). In many cases, a complex partial seizure can develop into a secondary generalized seizure. Because the majority of complex partial seizures originate in the temporal lobe, this type of seizure is often called temporal lobe epilepsy (TLE) (Alloy et al., 1999). Complex partial seizures with secondary generalization are often studied using the kindling technique (Mclntyre & Kelly, 1998; Mclntyre & Gilby, 2008), which is described below.

Comorbidity

Despite having a chronic seizure disorder, it is the interictal symptoms (those between ictal or rhythmic seizures) that are more powerful for predicting a poor quality of life than the epilepsy features. Thus, treating the comorbid disorders should be important in order to improve these patients' quality of life (Johnson et al., 2004).

Indeed, epileptic foci in the limbic system were linked to an increased risk for psychiatric disorders (Hermann & Blumer, 1996).

Typical comorbid conditions include problems with attention, impulsivity, aggression, anxiety, depression, cognitive functioning and learning (Anisman et al, 2000; Carlson, 1995; Devinsky & Vazquez, 1993; Hermann & Blumer, 1996; Kalynchuk et al., 1999; Mclntyre & Anisman, 2000; Mohapel & Mclntyre, 1998; Powell et al., 1997; Semrud-Clikeman & Wical, 1999; Trimble & van Elst, 1999; Weinberg et al., 1997). As suggested by this collection of behaviours, epilepsy and ADHD are highly correlated: approximately 1 in 5 ADHD patients can have this seizure disorder, whereas this ratio drops to 1 in 50 (Anisman & Mclntyre, 2002; Mclntyre et al, 2002b) or even 250 (Sato et al., 1990) within the general population. Fortunately for patients with ADHD and epilepsy, MPH can be used to treat the ADHD symptoms, as long as their seizures are under control (Gross-Tsur et al., 1997; Semrud-Clikeman & Wical, 1999; Weinberg et al., 1997).

While the psychological impact of epilepsy could underlie most of the comorbidities, there is evidence that biological mechanisms may contribute. Firstly, the location of the focus correlates with the comorbid disorder: depression is more common in left temporal lobe epilepsy, whereas right temporal lobe epilepsy is correlated with impulsivity and, possibly, aggression (Mclntyre & Anisman, 2000). Secondly, one disorder can predispose the individual to the other. For example, the pre-kindling anxiety levels can predict the animal's kindling rate: higher anxiety, slower kindling rate. Kindling itself, though, can trigger anxiety behaviour (Mohapel & Mclntyre, 1998) and increase the emotionality of the rats (Kalynchuk et al., 1999). Thirdly, rats that are

genetically prone or resistant to seizures tend to display these same comorbid disorders (Anisman et al., 2000; Anisman & Mclntyre, 2002; Mclntyre & Anisman, 2000; Mohapel & Mclntyre, 1998).

Studying Epilepsy and its Comorbid Disorders

Kindling

Often kindling is used as a model of TLE with secondary generalization, one that does not involve chemical confounds for its induction. Further, the changes associated with kindling are permanent (Goddard et al., 1969; Mclntyre et al., 1982). Through daily low intensity stimulation of a discrete forebrain site, complex electrographic and behavioural seizures progressively develop in response to daily stimulations, and eventually a generalized convulsion is triggered (Goddard et al., 1969). These generalized kindled convulsions are also known as stage 5 seizures, according to the following seizure classification scale: (1) eye closure and chewing, (2) head bobbing, (3) forelimb clonus (without rearing), (4) forelimb clonus (with rearing), and (5) generalized clonus with rearing and falling (Racine, 1972). Once the animal has experienced six stage-5 seizures following kindling stimulation, it is considered 'kindled' and has become permanently epileptic-prone. With continued stimulations, ultimately the animal will develop *spontaneous* seizures, i.e., those no longer provoked by the experimenter (Mclntyre et al., 1991).

Fast and Slow strains

Each subject in the kindling paradigm requires a certain number of stimulations to develop their first stage-5 seizure, also known as the kindling rate. Within all rat strains, including the Long Evan Hooded (LEH) and Wistar populations, it has been determined that there is strain-associated variability observed in the amygdala kindling rate. Using

this information, selective breeding was imposed on a hybridized parent population of the LEH and Wistar strains; those rats requiring relatively few stimulations to kindle the amygdala were bred together, as were those needing many stimulations. Eventually, the Fast and Slow strains were developed, so named because the subjects' amygdala kindled with a low and high number of stimulations, respectively (Mclntyre et al., 1999; Racine et al. 1999; Steingart, 1983). Apart from kindling rates, these strains also differ at various biological levels and in several behavioural tests.

Behavioural Differences

When breeding an animal for a certain trait, other traits may be emphasized or diminished. This occurs because a gene (or set of genes) may control more than one phenotype (i.e., pleiotrophy). Such is the case with the Fast and Slow strains; they were developed based on their rate of amygdala kindling, however, other comorbid amygdala traits were incidentally selected, including anxiety, impulsivity, hyperactivity, and altered learning and attention.

Anxiety

With respect to fear, the strains differed in their behavioural reactivity and amine changes, following specific stressors. Essentially, the Slow strain is more anxious (Anisman & Mclntyre, 2002) because it mostly adopts an immobile posture and makes more vocalizations during distress (Mclntyre & Anisman, 2000). The Slow strain is also consistently more fearful on the following behavioural tests: elevated plus-maze, open-field, inhibitory avoidance task, and one-way active avoidance task (Mohapel & Mclntyre, 1998). The behavioural profile, though, becomes more complicated when they are exposed to a startle stimulus, ferret or restraint.

The Fast strain has a greater fear-potentiated startle than the Slow strain. The opposite behavioural response is seen with an acoustic startling stimulus. Prepulse inhibition is similar, though, which suggests a similar ability to attend to the stimulus. With the acoustic stimulus considered to be a model of generalized anxiety and fear-potentiated startle associated with more stimulus-specific anxiety, the Slow strain could have a more generalized anxiety profile than Fast rats (Anisman et al., 2000).

While restrained, the Slow strain was immobile but the Fast strain moved about a lot. Exposure to a ferret will have the opposite effect (Anisman et al., 2000; Mclntyre et al., 1999b; Mclntyre & Anisman, 2000). Both stressors were associated with an increase of ACTH in the blood of the Slow rats. In contrast, the Fast strain had more ACTH from being restrained (Mclntyre & Anisman, 2000). The more vigorous responding in the different stressor scenarios was correlated with higher plasma corticosterone levels (Mclntyre et al., 1999b; Mclntyre & Anisman, 2000).

Impulsivity & Hyperactivity

The Fast strain is hyperactive, has poor response inhibition (Anisman & Mclntyre, 2002) and is sexually impulsive (Anisman & Mclntyre, 2002; Mclntyre & Anisman, 2000; Shin et al., 2002). Impulsivity, though, is a complex collection of behaviours; more tests are needed to understand the contributing behaviours.

Learning and Attention

Kindling and learning occur best in the same structures; the former is accomplished to the detriment of the latter, though (Mclntyre et al., 2002b). A similar effect is seen in rats that are not kindled, but are genetically predisposed to epilepsy. From cognitive functioning assessments, the Fast strain shows poor habituation in an

open field (Anisman & Mclntyre, 2002; Mclntyre & Anisman, 2000); poor working memory in the T-maze, especially when the time between trials was lengthened (Mclntyre & Anisman, 2000); and impaired working memory, long-term memory, and associative skills which led to poor performance on trials within one day, poor performance on the first trial on successive days, and improper use of cues, respectively, in the Morris Water Maze (Anisman & Mclntyre, 2002; Mclntyre & Anisman, 2000). Furthermore, when the platform was moved, the Fast rats persisted in searching in the previous position (e.g., perseverated) and then started to search randomly. The Slow strain, in contrast, adopted a new search strategy after they realized the platform was not in its previous spot (Anisman & Mclntyre, 2002).

As a result, it appears as though the Fast strain has altered memory and associated skills, which is also reminiscent of ADHD. Granted, their observed impulsive behaviour could be affecting their working memory. For example, the Fast rats typically chose the T-maze arm they were facing, rather than a 'thoughtful' choice (Mclntyre & Anisman, 2000). Alternatively, evidence suggests that attentional problems (Anisman & Mclntyre, 2002) could be contributing to and/or causing the learning/memory deficits. Similar to impulsivity, more tests are needed to more fully understand the underlying collection of behaviours.

Biological Differences

At the network level, the piriform cortex, perirhinal cortex and hippocampus show greater excitability (Mclntyre et al., 1999a; Anisman & Mclntyre, 2002), while there is reduced white matter in the temporal lobe and increased ventricle size (Gilby et al., 2002, 2007) in the Fast strain. At the neuron level, there are differences in

neurotrophic release during a seizure, granule cell sprouting in the hippocampus (Elmer et al, 1997; Anisman et al., 2000; Anisman & Mclntyre, 2002), hippocampal size (Gilby et al., 2002; Gilby & Mclntyre, 2002) and $GABA_A$ subunit expression (Poulter et al., 1999). These strains also differ in certain neurotransmitters, namely DA, NE, 5HT and GABA, which are discussed below.

DA

While there does not appear to be an overall change in the dopaminergic system (Mclntyre et al., 2002b), there are some isolated differences, both at baseline and following stressor exposure. At baseline, the Fast strain had more basal DA in the PFC (Anisman et al., 2000). However, the amygdala did not differ between strains (Shin et al., 2004). The PFC, though, reveals some differences between strains: exposure to a conditioned stimulus, ferret or restraint resulted in greater DA utilization in the PFC of the Slow strain, but no changes were evident in the Fast strain (Anisman et al., 2000; Mclntyre et al., 1999b). Thus, the strains' prefrontal DA neurons may react differently to stimuli. Interestingly, amygdala lesions have been shown to decrease DA release in the PFC following stressor exposure (Anisman et al., 2000) and to impair reinforcement learning (Robbins & Everitt, 2003). Presumably, selecting for amygdala kindling rate could result in disturbances in PFC DA functions, including attention or impulsivity (Anisman et al., 2000).

NE

No differences in NE levels have been found in control Fast and Slow strains (Mclntyre & Anisman, 2000; Mclntyre et al., 2002b; Poulter et al., 1999; Shin et al., 2004). However, there is evidence that the Fast strain may have fewer or less efficacious anti-convulsant a2 receptors (Shin et al., 2004). Interestingly, a decrease in the number

of a2 autoreceptors in the locus coeruleus has been linked to hyperactivity, decreased vigilance and poor sustained attention (Russell, 2002). Additionally, there is evidence that the strains' noradrenergic neurons in the amygdala (Anisman et al., 2000; Mclntyre & Anisman, 2000; Mclntyre et al., 1999b) and PFC may react differently to stimuli (Mclntyre et al., 1999b).

5HT

No differences have been found in control Fast and Slow 5HT levels (Mclntyre et al., 2002b; Shin et al., 2004). Furthermore, in general, it appears as though both strains may have similarly functioning serotonergic systems, with a potential for differences. More tests are needed.

GABA

The Fast and Slow strains' amygdala did not differ in baseline extracellular GABA levels (Shin et al., 2002, 2004). The strains, however, do differ in $GABA_A$ receptors and their sensitivity to GABA drugs. The Fast strain is more sensitive to GABA antagonists (bicuculline and picrotoxin), whereas the Slow strain is more responsive to GABA agonists (pentobarbital and diazepam) (Mclntyre et al., 2002b). Further distinguishing the strains, and the potential source for the kindling and pharmacological differences, the $GABA_A$ a subunits are expressed differently in the amygdala, as well as the piriform and perirhinal cortices; no differences were found in the dorsal hippocampus. The Fast strain shows a high concentration of subunits (a2, a3, and a5) typically found in the embryo and juvenile, but they underexpress the adult subunit, a1. The opposite pattern was evident in the Slow strain. This over-expression of embryonic/juvenile subunits provides some insight into the greater epileptogenecity

seen in the Fast strain. When activated, the embryonic subunits remain open much longer than the adult form (Mclntyre & Anisman, 2000; Poulter et al., 1999). Consequently, there is a greater opportunity for the circuitry to become synchronized, a synchronization that is crucial to developing a seizure (Mclntyre et al., 1999a, 2002a).

Additionally, the altered GABA function could contribute to altered DA and NE systems. GABA, within the NA and locus coeruleus, can modulate the release of DA (Rahman & McBride, 2002) and NE (Jones, 1991), respectively. Also, GABA-ergic neurons are the major output of the striatum (Viggiano et al., 2003a). One target of these projections is the VTA, to inhibit further release of DA (Rahman & McBride, 2002). Thus, the differences between these strains could be due to, or exacerbated by, poor GABA control of activity. This, in turn, could cascade into altered functions associated with these structures, namely the NA and VTA reward mechanisms, the striatum and inhibitory control, and their connections with the PFC and its function, executive control.

Glutamate, Aspartate and Acetylcholine

No differences have been found in glutamatergic systems (Mclntyre et al., 2002b) between control Fast and Slow strains, because they appear to have similar baseline NMDA and AMPA receptor binding in the temporal lobe structure (Mclntyre & Anisman, 2000; Poulter et al, 1999) and extracellular glutamate levels (Shin et al., 2002, 2004). Nor do the strains differ in baseline extracellular aspartate levels (Shin et al., 2004). Similarly, no differences have been found in the cholinergic systems (Mclntyre et al., 2002b), since they have similar baseline number of cholinergic neurons in the basal forebrain (Mclntyre & Anisman, 2000; Poulter et al., 1999).

Fast Strain: A new model for ADHD?

In summary, it is the seizure-prone Fast rats that are impulsive, hyperactive, sensitive to distractions and show poor habituation, symptoms that are reminiscent of ADHD (Mclntyre et al., 2002b). Furthermore, aside from the GABA pharmacology, the greatest differences between the strains are found in DA and NE, particularly in the PFC. Combining the data from Anisman et al. (2000), Mclntyre & Anisman (2000) and Mclntyre et al. (1999b), it appears as though the Fast strain's dopaminergic neurons in the PFC may be underactive, relative to the Slow strain. In addition, the noradrenergic neurons in the PFC (particularly in the right hemisphere) and amygdala appear to be hypoactive in this strain, possibly due to defective a2 NE receptors. Alternatively, the Slow strain's NE neurons may be overactive in these same structures. Additionally, the altered GABA function could contribute to the altered DA and NE levels.

Importantly, the selective breeding resulted in genotypes that differed by more than one gene, which contrasts with transgenic mice. While transgenic mice make it relatively easier to track the function of a gene, a disorder is rarely triggered by one gene. Indeed, the Fast and Slow rats show divergent and convergent gene expressions between and within the strains, respectively (Mclntyre et al., 1999a). Thus, these rats could provide further insight into the genetic sequences that cause seizures as well as comorbid disorders like ADHD, which may help identify potential therapeutic targets (Mclntyre et al., 2002b).

Purpose of the Thesis Experiments

With the need for alternative ADHD models, and a correlation between ADHD and epilepsy, the Fast strain has presented a unique opportunity to study this disorder. The potentially severe personal, social and economic implications of ADHD make this

investigation relevant to many people. As a result, tests were needed to assess strain differences in impulsivity (beyond their sexual impulsiveness) and attention.

Impulsivity, a core feature of ADHD, is a very complicated behaviour; quantifying it has also proven to be challenging. Since the goal of research is to measure behaviour, two useful definitions (more below) have emerged that are generally independent, but are not mutually exclusive; different tests were performed in order to address both definitions. These tests, though, require motivation through food restriction. Consequently, a motivation-independent test was needed in order to determine the extent of their attentional deficits. And finally, the drug manipulations of the relevant neurotransmitters (e.g., DA, NE, 5HT) were performed, and were geared to test all ADHD behaviours with the ADHD medication, AMPH. Importantly, this drug was administered in a manner similar to humans: orally.

Oral administration

For human ADHD symptom management, AMPH is administered orally at low doses. However, in ADHD research, AMPH is usually injected, an administration route that is too similar to drug addiction research. Indeed, even at doses considered to be within the therapeutic range, injected AMPH has produced drug addiction symptoms, namely stereotypy (Kuczenki & Segal, 1989, 2001; a more thorough report is available in Appendix 1). As a result, it would seem prudent to study the therapeutic application of d-AMPH by administering it orally. Unfortunately, the literature on low dose, orally administered AMPH was almost non-existent. Consequently, a study was warranted to investigate the pharmacokinetics of orally administered AMPH; these data would be used to time behavioural experiments. One such method is microdialysis, which allows for the

study of *in vivo* basal and changes in ADHD-implicated neurotransmitters (namely, norepinephrine, dopamine and serotonin) in an ADHD-implicated brain structure (namely, right prefrontal cortex) before and after oral AMPH administration.

Impulsivity Tests

As mentioned above, impulsivity has two definitions: (1) a preference for a shorter delay, even when this means a smaller reward, and (2) the inability to withhold a response. The former version was commonly tested in delay of reward (DOR) experiments. In DOR, the assumption was that impulsive behaviour will be reflected in the animal's preference for a reward size, depending on the reward delay (Monterosso & Ainslie, 1999). Here, in our case, the goal was to determine any Fast and Slow strain differences in delay preference depending on the reward size.

As for the second definition of impulsivity, differential reinforcement of low rate responding (DRL) was used. This experiment worked under the assumption that impulsive behaviour was seen in how drastically premature responding reduced the total reward amount. With DRL, an animal has to withhold a response (e.g., lever press, nose poke) for a certain amount of time before receiving a reward; premature responding will result in no (or a decreased) reward and the cycle re-starting (Bull et al., 2000; Monterosso & Ainslie, 1999; Peterson et al., 2003; Wiley et al., 2000). Here, the goal was to compare impulsive responding between Fast and Slow strains, whether it was punished by withholding or reducing the reward.

Attentional Test

Selective and/or sustained attention is a behaviour that is extensively studied. However, the main disadvantages to studying this form are that it requires (1) extensive

training and (2) food- and/or water-deprivation, in order to maintain high motivation levels (Aspide et al., 1998). In contrast, non-selective attention, which is quantified by the duration of rearing episodes, can be examined under different levels of motivation, including low levels (Aspide et al., 1998, 2000; Viggiano et al., 2003a). This latter advantage is particularly relevant to ADHD research because such patients tend to show their greatest attention deficits under low motivation; SHR and NHE also show similar deficits, as demonstrated by a high frequency of rearing episodes with short duration (Aspide et al., 1998,2000).

NSA is believed to be under the control of the frontal attentional aminergic systems (e.g., MCL DA system), which provide general attention, motivation and learning, and also the hippocampus (e.g., mossy fiber system), which allows an animal to explore and 'map' a novel environment (Aspide et al., 1998, 2000). Since the Fast and Slow strains may differ in the frontal attentional systems and mossy fibers in the hippocampus (Xu et al., 2004), these strains are expected to have different NSA profiles, as measured by the Lat Maze.

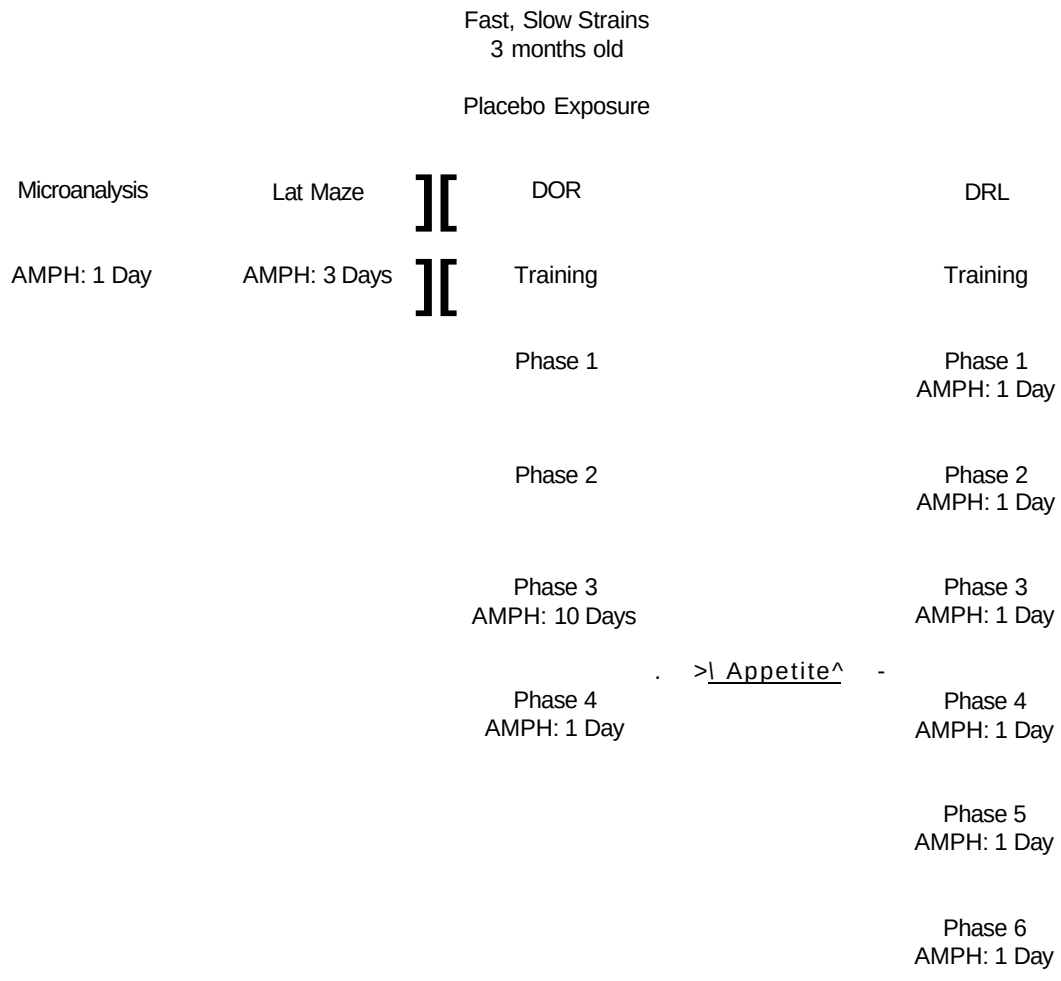
General Methods

The treatment of animals was conducted in accordance with the guidelines of the Canadian Council on Animal Care and all protocols were approved by the Carleton University Animal Care Committee.

Before the experiments started, each rat was exposed to the placebo (i.e., chocolate hazelnut spread) until consistent, rapid consumption occurred. As all of the oral AMPH administration experiments followed the same exposure/training and administration of the chocolate hazelnut treat methodology, this method was put into a separate section (see Appendix 2). After the familiarization occurred, the experiments started: Microdialysis, Lat Maze, DOR, and DRL. See Figure 1 for the flowchart of all the experiments.

In addition, it should be noted that AMPH can suppress appetite, which is a concern for the food reward-based experiments, DOR and DRL. Consequently, procedures were performed after some days' testing was finished, geared to elucidating whether there was a decrease in appetite or lack of interest in the chosen food reinforcer (chocolate pellets). Similar to the chocolate hazelnut administration methodology, these procedures were put into a separate section because they were used in multiple experiments (see Appendix 3).

Figure 1. Flowchart for the thesis experiments: Microdialysis, Lat Maze, Delay of Reward (DOR), and Differential Reinforcement of Low Rates of Responding (DRL). The number of amphetamine (AMPH) administration days is also provided, when relevant. Each experiment used the Placebo Exposure procedure, while the DOR and DRL experiments used the Appetite procedures; these procedures are presented in Appendices 2 and 3, respectively.


Fast, Slow Strains
3 months old
Placebo Exposure
Microanalysis
AMPH: 1 Day
Lat Maze
AMPH: 3 Days
DOR
Training
Phase 1
Phase 2
Phase 3
AMPH: 10 Days
Phase 4
AMPH: 1 Day
Appetite
DRL
Training
Phase 1
AMPH: 1 Day
Phase 2
AMPH: 1 Day
Phase 3
AMPH: 1 Day
Phase 4
AMPH: 1 Day
Phase 5
AMPH: 1 Day
Phase 6
AMPH: 1 Day

Experiment 1; Microdialysis

Synopsis

Microdialysis was used to study the *in vivo* basal and changes in ADHD-implicated neurotransmitters (namely, norepinephrine, dopamine and serotonin) in a brain structure (namely, the right PFC) suggested to be important to ADHD before and after oral AMPH administration in both the Fast and Slow strains. A dose of 5.0 mg/kg was administered orally, mixed in a chocolate hazelnut spread. The dose was extrapolated from human ADHD research on therapeutic blood levels of AMPH and injected AMPH pharmacokinetics in rats (see Appendix 1).

Materials and Methods

Animals

Subjects were six male Fast and six male Slow rats (Carleton University), weighing 300-400 g at the time of surgery. The rats were single-housed in standard opaque plastic cages (32 x 22 x 20 cm) and maintained on a 12-h light/dark cycle. Food and water were freely available.

Experimental Procedure

Chocolate Hazelnut Treat Consumption Training

Exposure to the chocolate hazelnut treat started at least one week before surgery. See Appendix 2 for the 'Chocolate Hazelnut Treat Consumption' methodology. Briefly, the rats were given a small amount of the treat on a dish, once a day, until they consumed it consistently and readily (within approximately 1 minute).

Surgery

The rats were anesthetised with isofluorane and Somnotol (60 mg/kg, i.p.). One cannula (Bioanalytical Systems, Inc, USA) was implanted into the right PFC using the

following coordinates, relative to bregma, on a Krieg stereotaxic: (a) Fast strain - 3.2 mm AP, 0.6 mm ML, and 3.0 mm DV; and (b) Slow strain - 3.7 mm AP, 0.6 mm ML, and 3.0 mm DV (the probe [Bioanalytical Systems, Inc, USA] would extend an additional 2 mm past cannula) (Pellegrino et al., 1979). The cannulae were sealed when not in use. After at least one week of recovery, the microdialysis experiment started.

Microdialysis

At least four hours before administering AMPH, the rat was lightly anesthetized with isofluorane, and the probe was inserted into the PFC cannula. The rat was placed in a Plexiglas box (26 x 36 x 34 cm) and the probe was attached to a peristaltic pump (MAB 20, SciPro) to pump artificial cerebral spinal fluid (ACSF) (Ringer solution: 120 mM NaCl, 3 mM KC1, 1.2 mM $CaCl_2$, 1.5 mM $MgCl_2$; pH 7.2) at a rate of 2.0 uL/min into the probe. Dialysates were collected in vials in an autosampler (820 Microsampler, Univentor), which automatically switched to the next vial every 20 minutes, and maintained the samples at 8°C. During the test, the rat was free to move around.

Three hours after probe insertion, three 20-minute baseline samples were collected. Then, four hours after probe insertion, 5.0 mg/kg AMPH (mixed in 1 g chocolate hazelnut spread) was administered (see 'Chocolate Hazelnut Treat Consumption' for detailed methodology). Dialysate samples were collected in 20-minute windows for the next hour. The vials were stored at -80°C until High-Performance Liquid Chromatography (HPLC) measurements were performed (below). The histological stage followed the end of testing.

Histological Evaluation

Under deep anesthesia, the rat was perfused intracardially with saline followed by fixation with 4% paraformaldehyde in a 100 mM phosphate buffer. The next day, the brain was removed and stored in 4% paraformaldehyde for a minimum of three days, followed by a 25% sucrose solution in a 100 mM phosphate buffer for another two days prior to sectioning. Frozen 40 urn-thick coronal slices were then mounted on slides and stained with Cresyl violet to verify dialysis probe placements.

High-Performance Liquid Chromatography

Levels (pg/40 uL) of DA, NE, 5HT, 5HIAA and DOPAC were determined using an HPLC system (Agilent Technologies, Waldbronn, Germany). Samples were injected (Agilent 1100 series Autosampler, Waldbronn, Germany) into the HPLC system equipped with a single-cell electrochemical detector (Antec Leyden Model Intro, Montreal, PQ, Canada) with an applied potential of 0.650 nA, filter of 1 s, and range of 0.1 nA/V. The separation of these analytes was achieved by their passage through an ESA, 4.6 x 150mm^2, 5 urn analytical column (Zorbax Eclipse XDB-C8, Agilent Technologies, Waldbronn, Germany). The mobile phase, consisting of 90mM sodium dihydrogen phosphate (monobasic), 1.7mM 1-octane sulfonic acid (sodium salt), 50nM EDTA, 10% (200 ml/21) acetonitrile, 50mM citric acid (monohydrate), and 5mM KC1 (final pH = 2.4), was delivered at a flow rate of 1.0 ml/min. Quantification of the various analytes was accomplished by comparing their area under the curve to those of known external standards (calibrated at 2.5 pg/50 ul, 5.0 pg/50 ul, and 50.0 pg/50 ul) using a computerized Agilent ChemStation chromatography data acquisition system (Agilent Technologies, Mississauga, Ontario).

Data Analysis

Baselines

To determine the baseline values for each rat, the hour preceding AMPH administration was averaged together. ANOVA was used to analyze the baseline concentrations of the neurotransmitters and metabolites, with Strain (2 levels: Fast, Slow) as the Between-Subjects variables.

Post-AMPH

Repeated Measures analysis was used to analyze the concentrations of the neurotransmitters and metabolites, with Strain (2 levels: Fast, Slow) as the Between-Subjects variables, and Sample (4 levels: Baseline and post-AMPH [0-20 min, 20-40 min, 40-60 min]) as the Within-Subjects variable.

Additionally, correlations were determined for each sample between all neurotransmitters and metabolites; these results are presented in Appendix 4.

Results

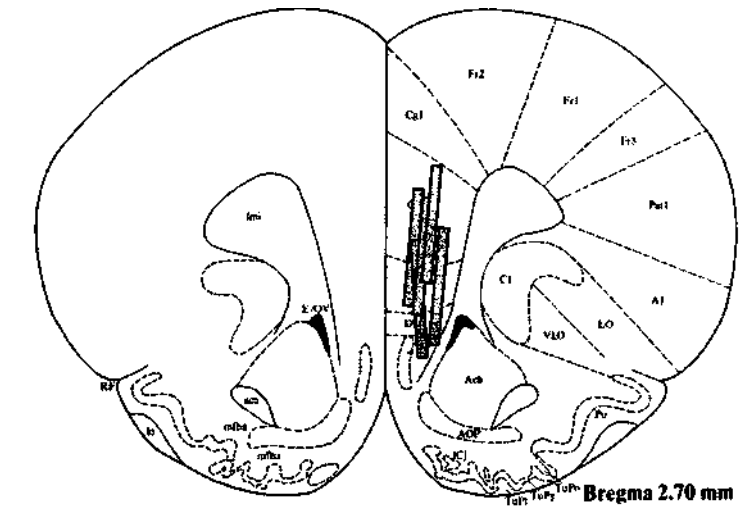
Histological results

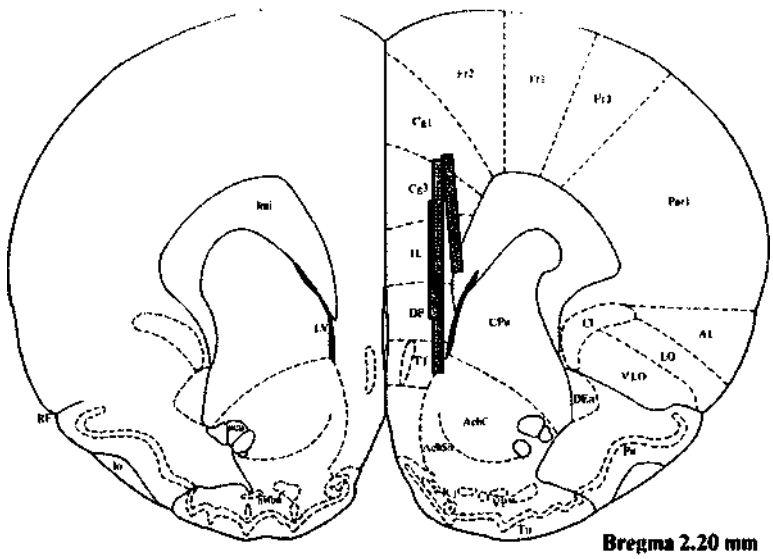
Histological evaluation confirmed microdialysis probe placements in the right PFC (Figure 2).

Baselines

The Slow strain had higher basal DA levels than the Fast strain ($F(1,9) = 6.6$, $p < .044$; one Fast outlier was removed). A trend for a similar strain difference was evident with basal NE but it approached significance ($F(1,10) = 3.9$, $p < .079$, observed power = .4). There were no strain differences in 5HT, DOPAC or 5HIAA. See Table 1.

Figure 2. Schematic diagram taken from Paxinos and Watson (1986) at bregma 2.70, 2.20 and 1.70 mm showing the microdialysis probe locations in the right prefrontal cortex.


Fr2
Fr1
Fr3
Par1
Cg1
lmi
C1
Al
LO
VLO
Acb
AOP
Pir
RF
Bregma 2.70 mm


Fr2
Fr1
Fr3
Cg1
Cg3
lmi
IL
DP
LV
CPu
Par1
G
Al
LO
VLO
RF
AcbC
Bregma 2.20 mm

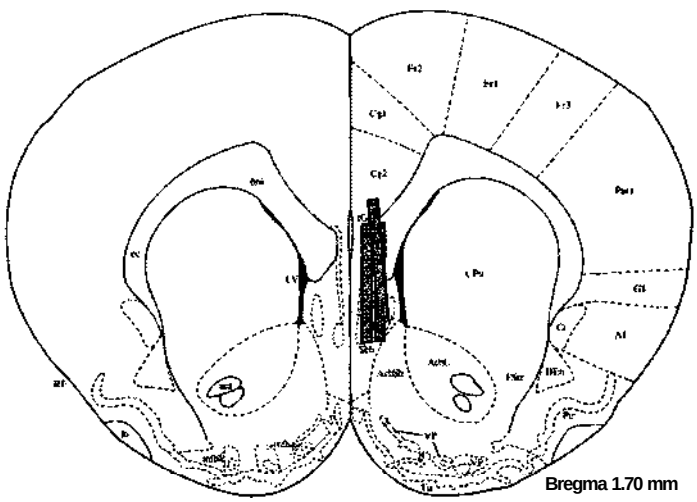

Fr2
Fr1
Fr3
Cg1
Cg2
lmi
LV
CPu
G1
G
Al
RF
Bregma 1.70 mm

Table 1. Baseline concentrations (pg/40 jal) of NE, DA, 5HT, DOPAC and 5HIAA in the Slow and Fast PFC.

Abbrev:
& - different from Slow strain
"&" - strain difference approaching significance ($p < .078$)

Neurotransmitter or Metabolite	Slow (pg/40 nl)	Fast (pg/40 nl)
NE	80.8 ± 8.3	62.8 ± 12.8"&"
DA	3.7 ±0.4	2.3 ±0.5*
5HT	21.3 ±2.5	23.9 ±3.8
DOPAC	101.0± 11.7	83.2 ±23.2
5HIAA	238.9 ± 66.5	286.9 ±83.9

Following amphetamine

Following oral amphetamine, NE had a significant Strain effect ($F(1,10) = 7.4$, $p < .023$; see Figure 3); there were no differences between samples. Additionally, there were no differences over time or between strains in DA, 5HT or the metabolites (data not shown).

Discussion

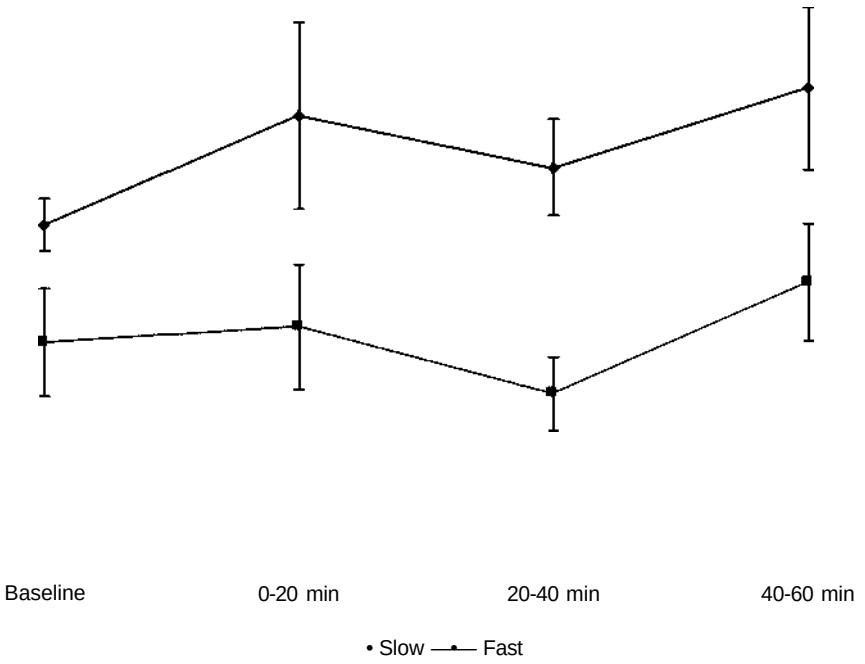
The Fast and Slow strains had different anterior-posterior coordinates for the right PFC, with the Slow strain's PFC being 5mm more rostral than the Fast strain. Previously, the Fast brain has been shown to have a smaller hippocampus, reduced white matter in the temporal lobe and increased ventricle size (Gilby et al., 2002, 2007). With the differing right PFC AP coordinates, this suggests that the frontal cortex also has altered volumes between the strains. Whether that difference is attributable to the PFC remains unclear at this point.

The strains' right prefrontal cortex differed in basal NE and DA; both neurotransmitters were higher in the Slow strain than the Fast strain. The strains' basal DA levels were similar to the PFC literature (see Appendix 5), however, but did not match post mortem results (Anisman et al., 2000). Aside from different methodologies, the reasons for the latter inconsistencies are unclear. Interestingly, decreased activity of the mesocortical DA projection has been implicated in ADHD (Boulougouris & Tsaltas, 2008) and schizophrenia (Boulougouris & Tsaltas, 2008; Jentsch et al., 1999), thereby lending weight to the Fast strain as a model for ADHD or even schizophrenia.

While the Slow strain's basal NE levels were almost significantly different from the Fast strain ($p < .078$), the moderate observed power (0.40) along with a previous

Figure 3. Average (± SEM) concentration (pg/40 |a.l) of norepinephrine during baseline and for the first hour after oral amphetamine, in 20-minute samples.

& - effect of Strain


Baseline
0-20 min
20-40 min
40-60 min
• Slow —•— Fast

report of a strain difference (Anisman et al., 2000), the post-AMPH strain differences seen in the current experiment, and the fact that the Slow strain's NE levels were higher than published studies (see Appendix 5), suggests that a larger sample size may result in a statistical significant strain effect. Indeed, when the NE baseline was analyzed together with the post-AMPH samples, the overall strain effect was significant. Additionally, the strain difference may extend beyond the PFC (see Appendix 6). The behavioural and neurological ramifications are speculative at this point. Since NE can have an anti-epileptic impact (Shin et al., 2004) and increases after stressors (Westerink , 1995), the higher concentrations in the Slow strain than both the Fast strain and published articles suggest that the Slow strain's NE levels are related to their seizure-resistant and/or anxious phenotype.

Another ADHD model, SHR, has an elevated level of basal NE in the frontal cortex (de Villiers et al., 1995), though, which directly contrasts with the Fast strain results. Granted, altered frontal NE function, in general, has been linked with ADHD, thus, this could be another manifestation of the pathology. The altered 5HT/DA interaction in the Fast strain, on the other hand, may be related to their impulsive/hyperactive phenotype since those systems are linked with those behaviours (Boulougouris & Tsaltas, 2008; Chudasama & Robbins, 2004; Dalley et al., 2002). Furthermore, it has been previously-suggested that the Fast strain's PFC DA neurons under-reacted to environmental stimuli (Anisman et al., 2000). In summary, the strains differ in NE and DA activity (and in the interaction between DA and 5HT; see Appendix 4). Thus, evidence is pointing to altered PFC monoamine circuitry and, by extension, their behavioural correlates of attention, learning and behavioural inhibition.

There were no differences in 5HT and 5HIAA, which supports other reports comparing impulsive rats to non-impulsive rats (Dalley et al., 2002; Puumala & Sirvio, 1998), but differing from another model of ADHD, SHR (de Villiers et al., 1995). Mysteriously, both strain's 5HT levels were higher than other reported values (see Appendix 5). The strains did not differ in serotonin or dopamine's metabolites, which agreed and didn't agree with a post mortem study (Anisman et al., 2000), respectively. The strains' comparison to the PFC metabolite literature, however, is unclear (see Appendix 5).

Following amphetamine

Following oral amphetamine, the only significant effect was between the strains with respect to NE levels; DA, 5HT or the metabolites showed no changes from baseline or strain effect. In the injected AMPH literature (see Appendix 5), though, there was an increase in NE, DA and DOPAC, but (potentially) no change in 5HT or 5HIAA. Thus, the experiment suggests that oral 5.0 mg/kg AMPH and injected AMPH (based on the literature) do not have the same effect on PFC catecholamines' systems, but do have a similar non-effect on PFC serotonin mechanisms.

The former comparison was unexpected. Not only are these compounds repeatedly linked with ADHD, but the most effective ADHD medications change their levels. This could suggest that the orally administration dose was too low. However, those monoamine results are from injected studies. Furthermore, in one (excluded) Fast rat with a probe in the caudate putamen, DA increased, while DOPAC had a delayed decreased, closely matching the expected profile (see Appendix 7). Additionally, the occurrence of stereotypy at this dose in the Lat Maze experiment counters that possibility.

Thus, the orally administered dose was sufficient to change non-PFC neurotransmitter and metabolite levels. Granted, this is the first study, with six rats per strain; thus, additional studies may produce a different conclusion. Regardless, any future investigations into the therapeutic effects of stimulants should consider oral administration, just like with human ADHD, in order to elucidate results more relevant to the human ADHD.

Apart from examining neurochemical differences between the strains, the other goal was to identify a time to run behavioural tests following oral administration. Unfortunately, there was a lack of significant changes from baseline. Based on the fact that many AMPH studies tested 20 minutes after injection, starting a test 20 minutes after oral administration would be suitable.

Conclusion

In conclusion, the strains differ not only in NE and DA activity but also in the interaction between DA and 5HT. Thus, evidence is pointing to altered PFC monoamine circuitry and, by extension, their behavioural correlates of attention, learning and behavioural inhibition. Orally administered 5.0 mg/kg AMPH did not affect PFC monoamines or the metabolites; rather, it affected non-PFC structure(s). This outcome differed sufficiently from published injected studies to indicate that investigating the therapeutic applications of AMPH should follow the therapeutic administration route: oral. Additionally, starting a test 20 minutes after oral administration would be reasonable.

Experiment 2: **Lat Maze**

Synopsis

The main disadvantage of most attention experiments is that they require food deprivation in order to maintain high motivation levels. With ADHD patients showing their greatest attention deficits under low motivation (Aspide et al., 1998), another test is needed to examine motivation-independent attentional deficits in the Fast strain. One such test is the Lat Maze, which provides a way to study a rat's natural tendency to rear up, scan its environment and detect stimuli (e.g., non-selective attention; NSA).

While the rat is in the 'maze', they are free to explore. The normal/expected behaviour will be rearings that increase in duration as testing progresses. Conversely, ADHD models have been shown to have a greater number of rearings of short duration. As a result, the goal was to assess how the Fast and Slow strains differ in this behavioural test. Furthermore, the ADHD drug, amphetamine (AMPH), was administered to see how it affected NSA.

Materials & Methods

Animals

Subjects were female Fast and Slow rats (Carleton University) were at least 3 months of age at the start of testing (sample sizes: (a) Slow strain - Placebo & 1.0 mg/kg p.o. = 4, 2.0 & 5.0 mg/kg p.o. = 5; (b) Fast strain - Placebo, 1.0 & 2.0 mg/kg p.o. = 4, 5.0 mg/kg p.o. = 5). At that time, the rats were placed on a restricted-quantity food diet; the goal was not to decrease weight, but to increase the rat's motivation to consume the daily treats. Thus, the ideal scenario was to find 1 g of food remaining at the start of each day's testing.

The rats were single-housed in standard opaque plastic cages (32 x 22 x 20 cm) and maintained on a 12-h light/dark cycle. On each testing day, any remaining food was removed at least 20 minutes before the chocolate hazelnut treat was administered. After the days' testing, which occurred during the light cycle, the rats were then fed between 15 to 30 g of food, depending on their weight; water was freely available. On non-testing days, food and water were freely available. The rats were weighed once a week.

Experimental Procedure

Chocolate Hazelnut Treat Consumption Training

Exposure to the chocolate hazelnut treat started at least one week before the Lat Maze procedure. See Appendix 2 for the 'Chocolate Hazelnut Treat Consumption' methodology. Briefly, the rats were given a small amount of the treat on a dish, once a day, until they consumed it consistently and readily (within approximately 1 minute).

Apparatus

The Lat maze was a 60x60x40 cm black acrylic box with 30x30x40 cm smaller glass box in the middle. The rat was able to explore the corridor between the boxes, 60x15x40 cm.

Lat Maze

Twenty minutes after consuming the Placebo (plain chocolate hazelnut spread) or oral AMPH (1.0, 2.0 or 5.0 mg/kg AMPH, mixed in with the chocolate hazelnut spread; see Appendix 2 for detailed methodology), the rat was placed in the Lat Maze. Their behaviour was video-recorded for 10 minutes. This was repeated on the two subsequent days. Before and after testing, the rats were observed for signs of taste aversion (e.g., incomplete consumption of AMPH-dosed treat) and stereotypy, respectively.

The videos were watched, off-line, for (1) number of rearings on each day and (2) duration of rearings on Days 1 and 3. With the duration of rearings data set, Day 2 was not examined due to the length of time to collect (e.g., save video to computer, convert file format, mark the start and end of each rearing) and input the rearing duration data. Essentially, it took approximately 2 hours per 10 min recording.

Analysis

The strains were examined for differences in five categories: rearing number, latency to first rearing, rearing duration, taste aversion and stereotypy. These categories were analyzed in different ways (see below), but all categories had two Between Group variables: Strain (2 levels: Slow, Fast) and Drug group (4 levels: Placebo, 1.0, 2.0, 5.0 mg/kg AMPH). The level of statistical significance was set at $p < .05$.

Latency to First rearing and Rearing Durations

Day 1 and Day 3 videos were copied onto a computer. The video files were marked with the start and end of each rearing using a multi-media program (Adobe Premiere Elements®). For the latency to first rearing, Repeated Measures analysis was used to investigate differences in the Between Group variables (as above) and the Within-Subjects variable, Day (2 levels: Day 1 vs. Day 3); latencies longer than 30 seconds were entered as 30 sec.

For rearing durations, the rearing ending marker time was subtracted from the starting marker time. This section was analyzed with the methods outlined in Aspide et al. (2000). First, the durations were rounded to the nearest quarter-sec to create the following categories: 1 sec or less, 1.25 s, 1.5 s, 1.75 s, 2 s, 4.5 s, 4.75 s, and 5 s or longer. The frequencies were then analyzed with the Kruskal Wallis nonparametric test.

With the Kruskal Wallis test, groups that have similar frequency patterns will have overlapping cumulative frequencies. However, if one group has more short or long duration rearings, then the cumulative frequency graph will appear to have shifted to the 'left' or 'right', respectively. Since differences were expected between the Strains, Drug groups and Days, pre-planned *post-hoc* tests were done with the Mann-Whitney test.

Rearing Number

The number of rearings was analyzed with the Repeated Measure design with Between Group variables (as above). The Within-Subjects variables depended on the analysis. To analyze the total number of rearings, there was one Within-Subjects variable: Day (3 levels: Day 1, Day 2, Day 3). Based on the means, the first three minutes had the most consistent and interesting differences; analysis focused on those intervals. As a result, the first three minutes had two Within-Subjects variables: Day (2 levels: Day 1, Day 3) and Minute (3 levels: Minute 1, Minute 2, Minute 3).

Taste Aversion and Stereotypy

The occurrence of taste aversion (incomplete consumption of treat) was analyzed with logistic regression. Incidentally, stereotypy happened in one group, thereby making logistic regression unnecessary.

Results

Latency to First Rearing

The latency to first rearing had a significant interaction between Day, Drug and Strain ($F(3,27) = 3.9$, $p < .020$). Further analyses found that, if there was a strain difference, the Slow strain always had a longer latency to first rearing than the Fast strain. This effect did not hold for all Drug groups nor on both Days, though. Only Day 1 had Drug effects, with the shortest latency in the 5.0 mg/kg groups. Additionally, on Day 3,

only the Slow 5.0 mg/kg group showed an increase from the first Day; in fact, all Day 3 first rearings occurred after 30 seconds. See Figure 4.

Rearing Number

Based on the means, the first three minutes had the most consistent and interesting differences; analysis focused on those intervals (see 'First Three Minutes' below). The Days' total number of rearings was also analyzed (see 'Total Rearings per Day'). Interestingly, the 1-minute interval and Days' total analyses did not always match up, indicating that the initial exposure and overall performance provide alternate ways to examine NSA.

First Three Minutes

The rearings were divided into 1-minute intervals for the first three minutes on Days 1 and 3. Repeated Measures analysis revealed a significant three-way interaction between Day, Strain and Drug ($F(3,27) = 4.4$, $p < .013$; Figure 5). The AMPH groups were different from Placebo and, within the AMPH groups, the highest dose was different from the two lowest doses. Additionally, whenever there was a Strain difference, the Fast strain had more rearings than the Slow strain.

Total rearings per Day

There was a significant three-way interaction between Day, Strain and Drug ($F(6,52) = 2.3$, $p < .046$) (Figure 6). Strain differences emerged when AMPH was 2.0 mg/kg (all three Days) and 5.0 mg/kg (Day 2 and 3). In all cases, the Fast strain had more rearings than the Slow strain. Isolated drug group differences were only found in the Fast strain, with more rearings in the 2.0 mg/kg group on Day 1 compared to Placebo and more rearings in the 5.0 mg/kg group on Day 3 compared to Placebo and 1.0 mg/kg.

Figure 4. Mean latency to first rearing in each strain's Drug group. Latencies 30 seconds or longer were entered as 30 sec.

Abbrev:
& - Strain effect
D - Day effect
P, 1,2- different from Placebo, 1.0 mg/kg, 2.0 mg/kg group, respectively

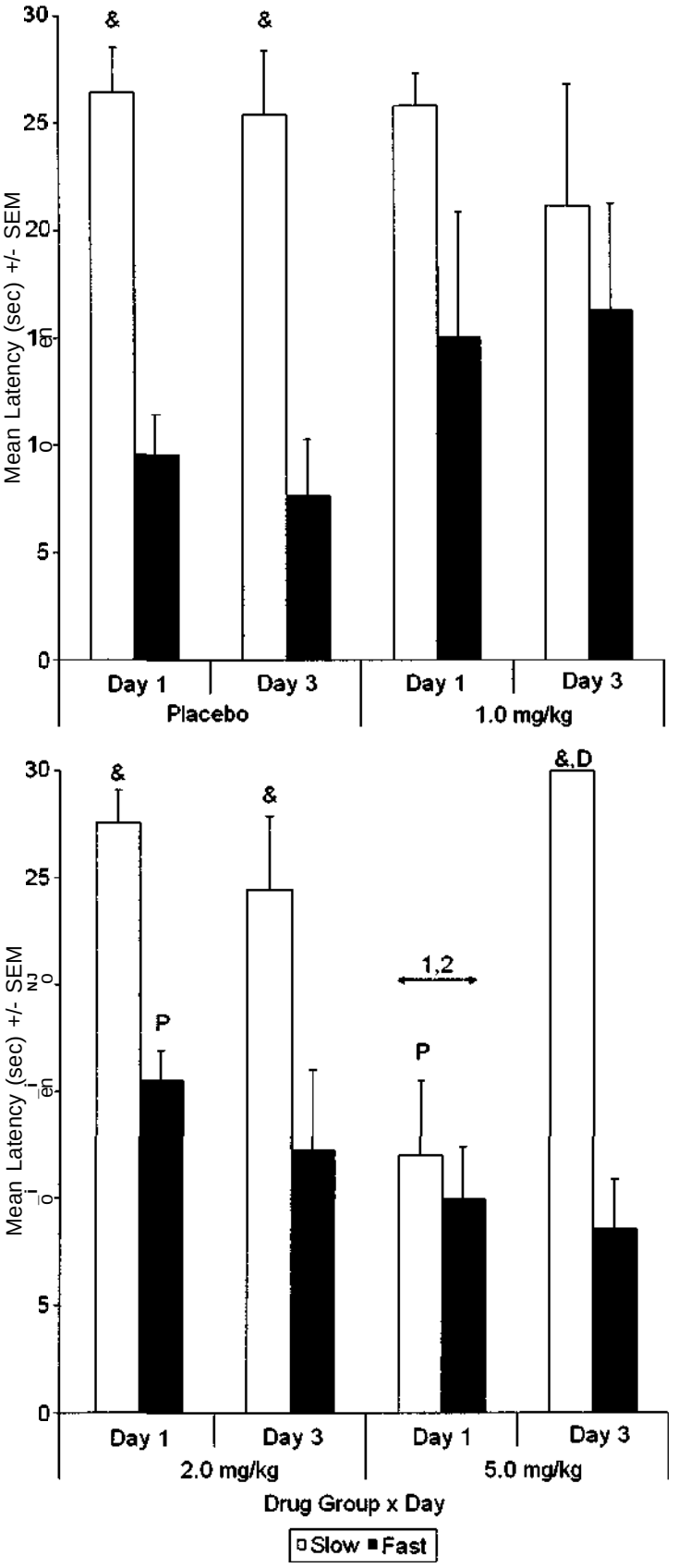
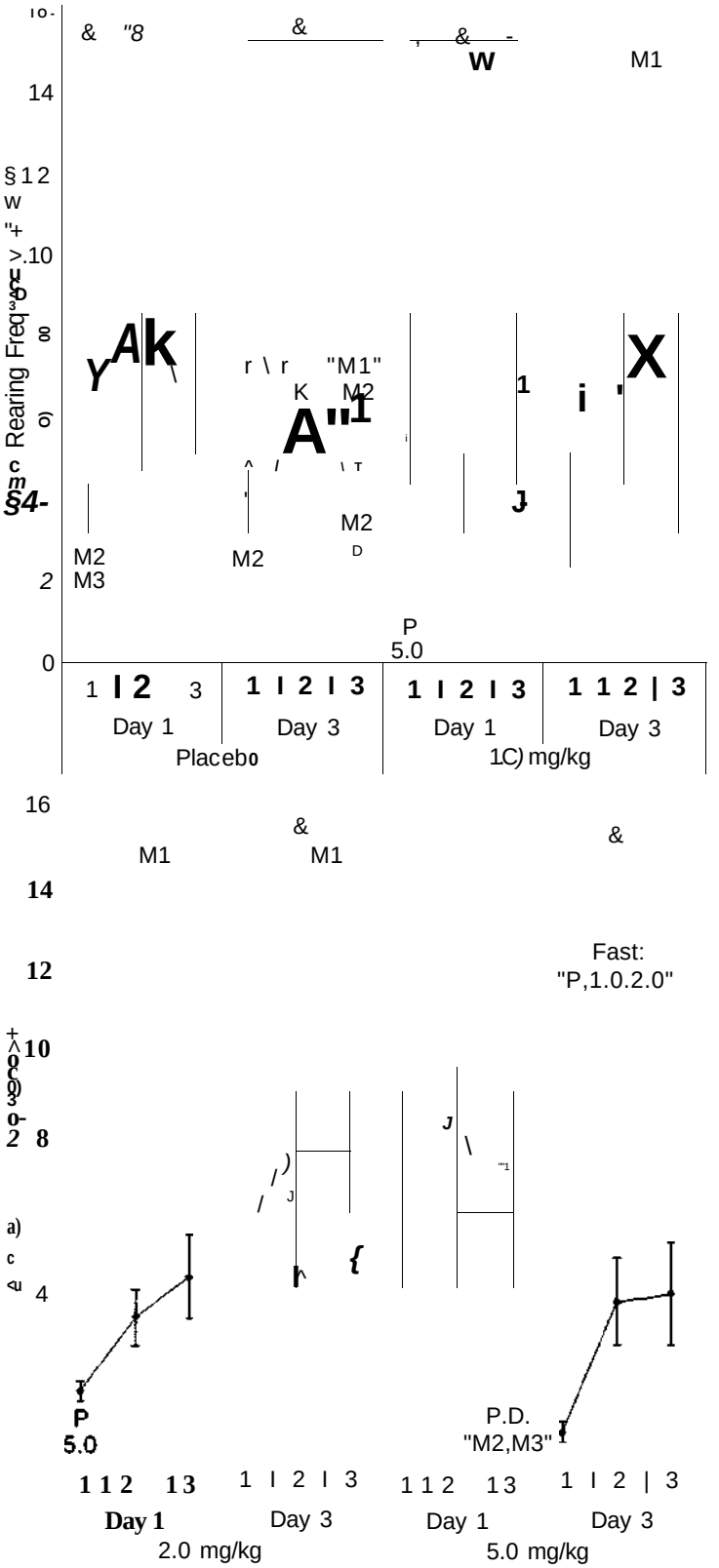

Mean Latency (sec) +/- SEM
30
25
20
15
10
5
0
&
&
Day 1
Day 3
Day 1
Day 3
Placebo
1.0 mg/kg
&
&
&,D
P
P
1,2
Day 1
Day 3
Day 1
Day 3
2.0 mg/kg
5.0 mg/kg
Drug Group x Day
Slow
Fast

Figure 5. The mean rearing frequency for each strain's drug group in the first 3 1-minute intervals, on Days 1 and 3.

Abbrev:
M# - Different from specified Minute
D - Day Effect
P, 1.0, 2.0, 5.0 - Different from Placebo, 1.0, 2.0, 5.0, respectively
& - Strain effect
" _" - Almost significant ($.061 < p < .083$)

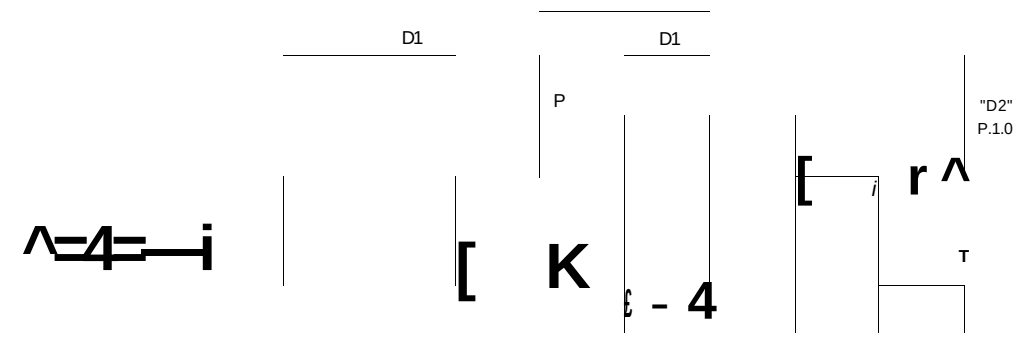


Strain x Drug x Day x Minute

a Slow • Fast

Figure 6. The mean rearing frequency for each strain's drug group on Days 1, 2 and 3.

Abbrev:
Dl - Different from Day 1
"D2" - almost significantly different from Day 2 ($p < .100$)
P, 1.0 - Different from Placebo, 1.0 mg/kg, respectively
& - Strain effect



Day 1 | Day 2 | Day 3 Day 1 | Day 2 | Day 3 Day 1 | Day 2 | Day 3 Day 1 | Day 2 | Day 3

Placebo 1.0 mg/kg 2.0 mg/kg 5.0 mg/kg

Drug Group x Day

- Slow • • Fast

Duration of Rearings

The article that provided the Lat Maze procedure (Aspide et al., 2000) used a non-parametric test to examine the frequencies of durations. If groups have similar frequency patterns, then their cumulative frequency graphs will overlap. However, if one group has more short or long duration rearings, then the cumulative frequency graph will appear to have shifted to the 'left' or 'right', respectively.

The Kruskal Wallis test was significant ($xis^2 = 196.7$, $p < lxlO''^{30}$). Mann-Whitney tests were then performed to make relevant comparisons: (1) drug groups within each strain for each day (for example, on Day 1, the Slow strain's drug groups were compared), (2) strains for each drug group for each day (for example, on Day 3, the 2.0 mg/kg dose was examined for differences between Fast and Slow), and (3) days within each strain's drug group (for example, in the Fast strain, the Placebo group's Day 1 was compared to Day 3). The cumulative percent of rearing duration frequencies are provided in Figures 7 and 8 for the Slow and Fast strains' drug groups, respectively, on Day 1 and 3; all indicated differences have absolute z-scores > 4.3, $p < .05$.

On Day 1, the Fast and Slow Placebo groups had overlapping frequencies; both groups, though, had frequency distributions significantly to the 'right' compared to the AMPH-dosed groups. In contrast, the Fast AMPH-dosed groups had a significant shift to the 'left' compared to the Slow AMPH-dosed groups.

On Day 3, the Slow and Fast 1.0 mg/kg and 2.0 mg/kg groups had a significant shift in rearing duration frequencies to the 'right' to overlap with Slow and Fast Placebo groups. However, the Slow Placebo, 1.0 mg/kg and 2.0 mg/kg groups frequency distributions were significantly to the 'right' compared to the Fast Placebo, 1.0 mg/kg

Figure 7. Cumulative percent frequency histograms of the different rearing duration categories in the Slow strain's drug groups on (a) Day 1 and (b) Day 3.

Abbrev:
D - difference between Day 1 and 3
P, 1.0, 2.0, 5.0 - different from Placebo, 1.0, 2.0, 5.0 mg/kg, respectively
& - different from Fast strain

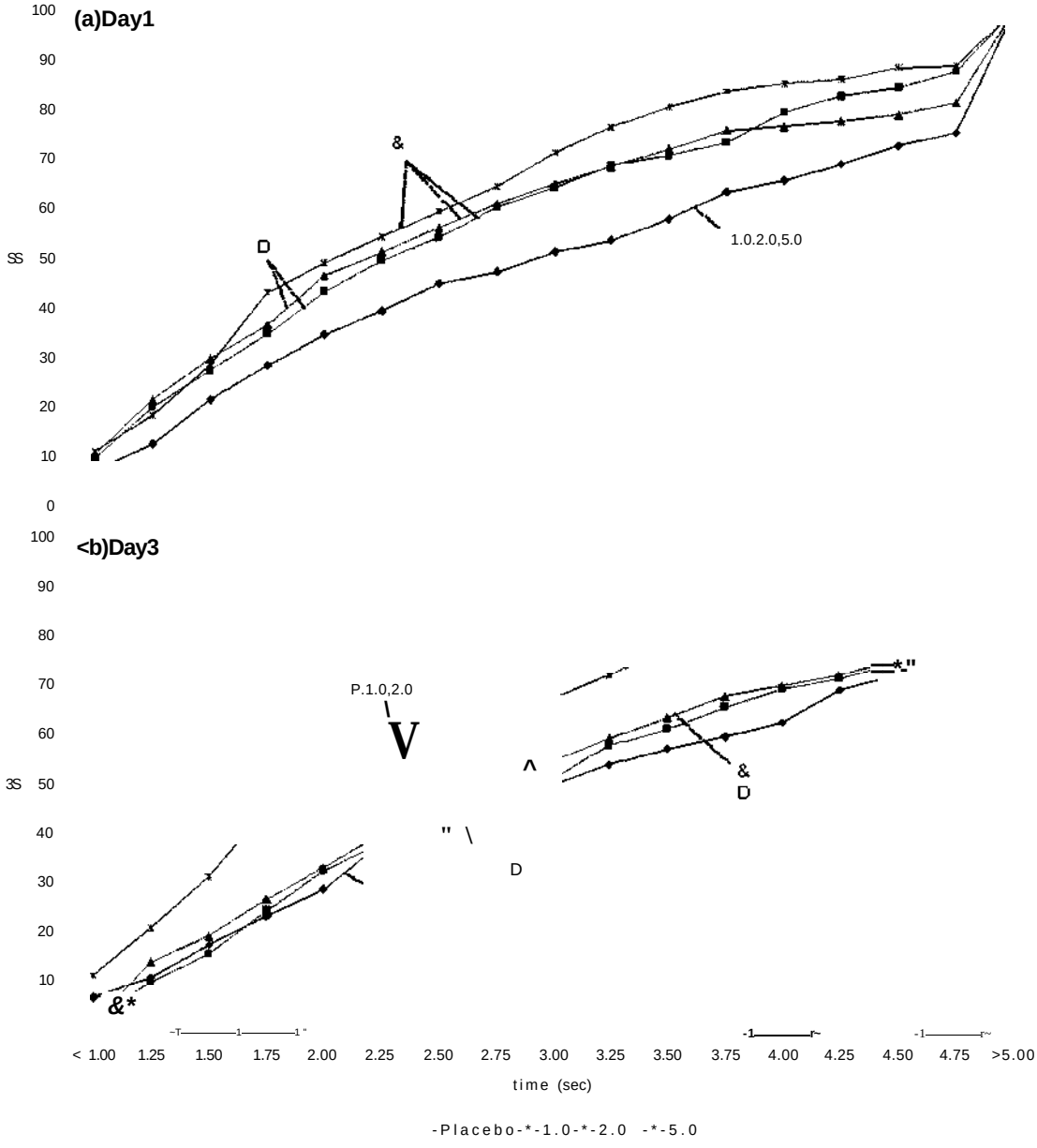
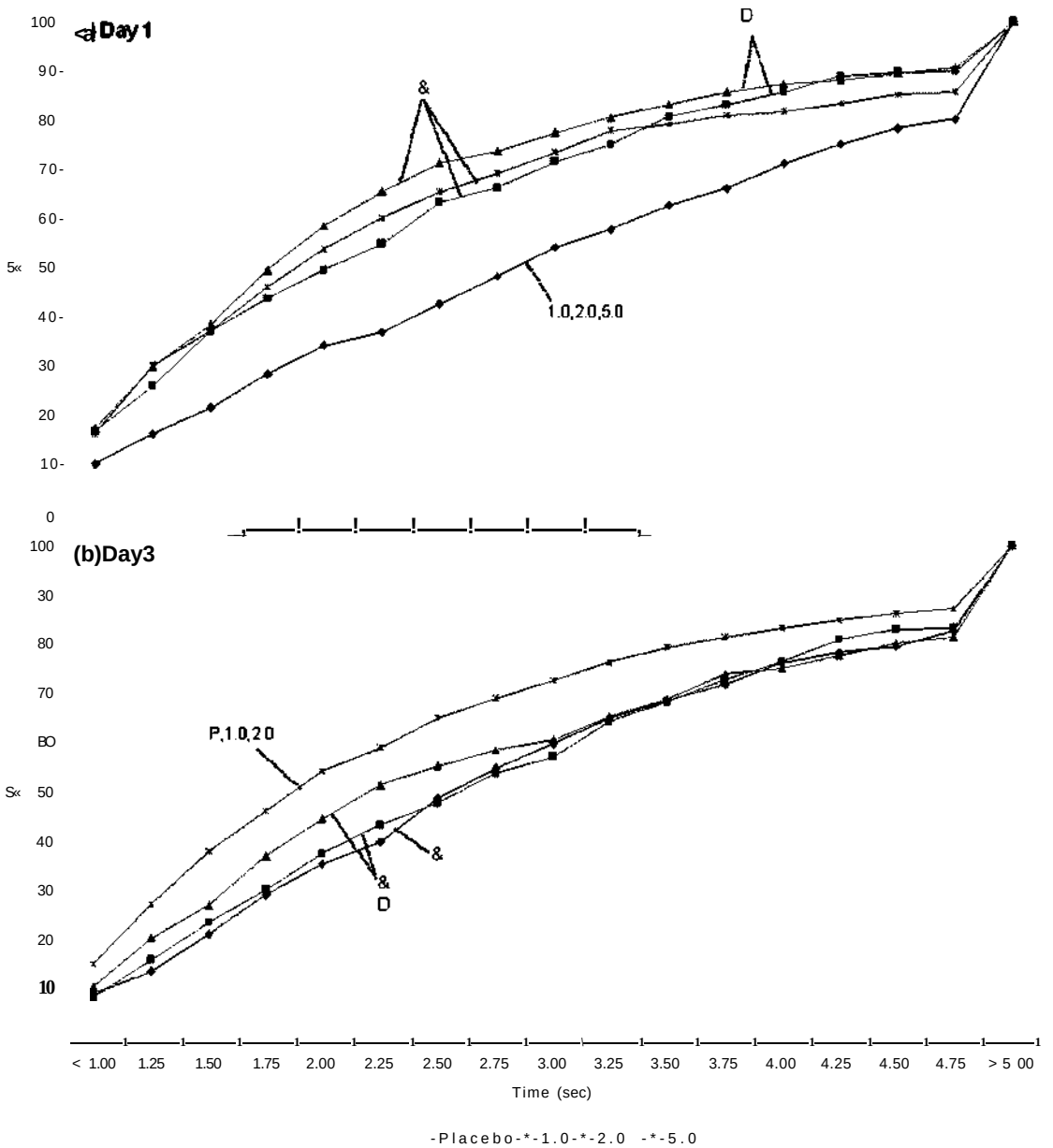

(a)Day1
100
90
80
70
60
SS 50
40
30
20
10
0
&
D
1.0.2.0,5.0
<b)Day3
3S
P.1.0,2.0
V
^
&
D
" \
D
&*
-T 1 1"
-1 r~
-1 r~
< 1.00 1.25 1.50 1.75 2.00 2.25 2.50 2.75 3.00 3.25 3.50 3.75 4.00 4.25 4.50 4.75 >5.00
time (sec)
-Placebo-*-1.0-*-2.0 -*-5.0

Figure 8. Cumulative percent frequency histograms of the different rearing duration categories in the Fast strain's drug groups on (a) Day 1 and (b) Day 3.

Abbrev:
D - difference between Day 1 and 3
P, 1.0, 2.0, 5.0 - different from Placebo 1.0, 2.0, 5.0 mg/kg, respectively
& - different from the Slow strain


(a)Day 1
100
90
80
70
60
50
40
30
20
10
0
D
&
1.0,2.0,5.0
(b)Day3
100
30
80
70
BO
50
40
30
20
10
P,1.0,2.0
&
&
D
< 1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
3.50
3.75
4.00
4.25
4.50
4.75
> 5 00
Time (sec)
-Placebo-*-1.0-*-2.0 -*-5.0

and 2.0 mg/kg groups. The highest-dosed Fast and Slow groups, though, had frequency distributions significantly to the 'left' compared to all other groups.

Taste Aversion and Stereotypy

Logistic regression was used to investigate the probability of taste aversion; this was unnecessary for stereotypy because only one group showed this behaviour. Indeed, following the test, only the Slow strain in the highest-dosed group were performing some odd behaviours repetitively (for example, smelling the side of the cage in circles, touching the cage lid then their nose), indicative of stereotypy.

As for taste aversion, the strains did not differ. However, drug dose was associated with increased probability of taste aversion ($X^3 = 15.4$, $p < .003$). None of the placebo group had any problems consuming their daily quantity of chocolate hazelnut spread. With the AMPH-dosed groups, the 1.0 and 2.0 mg/kg drug groups did not differ. However, the 5.0 mg/kg dose had a greater probability of developing taste aversion than the lower doses: 1.0 (approaching significance, $p < .075$) and 2.0 mg/kg.

Discussion

The Lat Maze distinguished the Fast and Slow rats' basal NSA, using measures of rearing number and duration. Furthermore, AMPH appeared to have dose-dependent effects on these measures, and could induce stereotypy even though the doses were low, administered orally and for a short time.

Placebo

The Fast strain had an innate tendency to rear more quickly than the Slow strain, tendencies that were not affected by repeated exposure. This shorter latency translated into more rearings during the first two minutes on the first and third days. When the

whole 10 minutes were examined together, the total number of rearings did not change over days or differ between strains. Interestingly, the strains started with the same frequency of rearing durations. By the third day, even though neither strain had a significant change over days, the Fast strain had more rearings of short duration than the Slow strain.

Consequently, the strains' initial reaction and habituation to the maze differed. With an NSA profile of more rearings of short duration indicating ADHD, the Fast strain's initially higher number of rearings and higher frequency of short rearing durations suggests that this strain has an ADHD-like NSA profile. Moreover, this alteration could be linked to an 'imbalance' in the strain's frontal attentional catecholamine systems and mossy fiber hippocampal network, supporting the microdialysis (see Experiment 1) and other published studies (e.g., Anisman et al, 2000; Xu et al., 2004).

1.0 mg/kg

The Fast strain had more rearings of shorter duration than the Slow strain and placebo after the first 1.0 mg/kg administration, indicative of an ADHD-like NSA profile. With repeated exposure, the rearing durations increased to placebo values. Importantly, though, the Fast strain's placebo on Day 3 had an ADHD-like NSA profile. Interestingly, the Slow strain also had more rearings of shorter duration than placebo, suggesting a shift to an ADHD-like NSA profile, but not as dramatically as the Fast strain. With repeated exposure, just like with the Fast strain, the NSA profile was at placebo values. Thus, subchronic 1.0 mg/kg did not improve their NSA profile, rather the negative effects decreased. Since the strains no longer differed on these rearing number measures by the

third day, except for a decrease in total number of reanngs, it appears that the strains had habituated to the environment and/or drug following subchronic administration. Granted, neither strain differed from Placebo, indicating that it is more likely that habituation to the drug occurred.

2.0 mg/kg

Just like with the 1.0 mg/kg dose, the increase in rearing number and higher frequency of short rearing durations in the Fast strain suggests that this strain, after the first 2.0 mg/kg administration, had an ADHD-like NSA profile. Similarly, the Slow strain had an ADHD-like NSA profile after acute AMPH, but not as dramatically as the Fast strain. Subchronic administration, likewise, only decreased the negative effects in both strains. The continued elevation in rearing numbers in the Fast strain, however, suggests that while the rearing durations were at placebo levels, this strain was still rearing a lot. Thus, the ADHD-like profile was more pronounced in the Fast 2.0 mg/kg group than in the 1.0 mg/kg group and/or they did not habituate to the 2.0 mg/kg dose, whereas the Slow strain had habituated to both doses.

5.0 mg/kg

Acute 5.0 mg/kg AMPH had a different effect on rearing frequency compared to the other doses, as demonstrated by the overlapping and unchanging strain means from the first day. This suggests that both strains' NSA was ADHD-like, but more pronounced in the Fast strain. Subchronic 5.0 mg/kg, on the other hand, had strain-dependent changes. For instance, in the Fast strain, there was an overall increase in rearing number that was higher than the other three groups and the Slow strain. In contrast, the Slow strain had a decrease, albeit primarily during the first minute, that was also lower than placebo. Aside from those findings, the strains had overlapping rearing duration

frequencies that, in turn, had a higher frequency of short durations than the other groups. Presumably, the increase and decrease in the Fast and Slow strains' rearing numbers reflect the stimulatory- and stereotypy-inducing aspect of AMPH, respectively. Indeed, the Slow strain, at this dose, was the sole group to portray stereotypical behaviours (more below). Furthermore, the overlapping rearing duration frequencies indicate that both strains' NSA was negatively affected.

Taste Aversion and Stereotypy

The Slow strain, at the highest dose, was the only group to show stereotypic behaviours. This suggests that the Slow strain could have a lower threshold for this hallmark of drug addiction or could be more sensitive to the addictive properties of stimulants. Aside from that, the occurrence of stereotypy was unexpected with the oral administration, because that dose was supposedly within the therapeutic range (see Appendix 1) and within the range of gavage AMPH used in Lobarinas & Falk (1999) that did not trigger stereotypy. One study, however, did have stereotypy, but that was after 9 days of gavage administration (Banjaw & Schmidt, 2005), not 3 days. Thus, this suggests that the therapeutic range claimed in rats is either too high, relevant only to injected AMPH studies or the Slow strain has an increased susceptibility/lower threshold.

In addition, taste aversion occurred, and it was correlated with AMPH dose. Fortunately, the low doses did not differ in the probability of developing taste aversion. Thus, while 5.0 mg/kg was the projected therapeutic dose based on human and rat pharmacokinetics (see Appendix 1), the development of stereotypy (in the Slow strain) and increased rearing/activity level (in the Fast strain) indicates that this dose was too

high. Therefore, the 1.0 and 2.0 mg/kg doses were used in subsequent behavioural tests (e.g., DRL and DOR), as they were relatively well-tolerated.

Conclusion

In conclusion, the strains differ in basal NSA, with the Fast strain appearing to have an NSA profile similar to other ADHD models, relative to the Slow strain. The ADHD drug, AMPH, did not have any positive effects on NSA; rather, any effects were negative. Furthermore, the stereotypy in the Slow strain suggests that oral AMPH may be addictive. More tests are recommended to investigate the strains' addiction profile.

Experiment 3: Delay of Reward

Synopsis

Impulsivity can be defined as a preference for a shorter delay, even if it means a smaller reward. ADHD patients are typically described with this behaviour pattern (e.g., quickly finishing a test without proof-reading, taking part in fast-paced games), thus, a model of ADHD should portray similar behaviour. In an experimental setting, this definition is commonly tested with delay of reward (DOR), where the assumption is that impulsive behaviour will be reflected in the animal's preference for a reward size, depending on the reward delay (Monterosso & Ainslie, 1999). Here, the goal is to determine any Fast and Slow strain differences in delay preference, depending on the reward size.

Amphetamine (AMPH), when given at low (therapeutic) doses, has been shown to reduce the impulsive behaviours in ADHD patients. Thus, AMPH, in the same dose range, is predicted to decrease the impulsive behaviour in the DOR experiment (e.g., increase the preference for the larger delayed reward). With the Fast strain showing signs of impulsivity in other tests, this strain was expected to perform the test and to respond to AMPH differently than the Slow strain.

Materials & Methods

Animals

Subjects were 22 Fast (15 male, 7 female) and 22 Slow (15 male, 7 female) rats, approximately 3 months of age at the start of testing (Carleton University). At that time, the rats were placed on a restricted-quantity food diet; the goal was not to decrease weight, but to increase the rat's motivation to consume the daily treats. Thus, the ideal scenario was to find 1 g of food remaining at the start of each day's testing.

The rats were single-housed in standard opaque plastic cages (32 x 22 x 20 cm) and maintained on a 12-h light/dark cycle. On each testing day, any remaining food was removed at least 20 minutes before the chocolate hazelnut treat was administered. After the days' testing, which occurred five days a week during the light cycle, the rats were then fed between 15 to 30 g of food, depending on their weight; water was freely available. On non-testing days, food and water were freely available. The rats were weighed once a week.

Experimental Procedure

Chocolate Hazelnut Treat Consumption Training

Exposure to the chocolate hazelnut treat started at least one week before lever-press training. See Appendix 2 for the 'Chocolate Hazelnut Treat Consumption' methodology. Briefly, the rats were given a small amount of the treat on a dish, once a day, until they consumed it consistently and readily (within approximately 1 minute).

Apparatus

The apparatus was an operant chamber (Model HI0-11R-TC; Coulbourn Instruments, Allentown, PA). The chamber, 28.5 x 25.5 x 27 cm, was fitted with two levers (Model H21-03R; Coulbourn Instruments, Allentown, PA) and a food pellet dispenser (Model H14-22R-45; Coulbourn Instruments, Allentown, PA). Chocolate food pellets (45mg, Product # F0299; Bio-Serv, Frenchtown, NJ) were delivered to a tray (Model H14-01R; Coulbourn Instruments, Allentown, PA) placed between the two levers, all approximately 1.5 cm above the floor. The tray was a recessed cup (width 3.4 cm x height 4.1 cm x depth 2.5 cm) that was illuminated overhead with a magazine light to signal the pellet delivery, and was equipped with a photocell and detector (Model H20-93; Coulbourn Instruments, Allentown, PA) to register entry. A second light placed just

below the roof of the chamber, centred above the tray, served as a houselight. Each chamber was housed in a separate soundproof box and controlled by the Graphic State Notation programming language.

Training

One week before the rats were exposed to the operant chamber, they had five days of exposure to the chocolate pellets by placing 5 chocolate pellets in each home cage. Following familiarization with the treats, the rats were placed in a lever-less operant chamber, with 20 pellets in the trough; they were free to explore for 20 minutes. On the next day, a pellet dropped every 30 seconds into the trough, for 20 minutes. Thereafter, the rats were trained to press the lever that delivered 1 pellet (1-lever) under continuous reinforcement (CRF); the other lever was not present. Training under CRF continued until a criterion of either (a) 2 consecutive days of 50 rewarded presses occurred within 10 minutes or (b) consistently close to 50 rewarded presses occurred over three or four days. Then, rats were trained to press the lever that delivered 3 pellets (3-lever) under CRF using the same criterion before moving on to the experimental procedure; the 1-lever was not present. Some rats had trouble learning how much force was required to press the lever; those rats received 'assistance' (see Appendix 8). Importantly, most of these rats needed just one day of 'assistance.'

Delay of Reward

In this experiment, the rat had a choice between two levers: 1-lever and 3-lever. The rat was exposed to different time settings: either the intertrial interval (ITI) or the delay increased every 12^{th} trial. In the former scenario, the rat had to wait for a certain amount of time before the next trial began; once a lever was pressed, the pellet(s) were delivered without delay. In contrast, with the delay scenario, the 3-lever delivered 3

pellets, but after a certain amount of time had passed; the 1-lever continued to deliver 1 pellet without delay. As a result, the same amount of time passed, but the immediacy of the larger reward can change. The main theory with DOR, then, is that an impulsive rat would be less likely to tolerate delays; thus, they would 'switch' sooner to the immediate small reward (1 pellet) rather than wait for the large reward (3 pellets).

To set this up, there were three main phases, followed by a fourth (control) phase. In Phase 1, both the ITI and Delay were 0 seconds - a lever press delivered the appropriate number of pellets without delay, and the next trial started immediately. In Phase 2, the first 12 trials had 0 sec ITI and Delay; the ITI then increased by 5 seconds every 12th trial, but a lever press continued to deliver the appropriate number of pellets without delay. For Phase 3, the first 12 trials has 0 sec ITI and Delay; the Delay then increased by 5 seconds every 12 trial for the 3 -lever only, but the 1-lever continued to deliver 1 pellet without delay. See Table 2.

A rat that switches to the smaller/immediate reward could be impulsive or it may have switched rewards because it is full. To rule out such a complication, the fourth phase was used. Phase 4 used the same Delay steps as Phase 3, except that the delay started at 20 seconds and decreased 5 seconds every 12th trial. The rats only experienced this Phase for one day after 10 days of Phase 3; thus, there was insufficient time to respond to the change in procedure. Should the rat continue with the same lever choices at the same Delay, then satiety could be ruled out. Otherwise, the same lever choices over time could indicate satiety.

Table 2. Each Phase had an ITI and Delay, in seconds. For Phase 1, both were 0 sec for all 60 trials. For Phases 2, 3 and 4, the ITI increased, the Delay increased and Delay decreased every twelfth trial by 5 sec, respectively. The first three Phases lasted for 10 days, whereas Phase 4 had one day of testing.

Phase	ITI(sec)	Delay (sec)	Duration (days)
1	0	0	10
2	0 for trials 1-12 5 for trials 13-24 10 for trials 25-36 15 for trials 37-48 20 for trials 49-60	0	10
3	0	0 for trials 1-12 5 for trials 13-24 10 for trials 25-36 15 for trials 37-48 20 for trials 49-60	10
4	0	20 for trials 1-12 15 for trials 13-24 10 for trials 25-36 5 for trials 37-48 0 for trials 49-60	1

Drug Administration

In Phases 3 (all ten days) and 4, Placebo or Amphetamine (AMPH), at 1.0 mg/kg p.o. and 2.0 mg/kg p.o. mixed in the chocolate hazelnut treat, was administered. Each strain had five rats in each dose group. See Appendix 2 for more detailed administration methods. Testing started 20 minutes after consuming the treat. Any unconsumed dosed-treat was weighed. After testing, the rats were observed for differences in activity level at 20-minute intervals. Note: only Male Fast and Slow rats were available for these drug administration phases.

Analysis

Days 1, 5 and 10 were selected for analysis. The variables analyzed were Responding Categories, Lever Press Categories, taste aversion and stereotypy. Additionally, the time to complete and the number of uneaten pellets at the end of each day of testing were analyzed in Appendix 3. The level of statistical significance was set at $p < .05$.

Responding and Lever Press Categories

For each phase, each day's testing was divided into five 12-trial sessions. Within each 12-trial session, there were two sections of analysis: (1) Responding Categories (responding [i.e., pressing either lever] or not), and (2) Lever Press Categories (for those that pressed a lever, the rat could press either the 3-lever or 1-lever). To calculate the Responding Categories' frequencies, each rats' sessions were scored according to the following continuum: (1) More Responding (i.e., receiving a reward at least 7 out of 12 trials), (2) Missed = Responding trials (i.e., receiving a reward exactly 6 out of 12 trials), and (3) More Missed (i.e., at least 7 out of 12 trials had no lever press). Then, to determine the Lever Press Categories' frequencies, each rats' sessions were scored as: (1)

more rewarded 3-lever presses, (2) 1-lever equals 3-lever rewarded presses, and (3) more rewarded 1-lever presses.

Days 1, 5 and 10 frequencies were analyzed with non-parametric tests. The Friedman Test was used to examine changes over Trials (5 levels: Trials 1-12, 13-24, 25-36, 37-48, 49-60) for each group. The Kruskal Wallis Test was used to identify differences between groups (Strain [2 levels: Slow, Fast] and, when appropriate, Drug [3 levels: Placebo, 1.0 mg/kg, 2.0 mg/kg]). Additionally, if there was a consistent trend for group differences, the Kruskal Wallis Test was redone to compare all six individual groups (6 levels: Slow Placebo, Fast Placebo, Slow 1.0 mg/kg, Fast 1.0 mg/kg, Slow 2.0 mg/kg, Fast 2.0 mg/kg). For any significant x statistic ($p < .05$), pre-planned *post-hoc* Mann-Whitney (i.e., 2-tailed z-statistic) tests comparing the trials or drug groups were done; all reported significant differences were $p < .05$.

It is important to note that (1) due to differing sample sizes, the males and females were analyzed separately; and, (2) repeated measures analysis was not a viable option due to a high degree of (a) variability and (b) correlation between independent variables in Phases 3 and 4.

Taste Aversion and Stereotypy

Taste aversion was defined by incomplete consumption of the dosed treat. Logistic regression was used to examine the odds of developing taste aversion depending on Strain (2 levels: Slow, Fast) and Drug (3 levels: Placebo [reference category], 1.0 mg/kg, 2.0 mg/kg). Stereotypy, or repetitive unusual behaviours, while expected at higher doses, remained a possibility with chronic administration. At regular intervals

after testing, the rats were observed for any unusual repetitive behaviours or hyperactivity. Since no stereotypy was observed, analyses were not done.

Results

Responding Categories

Phase 1 (0ITI, 0 Delay)

Females

On Day 1, 5 and 10, in Phase 1, neither female Slow nor Fast strains had any changes over trials. Additionally, there were no differences between strains (data not shown).

Males

Day 1

On Day 1, only the male Fast strain had a significant Friedman Test ($x4 = 20.5$, $p < .001$). Further testing showed that the last 12-trial session (Trials 49-60) had a greater frequency of missed trials than the three first 12-trials sessions (Trials 1-12, 13-24, 25-36). See Table 3.

Day 5

For the fifth day, both male strains had significant Friedman Tests (Slow: $X4 = 19.0$, $p < .002$; Fast: $y/ = 32.6$, $p < .001$). In the Slow strain, additional testing revealed that the last 12-trial session (Trials 49-60) had a greater frequency of missed trials than the three first 12-trials sessions (Trials 1-12, 13-24, 25-36). As for the Fast strain, this pattern was evident earlier and more dramatic: the second last (Trials 37-48) and last (Trials 49-60) 12-trial sessions had a greater frequency of missed trials than the first two (Trials 1-12, 13-24) and preceding (Trials 1-12, 13-24, 25-36, 37-48) 12-trial sessions, respectively. See Table 3.

Table 3. In Phase 1, the number of male Slow and Fast rats that had at least 7 rewarded presses ("Resp." column), 5 or fewer rewarded presses ("Miss" column), or exactly 6 rewarded presses ("50/50" column) out of 12 trials. These frequencies were calculated for Days 1, 5 and 10 in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a, b, c, d - significantly different from Trials 1-12, 13-24, 25-36, 37-48, respectively
& - significantly different from Slow strain

		Male Slow				Male Fast			
	Trials	Miss	50/50	Resp.	Diff.	Miss	50/50	Resp.	Diff.
Day 1	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	0	15	
	25-36	0	1	14		1	0	14	
	37-48	0	1	14		3	0	12	
	49-60	1	2	12		6	1	8	abc
Day 5	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	1	14	
	25-36	0	0	15		2	0	13	
	37-48	1	1	13		4	2	9	ab
	49-60	5	1	9	abc	9	2	4	abed
Day 10	1-12	0	0	15		0	0	15	
	13-24	0	1	14		0	0	15	
	25-36	0	1	14		3	0	12	
	37-48	2	0	13		7	1	7	abc,&
	49-60	4	0	11	a	11	0	4	abc,&

Day 10

As for the last day of Phase 1, both male strains continued to have significant Friedman Tests (Slow: *y/* = 11.6, p < .021; Fast: x/ = 32.6, p < .001). For the Slow strain, only the last 12-trial session (Trials 49-60) had a greater frequency of missed trials than the first 12-trials session (Trials 1-12). In contrast, the Fast strain's second last and last 12-trial session (Trials 37-48, 49-60) had a greater frequency of missed trials than the three first 12-trials sessions (Trials 1-12, 13-24, 25-36). Additionally, these last two 12-trial sessions had more missed trials in the Fast strain then the Slow strain ($xi^2 > 4.9$, p < .028). See Table 3.

Phase 2 (0-20ITI, 0 Delay)

Females

For Phase 2, on Days 1, 5 and 10, neither female Slow nor Fast strains had any changes over trials. Additionally, there were no differences between strains (data not shown).

Males

Day 1

On Day 1, both male strains had significant Friedman Tests (Slow: X4 = 30.4, p < .001; Fast: x/ = 36.5, p < .001). In the Slow strain, the last 12-trial session had more missed trials than the preceding four 12-trial sessions. As for the Fast strain, the middle, second last, and last 12-trial sessions had a greater frequency of missed trials than the preceding two, three and four 12-trial sessions, respectively. The middle and second last 12-trial sessions also had more missed trials in the Fast strain than the Slow strain (xi > 4.5, p < .036). Additionally, the last session was showing this trend, but it approached significant (p < .059). See Table 4.

Table 4. In Phase 2, the number of male Slow and Fast rats that had at least 7 rewarded presses ("Resp." column), 5 or fewer rewarded presses ("Miss" column), or exactly 6 rewarded presses ("50/50" column) out of 12 trials. These frequencies were calculated for Days 1, 5 and 10 in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff") column.

Abbrev:
a, b, c, d - significantly different from Trials 1-12, 13-24, 25-36, 37-48, respectively
& - significantly different from Slow strain

		Male Slow				Male Fast			
	Trials	Miss	50/50	Resp.	Diff.	Miss	50/50	Resp.	Diff.
Day 1	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	0	15	
	25-36	0	0	15		4	0	11	ab,&
	37-48	2	0	13		8	0	7	abc,&
	49-60	8	1	6	abed	13	0	2	abed
Day 5	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	0	15	
	25-36	0	0	15		1	4	10	ab,&
	37-48	1	0	14		6	1	8	abc,&
	49-60	4	0	11	abc	9	1	5	abc,&
Day 10	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	1	14	
	25-36	0	0	15		3	1	11	&
	37-48	2	0	13		6	0	9	ab
	49-60	6	1	8	abc	9	0	6	abc

Day 5

For the fifth day, both male strains continued to have significant Friedman Tests (Slow: $\%^2$ = 13.3, p < .011; Fast: X^2 = 31.6, p < .001). Likewise, the differences in frequencies between trials followed a similar pattern. For the Slow strain, the last 12-trial session (Trials 49-60) had a greater frequency of missed trials than the three first 12-trials sessions (Trials 1-12, 13-24, 25-36). In the Fast strain, the last three 12-trial sessions had more missed trials than the first two 12-trial sessions. The last two 12-trial sessions also had a greater frequency of missed trials than the middle 12-trial session. For the middle to last 12-trial session, the Fast strain had more missed trials than the Slow strain (xi^2 > 4.1,p<.043). See Table 4.

Day 10

As for the tenth day of Phase 2, the male strains continued to have significant Friedman Tests (Slow: $\%_A^2$ = 21.6, p < .001; Fast: *y/* = 25.5, p < .001). Just like Day 5, the Slow strain's last 12-trial session (Trials 49-60) had a greater frequency of missed trials than the first three 12-trials sessions. In the Fast strain, the second last and last 12-trial sessions (Trials 37-48, 49-60) had a greater frequency of missed trials than the two first 12-trials sessions (Trials 1-12, 13-24). Additionally, the last 12-trial sessions had more missed trials in the middle 12-trial session. The middle 12-trial session had the sole Strain difference: the Fast strain had more missed trials than the Slow strain (xi^2 = 4.4, p <.036). See Table 4.

Phase 3 (0ITI, 0-20 Delay)

Day I

During the first Day of testing, differences between trials depended, to a large extent, on the strain and the presence of AMPH. However, the overall trend was the

same: more missed trials as the Delay increased. Indeed, in the Placebo group, only the Slow strain had a significant Friedman Test on Day 1 (χ^2_4 = 13.3, p < .011). Further investigation revealed that the last 12-trial session had more missed trials than the first three 12-trial sessions. Then, in the AMPH-dosed groups, only the Fast strain had significant Friedman Tests. In the 1.0 mg/kg group, follow-up analyses of the significant Friedman Test (*j/* - 13.1, p < .012) showed that the last 12-trial session had more missed trials than the first and second 12-trial sessions. As for the 2.0 mg/kg group (Friedman Test: $X4^2$ = 13.1, p < .012), additional testing indicated that the last 12-trial session had more missed trials than the first and second 12-trial sessions. See Table 5.

Drug group-independent strain differences were evident on Day 1 only. During the middle and second last 12-trial session, the Fast strain had a higher frequency of missed trials than the Slow strain (xi > 4.0, p < .046); the last session had the same trend but it approached significance (p < .060). See Table 5.

Day 5

On the fifth day, neither strain in the Placebo, 1.0 mg/kg, or 2.0 mg/kg groups had any changes over trials (data not shown).

Day 10

For the tenth day, neither strain in the Placebo or 2.0 mg/kg groups had any changes over Trials. As for the 1.0 mg/kg group, the Slow strain had a significant Friedman Test (*y/* = 10.3, p < .037). Upon further testing, only the last 12-trial session had more missed trials than the first 12-trial session. The Fast strain, though, had a Friedman Test that was approaching significance (χ^2_4 = 9.1, p < .060); *post-hoc* testing found the same trend at the Slow strain. See Table 6.

Table 5. For Day 1 of Phase 3, the number of male Slow and Fast rats that had at least 7 rewarded presses ("Resp." column), 5 or fewer rewarded presses ("Miss" column), or exactly 6 rewarded presses ("50/50" column) out of 12 trials. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff") column.

Abbrev:
a, b, c - significantly different from Trials 1-12, 13-24, 25-36, respectively
& - significant difference between Strains (Drug groups combined)

		Male Slow				Male Fast			
	Trials	Miss	50/50	Resp.	Diff.	Miss	50/50	Resp.	Diff.
Placebo	1-12	0	0	5		0	0	5	
	13-24	0	0	5		0	0	5	
	25-36	0	0	5		1	0	4	&
	37-48	1	0	4		2	0	3	&
	49-60	4	0	1	abc	3	0	2	
1.0 mg/kg	1-12	0	0	5		0	0	5	
	13-24	0	0	5		0	0	5	
	25-36	0	0	5		1	1	3	&
	37-48	0	1	4		3	0	2	&
	49-60	1	1	3		4	1	0	ab
2.0 mg/kg	1-12	0	0	5		0	0	5	
	13-24	0	0	5		3	0	2	
	25-36	0	0	5		3	1	1	&
	37-48	2	0	3		4	0	1	a,&
	49-60	1	0	4		4	0	1	a

Table 6. For Day 10 of Phase 3, the number of male Slow and Fast rats that had at least 7 rewarded presses ("Resp." column), 5 or fewer rewarded presses ("Miss" column), or exactly 6 rewarded presses ("50/50" column) out of 12 trials. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a, b, c - significantly different from Trials 1-12, 13-24, 25-36, respectively
& - significant difference between Strains (Drug groups combined)
P, L - significantly different from Placebo, 1.0 mg/kg, respectively (Fast and Slow strains combined)

		Male Slow				Male Fast			
	Trials	Miss	50/50	Resp.	Diff.	Miss	50/50	Resp.	Diff.
Placebo	1-12	0	0	5		0	0	5	
	13-24	0	0	5		1	0	4	
	25-36	0	0	5		1	0	4	
	37-48	0	0	5		1	0	4	
	49-60	0	1	4		1	0	4	
1.0 mg/kg	1-12	0	0	5		0	0	5	
	13-24	2	0	3		1	0	4	
	25-36	2	1	2		3	0	2	
	37-48	3	0	2		2	0	3	
	49-60	4	0	1	a	4	0	1	a
2.0 mg/kg	1-12	1	0	4		1	1	3	
	13-24	1	1	3		3	1	1	
	25-36	3	0	2	P	5	0	0	P
	37-48	4	0	1	P	4	0	1	P
	49-60	3	2	0	P,L	5	0	0	P,L

Strain-independent drug group differences were evident only on the tenth day; this was from the middle 12-trial session onwards ($\chi^2 > 9.6$, $p < .008$). Follow-up tests for all three of these sessions showed that highest-dosed group had more missed trials than Placebo. Additionally, the lowest-dosed group had more missed trials than 2.0 mg/kg during the last 12-trial session. See Table 6.

Phase 4 (0ITI, 20-0 Delay)

Just like with Phase 3, the strains were combined for the Drug group analyses, as were the drug groups for the Strain analysis, and the Trials analyses were done for each individual group (i.e., Slow 1.0 mg/kg, Fast 1.0 mg/kg). Of all the individual groups, only the Fast 1.0 mg/kg group had a significant Friedman Test ($\chi^2 = 9.5$, $p < .050$) but further investigation did not reveal any differences between Trials. Differences between the Drug groups were evident from the middle 12-trial session (Delay 10s) onwards ($\chi^2 > 7.2$, $p < .027$). Starting in the middle session, the 2.0 mg/kg group had more missed trials than Placebo and 1.0 mg/kg. Then, for the second last and last sessions (Delay 5s and 0s), the 2.0 mg/kg continued to have a higher frequency of missed trials than Placebo only. Additionally, during the final 12-trial session, the Fast strain had more missed trials than the Slow strain ($xi^2 = 4.0$, $p < .046$). Granted, this effect is most likely attributable to the AMPH-dosed groups but statistical tests did not find a difference for these individual groups. See Table 7.

Lever Press Categories

Phase 1 (0 ITI, 0 Delay)

Females

On Day 1, 5 and 10, in Phase 1, neither female Slow nor Fast strains had any changes over trials. On Day 1, however, the female Slow strain had more rewarded 3-

Table 7. In Phase 4, the number of male Slow and Fast rats that had at least 7 rewarded presses ("Resp." column), 5 or fewer rewarded presses ("Miss" column), or exactly 6 rewarded presses ("50/50" column) out of 12 trials. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff") column.

Abbrev:
& - significant difference between Strains (Drug groups combined)
P, L - significantly different from Placebo, 1.0 mg/kg, respectively (Fast and Slow strains combined)

		Male Slow				Male Fast			
	Trials	Miss	50/50	Resp.	Diff.	Miss	50/50	Resp.	Diff.
Placebo	1-12	0	0	5		0	0	5	
	13-24	0	0	5		1	0	4	
	25-36	0	0	5		1	0	4	
	37-48	0	0	5		1	0	4	
	49-60		0	4		1	0	4	&
1.0 mg/kg	1-12		0	4		0	1	4	
	13-24		0	4		1	0	4	
	25-36		0	4		0	1	4	
	37-48		1	3		3	0	2	
	49-60	0	1	4		4	0	1	&
2.0 mg/kg	1-12	1	0	4		3	0	2	
	13-24	2	1	2		3	0	2	
	25-36	3	1	1	P,L	5	0	0	P,L
	37-48	4	0	1	P	2	2	1	P
	49-60	3	0	2	P	5	0	0	P,&

lever presses than the female Fast strain during the first 12-trial session (xi = 5.1, p < .026). See Table 8.

Males

Day 1

On Day 1, neither male strain had any changes in rewarded press frequencies over trials. However, there was a pervasive difference between strains. Indeed, for every 12-trial session (excluding the second session), the Slow strain had significantly more rewarded 3-lever presses than the Fast strain ($xi^2 > 4.7$, $p < .032$). The excluded second session, though, had the same trend but the difference approached significance (xi = 3.3, p<.070). See Table 9.

Day 5

For the fifth day, only the Slow strain had a significant Friedman Test (x4 =15.3, p < .004). Additional testing revealed that the last 12-trial session (Trials 49-60) had more rewarded 1-lever presses than the three first 12-trials sessions (Trials 1-12, 13-24, 25-36). The Slow strain also had more rewarded 3-lever presses than the Fast strain during the second and third 12-trial session ($_{Xl}^2 > 4.4$, $_p < .036$). See Table 9.

Day 10

As for the last day of Phase 1, only the Fast strain had a significant Friedman Test ($X4^2 = 22.3$, $p < .001$). Further analyses found that the second last 12-trial session had less rewarded 3-lever presses than the first. This trend continued for the last 12-trial session, which also had fewer 3-lever presses than the second and third 12-trial session. The second last and last 12-trial sessions also had more rewarded 3-lever presses in the Slow strain than Fast strain ($xi^2 > 4.0$, $p < .047$). See Table 9.

Table 8. In Phase 1, the number of female Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for Days 1, 5 and 10 in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
& - significantly different from Slow strain

		Female Slow				Female Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Day 1	1-12	0	0	7		2	2	3	&
	13-24	0	0	7		2	1	4	
	25-36	0	2	5		1	1	5	
	37-48	1	1	5		1	0	6	
	49-60	1	0	6		1	0	6	
Day 5	1-12	0	0	7		0	0	7	
	13-24	0	0	7		0	0	7	
	25-36	0	0	7		0	0	7	
	37-48	0	0	7		1	0	6	
	49-60	0	1	6		0	0	7	
Day 10	1-12	0	0	7		0	0	7	
	13-24	0	0	7		0	0	7	
	25-36	0	0	7		0	0	7	
	37-48	1	0	6		0	0	7	
	49-60	0	1	6		0	1	6	

Table 9. In Phase 1, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for Days 1, 5 and 10 in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff") column.

Abbrev:
a, b, c - significantly different from Trials 1-12, 13-24, 25-36, respectively
& - significantly different from Slow strain

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Day 1	1-12	1	1	13		11	3	1	&
	13-24	5	3	7		10	2	3	
	25-36	4	0	11		10	0	5	&
	37-48	4	0	11		12	1	2	&
	49-60	5	0	10		11	2	2	&
Day 5	1-12	0	1	14		3	1	11	
	13-24	0	0	15		4	1	10	&
	25-36	0	0	15		1	3	11	&
	37-48	1	2	12		3	1	11	
	49-60	4	1	10	abc	4	7	4	
Day 10	1-12	0	0	15		0	0	15	
	13-24	0	0	15		1	0	14	
	25-36	0	0	15		1	0	14	
	37-48	1	0	14		2	4	9	a,&
	49-60	2	1	12		3	7	5	abc,&

Phase 2 (0-20ITI, 0 Delay)

Females

For Phase 2, on Days 1, 5 and 10, neither female Slow nor Fast strains had any changes over trials. Additionally, there were no differences between strains (data not shown).

Males

Each day followed similar patterns of differences. First, the second last 12-trial session also had more rewarded 3-lever presses in the Slow strain than the Fast strain on the first, fifth and tenth days ($xi^2 > 4.0$, $p < .047$). Second, both strains had fewer rewarded 3-lever presses as each day progressed through the trials, but in a day- and strain-dependent manner (more below). Third, the change over trials was more pronounced/prolonged in the Fast strain, relative to the Slow strain (more below).

Day 1

On Day 1, both male strains had significant Friedman Tests (Slow: $\%4^2 = 26.1$, $p < .001$; Fast: $X4^2 = 32.6$, $p < .001$). In the Slow strain, the last 12-trial session had more rewarded 1-lever presses than the preceding four 12-trial sessions. As for the Fast strain, the second last and last 12-trial sessions had more rewarded 1-lever presses than the first three 12-trial sessions. See Table 10.

Day 5

For the fifth day, both male strains continued to have significant Friedman Tests (Slow: $x/ = 14.4$, $p < .007$; Fast: $X4^2 = 16.3$, $p < .004$). Significant differences between Trials, though, were found only in the Fast strain: the last two 12-trial sessions had fewer rewarded 3-lever presses than the first two 12-trial sessions. As for the Slow strain, there

Table 10. In Phase 2, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for Days 1, 5 and 10 in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a, b, c, d - significantly different from Trials 1-12, 13-24, 25-36, 37-48 respectively
& - significantly different from Slow strain

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Day 1	1-12	0	0	15		0	1	14	
	13-24	0	0	15		0	0	15	
	25-36	0	0	15		0	0	15	
	37-48	0	1	14		3	4	8	abc,&
	49-60	5	2	8	abed	6	5	4	abc
Day 5	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	0	15	
	25-36	0	0	15		1	1	13	
	37-48	0	1	14		1	5	9	ab,&
	49-60	3	1	11		2	4	9	ab
Day 10	1-12	0	0	15		0	0	15	
	13-24	0	0	15		0	0	15	
	25-36	0	0	15		0	1	14	
	37-48	1	0	14		2	4	9	abc,&
	49-60	3	0	12		1	6	8	abc

was a trend towards fewer rewarded 3-lever presses in the last 12-trial session compared to the previous four sessions ($p < .064$). See Table 10.

Day 10

For Day 10, both male strains again had significant Friedman Tests (Slow: $j/ = 9.7$, $p < .047$; Fast: $\%4^2 = 22.6$, $p < .001$). In the Fast strain, the last two 12-trial sessions had fewer rewarded 3-lever presses than the first three 12-trial sessions. The Slow strain, however, had a trend towards fewer rewarded 3-lever presses in the last 12-trial session compared to the first three sessions ($p < .084$). See Table 10.

Phase 3 (0ITI, 0-20 Delay)

Similar to the Lever Press analyses, the Fast and Slow data was combined for the Drug group analyses, the Placebo, 1.0 mg/kg and 2.0 mg/kg data was combined for the Strain group analyses, and the Trials analyses were done for each individual group (e.g., Slow 2.0 mg/kg, Fast 2.0 mg/kg).

*Day **1***

On Day 1, both strains of the Placebo and 1.0 mg/kg groups had significant Friedman Tests ($x4 > 9.4$, $p < .05$). However, only the Slow Placebo group showed a significant increase in rewarded 1-lever presses during the final 12-trial session compared to the first three 12-trial sessions. The Slow and Fast 2.0 mg/kg groups did not change over Trials. There were no strain or Drug group differences. See Table 11.

*Day **5***

For the fifth day, significant Friedman Tests were found in the Slow Placebo, Fast Placebo, and Fast 1.0 mg/kg groups ($x4^2 > 9.5$, $p < .05$). Of these three groups, though, the Slow Placebo and Fast 1.0 mg/kg groups had an increase in rewarded 1-lever presses during the final 12-trial session compared to the first 12-trial session. Just like Day 1, the

Table 11. On the first day of Phase 3, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff") column.

Abbrev:
a, b, c - significantly different from Trials 1-12, 13-24, 25-36, respectively

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Placebo	1-12	0	0	5		0	0	5	
	13-24	0	0	5		0	0	5	
	25-36	0	0	5		0	0	5	
	37-48	1	0	4		1	1	3	
	49-60	4	0	1	abc	0	3	2	
1.0 mg/kg	1-12	0	0	5		0	0	5	
	13-24	0	0	5		0	0	5	
	25-36	0	0	5		0	1	4	
	37-48	2	1	2		3	0	2	
	49-60	2	1	2		2	1	2	
2.0 mg/kg	1-12	0	0	5		0	0	5	
	13-24	0	0	5		1	1	3	
	25-36	1	0	4		2	0	3	
	37-48	1	1	3		1	3	1	
	49-60	1	0	4		3	1	1	

Slow and Fast 2.0 mg/kg groups did not change over Trials nor were there any strain or Drug group differences. See Table 12.

Day 10

Following the tenth Placebo or AMPH administration, the Slow Placebo, Fast Placebo, and Fast 1.0 mg/kg groups continued to have the significant Friedman Tests ($\chi^2_4 > 12.7$, $p < .014$). All three of these groups had a significant decrease in 3-lever presses compared to the first 12-trial session but timing of this difference depended on the group. For instance, in the Slow Placebo group, the decrease was found during the last two 12-trial sessions while the Fast Placebo showed the decrease for all 12-trial sessions following the first session. In contrast, the Fast 1.0 mg/kg group had the decrease in the middle and fourth 12-trial sessions.

Different from the previous days, though, there were significant Strain and Drug group comparisons. Indeed, during the middle session, the Slow strain had more rewarded 3-lever presses than the Fast strain (all Drug groups combined; $\chi^2_1 > 6.2$, $p < .014$). Then, during the last 12-trial session, both AMPH groups had more rewarded 3-lever presses than Placebo (both strains combined; $\chi^2_2 > 7.2$, $p < .028$). See Table 13.

Phase 4 (0ITI, 20-0 Delay)

For the Trials examinations, the Slow Placebo and 1.0 mg/kg and Fast 1.0 mg/kg and 2.0 mg/kg groups had significant Friedman Tests ($\chi^2_4 > 13.2$, $p < .012$). Further comparisons revealed that, when significant differences were found, the difference was always more 1-lever presses relative to the first 12-trial session, albeit in a group-dependent manner. In the Slow Placebo group, all 12-trial sessions had more 1-lever presses than the first session. A similar trend was evident in the Slow and Fast 1.0 mg/kg

Table 12. On the fifth Day of Phase 3, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a - significantly different from Trials 1-12

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Placebo	1-12	0	0	5		1	0	4	
	13-24	1	0	4		1	1	3	
	25-36	2		2		2	2	1	
	37-48	3		1		3	2	0	
	49-60	4		0	a	3	2	0	
1.0 mg/kg	1-12	1		3		0	0	5	
	13-24	3		1		1	1	3	
	25-36	4		0		3	0	2	
	37-48	3	2	0		2	1	2	
	49-60	3	2	0		4	0	1	a
2.0 mg/kg	1-12	0	0	5		2	1	2	
	13-24	2	0	3		3	1	1	
	25-36	2	0	3		2	1	2	
	37-48	1	1	3		1	2	2	
	49-60	3	0	2		3	1	1	

Table 13. On the tenth day of Phase 3, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a - significantly different from Trials 1-12
& - significant difference between Strains (Drug groups combined)
P - significantly different from Placebo (Fast and Slow strains combined)

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Placebo	1-12	0	0	5		0	0	5	
	13-24	3	0	2		4	0	1	a
	25-36	3	0	2		4	1	0	a,&
	37-48	4	0	1	a	4	1	0	a
	49-60	5	0	0	a	4	1	0	a
1.0 mg/kg	1-12	1	1	3		0	0	5	
	13-24	1	1	3		1	1	3	
	25-36	1	1	3		3	2	0	a,&
	37-48	2	2	1		4	0	1	a
	49-60	2	2	1	P	2	2	1	P
2.0 mg/kg	1-12	0	0	5		3	0	2	
	13-24	3	0	2		0	2	3	
	25-36	1	2	2		3	2	0	&
	37-48	2	1	2		3	2	0	
	49-60	2	1	2	P	2	0	3	P

groups but delayed by one 12-trial session. As for the Fast 2.0 mg/kg group, the middle and second last sessions had more 1-lever presses than the Trials 1-12 session. See Table 14.

Unlike the other drug administration phase analyses, the Lever Press analysis for Phase 4 had more comparisons between the individual groups. For the group comparisons, the Kruskal Wallis test was significant for Trials 1-12, 37-48 and 49-60 *(%5* > 11.6, $p < .042$). Additional analyses found that the Fast Placebo had more rewarded 1-lever presses than Slow Placebo and Fast 2.0 mg/kg groups during the first 12-trial session; the Fast 1.0 mg/kg had a tendency to have fewer rewarded 1-lever presses than Fast Placebo, as well ($p < .057$). Then, in the second last session, the Slow Placebo and 1.0 mg/kg had more rewarded 1-lever presses than Slow 2.0 mg/kg. And, finally, during the last 12-trial session, the Fast 1.0 mg/kg had more rewarded 1-lever presses than Fast 2.0 mg/kg. See Table 14.

Taste Aversion and Stereotypy

While only the 2.0 mg/kg-dosed rats (2 Slow, 2 Fast) failed to complete their entire chocolate hazelnut spread on the tenth Day of Phase 3 and Day 1 of Phase 4, logistic regression did not find any difference between Drug groups. Additionally, none of the rats showed signs of stereotypy and/or hyperactivity.

Discussion

Females - Phases 1 and 2

In Phase 1, all females had more responding trials. Initially, the Slow females had more rewarded 3-lever presses that Fast females but that difference no longer existed from the 13* trial onwards. Then, in Phase 2, the female strains did not differ in

Table 14. In Phase 4, the number of male Slow and Fast rats that had more rewarded 1-lever presses ("More 1L" column), more rewarded 3-lever presses ("More 3L" column) presses, or no difference ("50/50" column) for each 12-trial session. These frequencies were calculated for the Placebo, 1.0 mg/kg or 2.0 mg/kg Drug groups in five 12-trial sessions. Any significant differences in the frequencies are noted in the Difference ("Diff.") column.

Abbrev:
a - significantly different from Trials 1-12 respectively
& - significant difference between Strains
P, L - significantly different from Placebo, 1.0 mg/kg, respectively

		Male Slow				Male Fast			
	Trials	More 1L	50/50	More 3L	Diff.	More 1L	50/50	More 3L	Diff.
Placebo	1-12	0	1	4		3	2	0	&
	13-24	4	1	0	a	4	0	1	
	25-36	5	0	0	a	4	1	0	
	37-48	5	0	0	a	4	1	0	
	49-60	4	1	0	a	4	1	0	
1.0 mg/kg	1-12	1	0	4		1	0	4	
	13-24	4	0	1		2	0	3	
	25-36	5	0	0	a	5	0	0	a
	37-48	5	0	0	a	5	0	0	a
	49-60	5	0	0	a	5	0	0	a
2.0 mg/kg	1-12	0	0	5		0	0	5	P
	13-24	2	0	3		2	0	3	
	25-36	2	0	3		4	1	0	a
	37-48	1	1	3	P,L	3	2	0	a
	49-60	3	0	2		1	2	2	L

responding or lever press categories. Interestingly and importantly, these response patterns differed from males, indicating different impulsivity profiles from males.

Males - all Phases

Phase 1 (0ITI, 0 Delay)

For Phase 1, both of the male strains had more responding trials; towards the end of the tenth day, however, the male Fast strain had more missed trials than the male Slow strain. Interestingly, the male Slow strain had more rewarded 3-lever presses than the male Fast strain on the first day. As the days progressed, if there was a strain difference, the same pattern happened but its occurrence changed (i.e., Trials 13-24 and 25-36 on Day 5 but Trials 37-48 and 49-60 on Day 10). In the vast majority of those occasions, though, the lower number of rewarded 3-lever presses in the Fast strain could be attributable to a greater number of 50/50 (i.e., same number of rewarded 1-lever and 3-lever presses). This differed from Day 1 in which the Fast strain had more rewarded 1-lever presses. Presumably, the increase in rewarded 1-lever presses on Day 1 may be due to the reintroduction of the 1-lever following 3-lever training. Then, for the subsequent analyzed days, the lower number of rewarded 3-lever presses may be due to more Fast rats not biasing their lever pressing for one lever or the other.

Phase 2 (0-20s ITI, 0 Delay)

For Phase 2, the Male Slow strain typically had a greater number of responding trials compared to the Male Fast strain when ITI was 10s or more, suggesting that the Fast strain's participation rate dropped as the days' testing progressed. When the ITI was 15 s, the Slow strain had more rewarded 3-lever presses than the Fast strain on each of the examined days, indicating an overall greater ability for the Slow strain to maintain a

preference for the larger-reward lever. Indeed, this is echoed in the minimal change over trials in the Slow strain but a shift in the Fast strain towards more 1-lever or more 50/50.

Phase 3 (0ITI, 0-20s Delay)

Placebo and Group-Independent Analyses

Aside from a shift to more missed trials on the first Day of Phase 3 in the Slow Placebo group, the Placebo groups primarily had more responding trials compared to missed trials. Closely paralleling the Day 1 Response Categories pattern, the Slow strain showed a shift to more rewarded 1-lever presses during the last 12-trial session whereas the Fast strain did not. This occurred again on Day 5. Since an earlier bias for the smaller/immediate reward was assumed to reflect increased impulsivity, this could imply that the Slow strain had an 'impulsive' profile, relative to the Fast strain. Granted, this difference was found during one (and the last) 12-trial session so an indication of a trend was not feasible.

The tenth day's pattern was very different, though. Indeed, in the Fast strain, any session with a delay had more rewarded 1-lever presses than the first session. In contrast, this shift wasn't evident until the Delay was 15s in the Slow strain. Thus, the Fast strain had a much earlier and long-term bias for the smaller/immediate reward, a bias that emerged whenever any delay occurred between the large-reward lever press and reward delivery. Consequently, the Fast strain had a stronger 'impulsive' profile, relative to the Slow strain, but this profile took over five days to emerge.

Additionally, the Strains and Drug groups did not differ in the occurrence of taste aversion, which closely matched their chocolate hazelnut consumption speeds (see 'Chocolate Hazelnut Treat Consumption Times'); none showed signs of stereotypy.

1.0 mg/kg

The low dose of AMPH had a slight effect on responding; primarily, shifts to more missed trials did not occur until the last 12-trial session (Delay 20s), relative to the first 12-trial session or Placebo. For the Lever Press Categories, the Slow 1.0 mg/kg did not have a shift to more rewarded 1-lever presses, differing from Slow Placebo. In the Fast 1.0 mg/kg group, the shift to more rewarded 1-lever presses occurred but it was less dramatic compared to the Fast Placebo group on the tenth day. Interestingly, during the longest delay (20s) on the tenth day, this dose group had more 3-lever rewarded presses compared to Placebo. Consequently, with minimal changes in participation (i.e., number of lever presses vs. number of missed trials), the low AMPH dose removed or decreased the bias for the smaller/immediate reward relative to Placebo in both strains. In other words, 1.0 mg/kg decreased impulsivity in a strain-independent manner.

2.0 mg/kg

Following the 2.0 mg/kg dose, both strains showed a decrease in responding relative to Placebo, particularly after the tenth administration when the delay was 10s or more. Despite that decrease in responding, the high dosed group had more rewarded 3-lever presses compared to Placebo when the delay was 15 and 20s on the fifth and tenth day, respectively. Thus, when the rats in the 2.0 mg/kg group pressed a lever, more pressed the larger/delayed reward lever than the smaller/immediate lever, relative to Placebo, when the delay was long (15s or more), but this effect required subchronic and chronic administration. Consequently, this dose decreased impulsivity in a strain-independent manner.

Phase 4 (0ITI, 20-0s Delay)

Phase 4 was used as a control measure. While a shift in a preference to the immediate reward as the delay increased could reflect impulsivity, it is also possible that this switch could be due to satiation. To rule this out, the Delay schedule was reversed.

Placebo

Like the tenth day of Phase 3, the Strains continued to not differ in Response Categories in the Placebo group. Unlike Phase 3, though, the Slow strain had a greater number of rewarded 3-lever presses during the first 12-trial session (Delay 20s) than the Fast strain. Thereafter, the Slow strain had a shift to more rewarded 1 -lever presses and the Strains did not differ. Thus, the Fast strain had an early indication of impulsivity when the challenge was presented at the start.

1.0 mg/kg

Unlike the last day of Phase 3, the 1.0 mg/kg group had no significant shift towards missed trials over sessions, nor was there a difference from Placebo. Additionally, the strains had a shift to the smaller/immediate lever during the same sessions. Interestingly, though, the strain difference in Lever Press Categories was absent in the first session, relative to Placebo; indeed, the Fast strain had a trend towards a significant difference from Placebo ($p < .057$). Consequently, the low AMPH dose may have decreased the early impulsivity in the Fast strain.

2.0 mg/kg

The 2.0 mg/kg AMPH dosed decreased the number of responding trials relative to Placebo, especially during the last half of the day's testing, just like the last half of the last day of Phase 3. Similar to the 1.0 mg/kg dose, the Fast 2.0 mg/kg group had more rewarded 3-lever presses than Placebo during the first session, further indicating that

AMPH may have decrease the early impulsivity in this strain. Later on, in the Slow strain, when the delay was short (5s), this dose had more rewarded 3-lever presses than the other groups. As for the Fast strain, there were more rewarded 3-lever presses than the low AMPH group during the last session (Delay Os). Thus, the high AMPH dose had a more pervasive decrease in impulsivity relative to both Placebo and the lower AMPH dose. A summary of amphetamine's effects on impulsivity are provided in Table 15.

Satiety

The decreased responding in both Phase 3 and 4 could indicate satiation. However, there were some differences between Phases 3 and 4. First, in Phase 3, the Slow strain had more responding trials than the Fast strain during almost all sessions while, in Phase 4, this strain difference did not emerge until the last session. Second, satiety could be inferred from the number of uneaten pellets and the duration of the Day's testing (e.g., satiety could decrease participation which would increase the duration of the test; see Appendix 3). However, the number of uneaten pellets in each strain did not correlate with test duration in Phase 3 or 4 (data not shown).

Interestingly, in fixed consecutive number (FCN) tests, the rat has to press a lever a fixed number of times then press an alternate lever to receive a chocolate pellet reward after completing the correct sequence. Presumably, this experiment studies an alternate definition of impulsivity, namely premature task completion. Thus, an impulsive rat would prematurely end the sequence and, as a result, press the reward lever too soon. The key feature of this procedure, then, is the clear request for a reward, whereas DOR did not distinguish between the action and the request. Of particular relevance to the current DOR study, though, is that the (injected) AMPH, while within the therapeutic

Table 15. A summary of the positive (green upward arrow) and negative (red downward arrow) changes in DOR performance, indicating a decrease or increase in impulsivity, respectively, in the Fast and Slow strain following either the tenth administration of 1.0 mg/kg or at least five administrations of 2.0 mg/kg oral amphetamine doses. The changes depended on, to a certain extent, whether the delay time was increasing (Phase 3) or decreasing (Phase 4) and the strain.

Abbrev:
0 - no effect

Delay time	Fast		Slow	
	l.Omg/kg	2.0 mg/kg	1.0 mg/kg	2.0 mg/kg
Increasing	**ft**	**ft**	**ft**	**ft**
Decreasing	**ft**	**ft**	0	**ft**

dose range (0.2-0.8 mg/kg), increased impulsivity and decreased response rate (Charrier & Thiebot, 1996; Evenden, 1998a,b). Consequently, the AMPH-dosed rats in the FCN procedure continued to request the food reward, thereby indicating that appetite was not affected. Taken together, satiation may not underlie the decrease in response probability nor lever preference seen in the current DOR experiment.

Conclusion

In conclusion, females were different from males. Within the males, the Fast strain tended to lose interest in participation when all the trials had the same delay. Changing the delay, though, elucidated their impulsive profile, albeit after ten days of testing or when the challenge was immediately presented. Regardless, the Delay of Reward procedure adds more evidence that the Fast strain is more impulsive than the Slow strain. AMPH, while affecting the probability of participation, actually decreased impulsivity in both strains provided it was administered at least subchronically. The latter finding was quite interesting. Not only have ADHD medications been reported to improve attention and calm activity levels of ADHD and non-ADHD individuals, but it also suggests that the decrease in impulsivity requires repeated administration. Furthermore, amphetamine's appetite suppression side effect may not underlie these response patterns.

Experiment 4: Differential Reinforcement of Low Rates of Responding

Synopsis

One other way to define impulsivity is an inability to withhold a response even if it means punishment or reward reduction. ADHD patients are typically described with this problem (e.g., blurting out answers, butting in line), thus, a model of ADHD should portray similar patterns of behaviour. The Differential Reinforcement of Low Rates of Responding (DRL) procedure was chosen to investigate this impulsivity definition in Fast and Slow rats. Essentially, the rat must withhold pressing a lever for a certain amount of time. If the rat pressed the lever before the required amount of time had passed, then they either did not get a reward or the reward size was reduced. An impulsive rat, then, would find it challenging to refrain from responding as the withholding time increased or would prefer the smaller reward size, respectively.

Amphetamine (AMPH), when given at low (therapeutic) doses, has been shown to reduce the impulsive behaviours in ADHD patients. Thus, AMPH, in the same dose range, is predicted to decrease impulsive behaviour seen in the DRL experiment. With the Fast strain showing signs of impulsivity in other tests, this strain was expected to perform the DRL test and to respond to AMPH differently than the Slow strain. Based on pilot study results, AMPH had different effects on behaviour depending on the withholding times; thus, AMPH was administered in each phase of the experiment.

Materials and Methods

Animals

Subjects were 24 male Fast and 24 Slow rats (Carleton University), approximately 3 months of age at the start of testing. At that time, the rats were placed on a restricted-quantity food diet; the goal was not to decrease weight but to increase the rat's

motivation to consume the daily treats. Thus, the ideal scenario was to find 1 g of food remaining at the start of each day's testing.

The rats were double-housed until 4 months of age then single-housed in standard opaque plastic cages (32 x 22 x 20 cm) and maintained on a 12-h light/dark cycle. While double- or single-housed, on each testing day, the rats were separated into individual (foodless) cages or the food was removed, respectively, at least 20 minutes before the chocolate hazelnut treat was administered. After the days' testing, which occurred five days a week during the light cycle, the double-housed rats were returned to their home cages; this step was not necessary when single-housed. The rats were then fed between 15 to 30 g of food, depending on their weight; water was freely available. On non-testing days, food and water were freely available. The rats were weighed once a week, on the day before AMPH administration.

Experimental Procedure

Chocolate Hazelnut Treat Consumption Training and Chocolate Pellet Exposure

Exposure to the chocolate hazelnut and chocolate pellet treats started at least one week before lever-press training. See Appendix 2 for the 'Chocolate Hazelnut Treat Consumption' methodology. Briefly, the rats were given a small amount of the treat on a dish, once a day, until they consumed it consistently and readily (within approximately 1 minute). Similarly, 5 to 10 pellets were placed in the home cage, once a day.

Apparatus

The apparatus was an operant chamber (Model H10-11R-TC; Coulbourn Instruments, Allentown, PA). The chamber, 28.5 x 25.5 x 27 cm, was fitted with one lever (Model H21-03R; Coulbourn Instruments, Allentown, PA) and a food pellet dispenser (Model H14-22R-45; Coulbourn Instruments, Allentown, PA). Chocolate food

pellets (45g, Product # F0299; Bio-Serv, Frenchtown, NJ) were delivered to a tray (Model H14-01R; Coulbourn Instruments, Allentown, PA) placed to the left of the lever, both approximately 1.5 cm above the floor. The tray was a recessed cup (width 3.4 cm x height 4.1 cm x depth 2.5 cm) that was illuminated overhead with a magazine light to signal the pellet delivery (45mg chocolate pellet, Product # F0299; Bio-Serv, Frenchtown, NJ), and was equipped with a photocell and detector (Model H20-93; Coulbourn Instruments, Allentown, PA) to register entry. A second light placed just below the roof of the chamber, centred above the tray, served as a houselight. Each chamber was housed in a separate soundproof box and controlled by the Graphic State Notation programming language.

Training

Following familiarization to both treats, the rats were placed in a lever-less operant chamber with 20 pellets in the trough; they were free to explore for 20 minutes. On the next day, a pellet dropped every 30 seconds into the trough for 20 minutes. Thereafter, the rats were trained to press the lever, which delivered 1 pellet, under continuous reinforcement (CRF). Training under CRF continued until a criterion of either (a) 2 consecutive days of 50 rewarded presses within 10 minutes or (b) consistently close to 50 rewarded presses over three or four days. As in Experiment 3, some rats had trouble learning how much force was required to press the lever; those rats received 'assistance' (see Appendix 8). Importantly, most of these rats needed just one day of 'assistance.'

Differential Reinforcements of Low Rates of Responding

The DRL procedure was based on that used by Bull et al. (2000). After training, each rat went through 5 five-day phases of increasing withholding (WH) times: 0, 2, 5, 10

and 20 seconds each on subsequent weeks. If the rat pressed the lever after the WH time had passed, then one pellet was delivered. Otherwise, the chamber's houselight turned off for a 2-sec time-out and the clock was reset back to zero. Each day's testing lasted 20 minutes.

Following those five phases, the sixth phase was designed to see if the reward size was affected by the amount of time the rat withheld a response. Essentially, there was no reward (plus a 2-sec time out) if the lever was pressed before 10 sec had passed, 1 pellet was delivered (after a 2-sec time out) if they pressed the lever between 10 and 20 sec, or they were rewarded with 3 pellets if they withheld responding for 20 sec or more. On Day 1 of this phase, the rats learned that withholding a response delivered 3 pellets (i.e., same procedure as Phase 5 was used except that a rewarded press delivered 3 pellets, not 1). Then, from Days 2 to 5, the rat experienced the 1 vs. 3 pellet procedure.

Drug Administration

Placebo (plain chocolate hazelnut spread) was administered to all rats on Days 1 to 4. On the fifth day of each phase, Placebo or Amphetamine (AMPH), at 1.0 mg/kg p.o. and 2.0 mg/kg p.o. mixed in the chocolate hazelnut treat, was administered. See Appendix 2 for the 'Chocolate Hazelnut Treat Consumption' methodology. Testing started 20 minutes after consuming the treat. Any unconsumed dosed-treat was weighed. After testing on Day 5, the rats were observed for differences in activity level at 20-minute intervals.

Analysis

The level of statistical significance was set at $p < .05$.

Taste Aversion

Taste aversion was defined by incomplete consumption of the dosed treat. In those cases, the rat was placed into the drug group that most closely matched their consumed AMPH dose. Logistic regression was used to examine the odds of developing taste aversion depending on the Strain and Drug group.

Lever Presses

The variables examined fell into two general categories: unmodified and calculated. For the unmodified category, the variables were (a) total number of lever presses, (b) total number of time outs, and (c) total rewarded presses. In the calculated category, the variables were determined by comparing the unmodified variables. For instance, the proportion of rewarded presses was calculated from the presses that produced a reaction (i.e., a time out or a reward): 100%*number of rewarded presses/(number of rewarded presses + number of time outs). In contrast, those lever presses that produced no reaction were entered as extraneous lever presses. These variables were combined in ways to reflect participation (i.e., willingness to press the lever) and performance (i.e., correctly press the lever for a reward, inferring impulsivity). For example, a decrease in rewarded presses with no change in extraneous presses would indicate a decrease in performance, but decreases in both variables represented a decrease in participation.

The Between-groups variables were Strain (2 levels: Slow and Fast) and, when appropriate, Drug (3 levels: Placebo, 1.0 mg/kg and 2.0 mg/kg). The primary Within-subjects variables were Phase and Day, the levels of which depended on the analysis. For the most part, Phase had 5 (Phases 1 to 5 or Phases 2 to 6) or 6 (Phases 1 to 6) levels, while Day had 2 (Days 4 and 5) or 4 (Days 1, 2, 3 and 4) levels. Then, for the final phase

(Phase 6), an additional Within-subjects variable, Reward size (2 levels: 1 pellet and 3 pellets), and the Day variable had either 2 (Days 4 and 5), 3 (Days 2, 3, and 4) or 4 (Days 1 to 4) levels. Unfortunately, due to changing drug group membership between phases (see Table 16; more below), the Phase variable could not be a Within-Subjects variable for the drug analyses.

Data distribution

Outliers were removed, when necessary. Some variables' data were not normally distributed. For those variables, the data set was transformed with either square root or log 10 arithmetic, as indicated in each section, when relevant.

Results

Taste Aversion and Sample sizes

While there were 24 rats of each strain, not every rat consumed their entire dosed treat. In those cases, the rat was placed into the drug group that most closely matched their consumed amphetamine dose; thus, not each drug group had n = 8. As expected, none of the Placebo rats showed taste aversion. Logistic regression revealed a significant interaction between the AMPH groups and Strain (xi = 11.5, $p < .002$). Interestingly, none of the Slow 1.0 mg/kg group showed taste aversion. Further testing showed that the AMPH-dosed Fast strain had greater odds of taste aversion than the Slow strain ($x2^2$ = 21.2, x^2 $p < -001$, odds ratio = 2.7). Then, within the Fast strain, the 2.0 mg/kg group had a greater odds of taste aversion than 1.0 mg/kg group (xi = 6.6, $p < .011$, odds ratio = 3.9).

Total Number of Lever Presses

For Days 1 to 4, there were two significant interactions: Phase by Strain ($F(5,42) = 7.5$, $p < .001$) and Phase by Day ($F(15,32) = 2.8$, $p < .008$). Analyses were separated

Table 16. The number of rats, per Strain, in each Drug group for the six withholding time (WH) phases. Not every rat consumed their entire dosed treat. In those cases, the rat was placed into the drug group that most closely matched their consumed amphetamine dose.

* - not included in analyses

Phase	WH time	Drug Group	Slow	Fast
1	Os	Placebo	8	8
		l.Omg/kg	8	8
		2.0 mg/kg	8	8
2	2s	Placebo	8	8
		1.0 mg/kg	8	8
		2.0 mg/kg	8	8
3	5s	Placebo	8	9
		l.Omg/kg	9	8
		2.0 mg/kg	7	7
4	10s	Placebo	9	8
		1.0 mg/kg	8	14
		2.0 mg/kg	7	2*
5	20s	Placebo	8	9
		l.Omg/kg	9	8
		2.0 mg/kg	7	7
6	10 or 20s	Placebo	11	11
		1.0 mg/kg	8	9
		2.0 mg/kg	5	4

into each individual phase. Each phase was examined, first, for strain differences over Days 1 to 4 and, second, for Strain and Drug differences from Day 4 to Day 5.

Phase 1: Withholding Os

Days 1-4

Initially, the number of presses started off low then increased to a plateau from Days 2 to 4. On each of the four days, the Slow strain pressed the lever more than the Fast strain. See Figure 9.

Days 4 vs. 5

Further analyses found significant decreases from Day 4 only in the AMPH-dosed groups, in a strain-dependent manner. In the Slow strain, both dosed groups had fewer lever presses than Day 4 but there was no difference between Drug groups. As for the Fast strain, the decrease was seen at the highest dose; this group was also significantly lower than the Slow strain, Placebo and (approaching significance, $p < .063$) 1.0 mg/kg. See Figure 9.

Phase 2: Withholding 2s

Days 1-4

Both strains started with an initially high number of presses on Day 1 and decreased to a minimum on Day 3. There were no Strain differences. See Figure 9.

Days 4 vs. 5

Further analyses found significant Day effects only in the AMPH-dosed groups but in a strain-dependent manner. At the lowest dose, the Fast strain had fewer lever presses than both the previous day and the Slow strain. With the highest dose, both strains had a decrease from Day 4 that was also lower than Placebo and (almost significant, $p < .072$) 1.0 mg/kg. See Figure 9.

Figure 9. Total number of lever presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) Os, (b) 2s and (c) 5s. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine.

Abbrev:
& - effect of Strain
D1, D3, D4 - different from Day 1, 3 or 4, respectively
P - different from Placebo
1.0- different from 1.0 mg/kg
"__" - approaching significance, $p < .075$
* - Day 3 was almost significantly lower than Day 1 when WH was 5 s ($p < .075$)

Total Lever Press +/- SEM
Total Lever Press +/- SEM
Total Lever Press +/- SEM
D1. D3
Both Strains:
D4,P
2.0
2

Phase 3: Withholding 5s

Days 1-4

More presses were evident on the first day compared to the Day 2, Day 3 (approaching significance, $p < .075$) and Day 4. The means suggested a Strain effect on Day 4. Indeed, while the Repeated Measures' Strain effect was not significant, a one-way ANOVA found a significant Strain effect on Day 4. See Figure 9.

Days 4 vs. 5

The Slow strain pressed the lever more than the Fast strain. Both AMPH-dosed groups had a significant decrease in lever pressing from the fourth Day, in both strains, that was lower than Placebo. See Figure 9.

Phase 4: Withholding 10s

Days 1-4

The number of lever presses did not differ between strains or over days. See Figure 10.

Days 4 vs. 5

For this phase, the Fast strain had two rats in the 2.0 mg/kg drug group; it was excluded from analyses but the means were included in the graph. For the remaining Fast strain drug groups, there were no significant differences. In the Slow strain, only the Slow 2.0 had an almost significant decrease from Day 4 ($p < .059$). See Figure 10.

Phase 5: Withholding 20s

Days 1-4

The Fast strain pressed the lever more on the second and fourth days compared to the first day; the Slow strain did not change over days. From Days 2 to 4, the Fast strain pressed the lever more than the Slow strain. See Figure 10.

Figure 10. Total number of lever presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 10s, (b) 20s and (c) 10 vs. 20s. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine. For the WH 10s Phase, the Fast strain had two rats in the 2.0 mg/kg Drug group; it was excluded from analyses but the means were included in the graph.

Abbrev:
& - effect of Strain
D1, D2, D4 - different from Day 1, 2, or 4, respectively
P - different from Placebo
1.0 - different from 1.0 mg/kg
"__" - approaching significance, $p < .085$

Total Lever Press +/- SEM
Total Lever Press +/- SEM
Total Lever Press +/- SEM
Slow
Placebo
1.0
2.0

Days 4 vs. 5

The Fast strain pressed the lever more than the Slow strain; Day and Drug effects were not significant. This conclusion did not match up with the means, though; thus, analysis was redone by focusing on each data point. Indeed, one-way ANOVA found that the Strain effect continued in the Placebo group on Day 5. In the AMPH-dosed groups, the strains had similar number of lever presses. The only Day effect was found in the Slow strain's 1.0 mg/kg, albeit approaching significance ($p < .085$). See Figure 10.

Phase 6: Withholding 10 or 20s

Days 1-4

Only the Slow strain had changes over Days, with a lower number of presses on Day 1 and an almost significant increase from Day 2 to Day 4 ($p < .060$). On Day 3, the Slow strain had more lever presses than the Fast strain. See Figure 10.

Days 4 vs. 5

Both strains' high dose groups showed a decrease in the number of lever pressing from Day 4 that was also lower than Placebo and (almost significantly, $p < .067$) 1.0 mg/kg groups. See Figure 10.

Total Number of Time Outs

Time outs (TO), when the house light was turned off for 2 sec, were triggered if the rat pressed the lever before the withholding time had elapsed. Consequently, only WH times of 2 sec or more were examined for differences in TO. Repeated Measures analysis of Days 1 to 4 revealed a significant three-way interaction between Phase, Strain and Day ($F(12,35) = 2.8$, $p < .010$). Analyses were separated into each individual phase. Each phase was examined, first, for strain differences over Days 1 to 4 and, second, for Strain and Drug differences from Day 4 to Day 5.

Phase 2: Withholding 2s

Days 1-4

Both strains followed a similar pattern of decrease in the number of TO over Days but the overall timing differed. The Slow strain started off with the highest number of TO on the first day, followed by a daily decrease to a low on the third Day then a slight increase on Day 4. With the Fast strain, the decrease took an extra day to become evident: Days 1 and 2 had the highest number of TO and Days 3 and 4 had the lowest number. On Day 3, the Fast strain had almost significantly more TO than the Slow strain ($p<.095$). See Figure 11.

Days 4 vs. 5

Significant decreases in TO were seen only in the AMPH-dosed groups: at both doses in the Fast strain and at the high dose in the Slow strain. The other Slow dose-group, 1.0 mg/kg, though, had significantly more TO than the Fast strain and (almost significantly, $p < .092$) Placebo group. See Figure 11.

Phase 3: Withholding 5s

Days 1-4

The Slow strain had more overall TO than the Fast strain when WH was 5s. There was no change in TO numbers over Days. See Figure 11.

Days 4 vs. 5

Significant decreases in TO were seen only in the AMPH-dosed groups in both strains. In the Fast strain, the high dose was also significantly lower than Placebo. See Figure 11.

Phase 4: Withholding 10s

Days 1-4

Neither Day nor Strain had a significant effect. See Figure 11.

Figure 11. Total number of Time outs on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 2s, (b) 5s, (c) 10s, (d) 20s or (e) 10s vs. 20s. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine. NOTE: the Fast 2.0 mg/kg group had 2 rats in the WHIOs Phase, thus, this group was excluded from analysis but the means are provided.

Abbrev:
& - effect of Strain
D1, D2, D4 - different from Day 1, 2 or 4, respectively
P - different from Placebo
1.0 - different from 1.0 mg/kg
"__" - approaching significance ($p < .096$)

Mean Time outs +/- SEM
Mean Time outs +/- SEM
Mean Time outs +/- SEM
D1
D1,D2
D1,D2
Day
Day
Both
B

Days 4 vs. 5

The Fast 2.0 mg/kg group had 2 rats, thus, this group was excluded from analysis but the means are provided on Figure 11. The remaining Fast Drug groups (Placebo, 1.0 mg/kg) did not have any significant Day or Drug group effects. As for the Slow strain, only the high dose had a significant decrease from Day 4.

Phase 5: Withholding 20s

Days 1-4

There was a significant increase in the number of TO from Day 1 to Day 2 and to Day 4 in the Fast strain only. This increase was matched with more TO than the Slow strain on Days 1, 2 (approaching significance, $p < .087$) and 3. See Figure 11.

Days 4 vs. 5

Further testing revealed that only the Slow strain's 1.0 mg/kg Drug group (1) had a significant increase in TO from Day 4 and (2) was almost significantly higher than the Placebo group ($p < .096$). See Figure 11.

Phase 6: Withholding 10 or 20s

Days 1-4

The first Day's TO mean was higher than Days 2 to 4. The Strain effect was not significant. See Figure 11.

Days 4 vs. 5

The 2.0 mg/kg dose group was associated with a decrease in the number of TO in both strains relative to Day 4, Placebo and 1.0 mg/kg. See Figure 11.

Total Number of Rewarded Presses

Provided the rat pressed the lever after the WH elapsed, they received a reward. The number of rewarded presses was examined, both for changes over time (i.e., over Days and Phases) and for differences between Strain and, when applicable, Drug groups.

With Phases 1 to 5, the total number of rewarded presses was straightforward. In contrast, with Phase 6, there were two kinds of rewarded presses: those that delivered 1 pellet or 3 pellets. However, in order to include Phase 6 in the phase comparisons analysis, the two kinds of rewarded presses were added together to reflect the overall total of rewarded presses. Then, for the Phase 6 analysis, the two reward sizes were analyzed separately.

For Days 1-4, there were significant interactions between Phase and Strain ($F(5,42) = 6.1$, $p < .001$), Phase and Day ($F(15,32) = 30.7$, $p < .001$) and Day and Strain ($F(3,44) = 2.9$, $p < .047$). Analyses were separated into each individual phase. Each phase was examined, first, for strain differences over Days 1 to 4 and, second, for Strain and Drug differences from Day 4 to Day 5. As for Phase 6, there was a slightly different analysis procedure, due to the addition of the Reward variable (more below).

Phase 1: Withholding 0s

Days 1-4

The Slow strain successfully pressed the lever more than the Fast strain. See Figure 12.

Days 4 vs. 5

The Strain effect remained in the Placebo (approaching significance, $p < .063$) and 2.0 mg/kg groups. Significant decreases from Day 4 performances were found only in the AMPH-dosed groups. In the Slow strain, this decrease was evident at the low dose. In contrast, at the highest dose, both strains had a significant decrease from Day 4 that was lower than Placebo; the Fast 2.0 mg/kg group was also lower than 1.0 mg/kg. See Figure 12.

Figure 12. Total number of Rewarded presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) Os, (b) 2s and (c) 5s. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine.

Abbrev:
& - effect of Strain
D1, D2, D3, D4 - different from Day 1, 2, 3 or 4, respectively
P - different from Placebo
1.0 - different from 1.0 mg/kg
"_" - approaching significance ($p < .068$)

Mean Reward Press +/- SEM
Mean Reward Press +/- SEM
Mean Reward Press +/- SEM
Placebo
1.0
2.0
Both strains: P

Phase 2: Withholding 2s

Days 1-4

The Slow strain successfully pressed the lever more than the Fast strain. See Figure 12.

Days 4 vs. 5

The Slow strain continued to have more rewarded presses than the Fast strain. All of the AMPH-dosed groups had a significant decrease from Day 4. Of these drugged groups, however, only the high dose was almost significantly lower than Placebo ($p < .057$). Additionally, the Fast 2.0 mg/kg group was lower than 1.0 mg/kg.

Only the Fast 2.0 mg/kg had significantly fewer rewarded presses than the other Drug groups. While the Slow 2.0 mg/kg mean suggested similar comparisons, this did not appear to be the case. However, if the Slow and Fast strain's were combined, then the 2.0 mg/kg dose was almost significantly lower than Placebo ($p < .057$). See Figure 12.

Phase 3: Withholding 5s

Days 1-4

There was a general trend towards increased successful presses in both strains, but in a Strain-dependent manner. In the Slow strain, the lowest number of rewarded presses occurred on the first Day; by the fourth Day, the number had increased to be higher than Day 2 (almost significant, $p < .068$) and Day 3. As for the Fast strain, the increase was not evident until the third Day; both the third and fourth Days were significantly higher than the first two Days. Overall, the Slow strain had more rewarded presses than the Fast strain. See Figure 12.

Days 4 vs. 5

Significant decreases from Day 4 performances occurred in the Fast and Slow AMPH-dosed groups only. The decrease in the 2.0 mg/kg groups was also significantly lower than Placebo. The overall Strain effect was significant, too, with the Slow strain successfully pressing the lever more than the Fast strain. See Figure 12.

Phase 4: Withholding 10s

Days 1-4 & Days 4 vs. 5

There were no significant changes over Days, between Strain or between Drug groups (even when the Fast 2.0 mg/kg group was excluded form analysis). See Figure 13.

Phase 5: Withholding 20s

Days 1-4

There was an isolated change over Days in the Slow strain: the second Day had significantly more rewarded presses than the first Day. See Figure 12.

Days 4 vs. 5

Only the 1.0 mg/kg groups had a significant decrease from Day 4. See Figure 13.

Phase 6: Withholding 10 or 20s

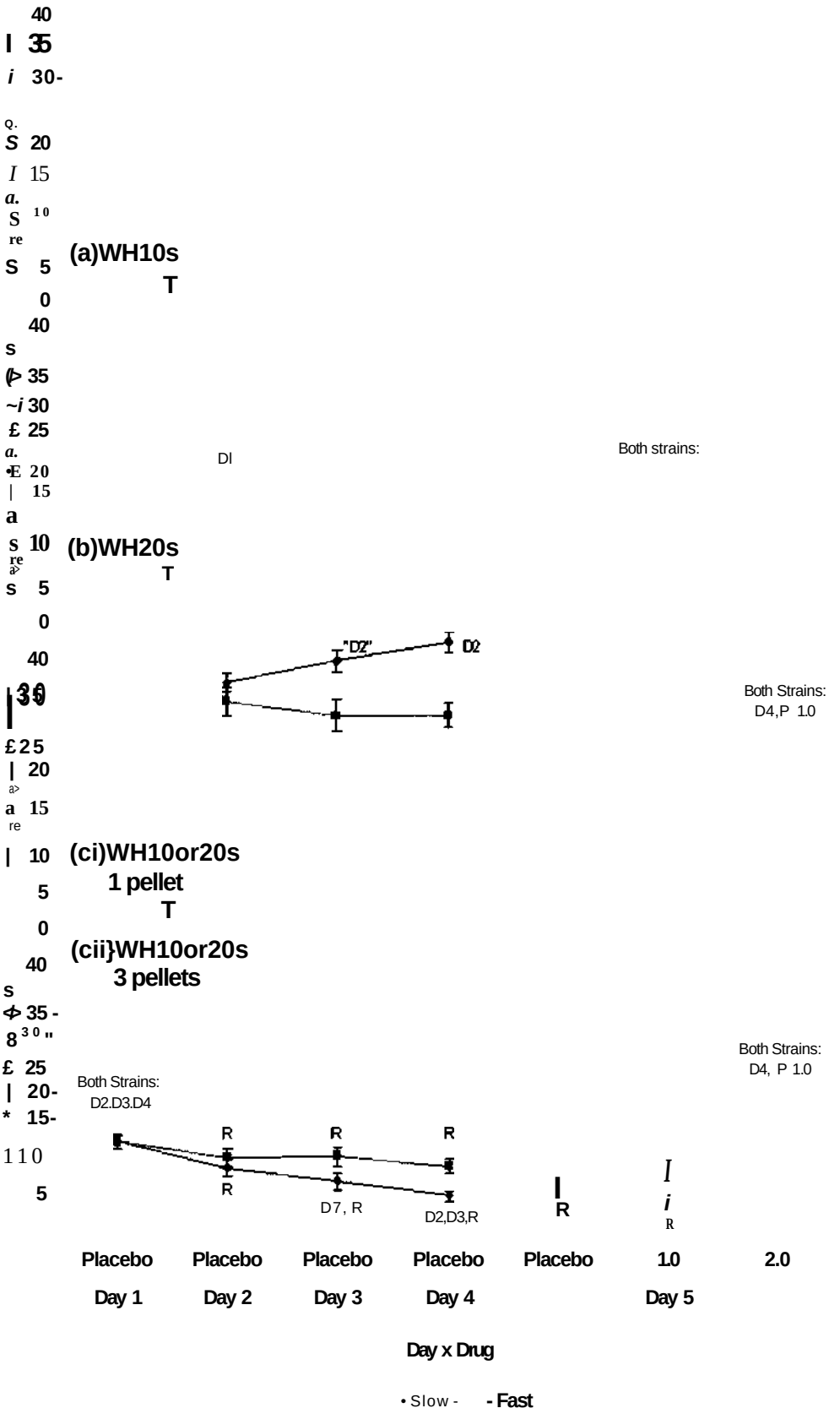
The first Day of this Phase was considered a training day since it was designed for the rat to learn that a correctly withheld press would deliver 3 pellets, not 1. As a result, Day 1 was kept separate from analyses, apart from a comparison with the following three Days to check for differences over time. Days 2 to 5, however, were investigated for changes in presses that delivered 1 or 3 pellets.

Days 1-4: 3-pellets

For the 3-pellet presses, there was a significant interaction between Day and Strain ($F(3,44) = 4.7$, $p < .008$). Initially, both strains had the highest number of 3-pellet

Figure 13. Total number of Rewarded presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 10s and (b) 20s. In Phase 6, when the reward size was correlated with withholding time (WH 10 vs. 20s), the graphs are separated into 1-pellet (ci) and 3-pellet presses (cii). For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine. Fast 2.0 mg/kg had two rats when WH time 10s, thus, it was excluded from the analysis but the means are provided.

Abbrev:
& - effect of Strain
D1, D2, D3, D4 - different from Day 1, 2, 3, and 4, respectively
R - effect of Reward
P - different from Placebo
1.0- different from 1.0 mg/kg
"__" - approaching significance ($.070 < p < .083$)


(a)WH10s
T
(b)WH20s
T
(ci)WH10or20s
1 pellet
T
(cii}WH10or20s
3 pellets
DI
Both strains:
"D2"
D2
Both Strains:
D4,P 1.0
Both Strains:
D2.D3.D4
Both Strains:
D4, P 1.0
R
D7, R
D2,D3,R
Placebo
Placebo
Placebo
Placebo
Placebo
1.0
2.0
Day 1
Day 2
Day 3
Day 4
Day 5
Day x Drug
• Slow - - Fast

presses on Day 1. Further results are outlined in the Days 2-4 section (below). See Figure 13.

Days 2-4:1 pellet vs. 3 pellets

There was a significant three-way interaction between Reward, Day and Strain ($F(2,45) = 9.1$, $p < .001$). Initially, the strains had the same number of presses resulting in 1 pellet; likewise for the 3-pellet presses. On Day 3, though, the strains started to differ. In the Slow strain, there was a gradual decrease in 3-pellet presses (Day 3 was almost significantly lower than Day [$p < .083$], and Day 4 was significantly lower than Days 2 and 3) with a corresponding increase in 1-pellet presses (Days 3 [approaching significance, $p < .070$] and 4 were higher than Day 2). The Fast strain, in contrast, did not change their number of 1- and 3-pellet presses from Days 2 to 4. Consequently, the Slow strain had more 1-pellet but less 3-pellet presses than the Fast strain on Days 3 and 4. As for Reward size, both strains, on all three Days, had more levers presses delivering 1 pellet than 3 pellets. See Figure 13.

Days 4 vs. 5

There were significant interactions between Reward size and Strain ($F(1,42) = 13.2$, $p < .002$) and Reward size, Day and Drug ($F(2,42) = 13.8$, $p < .001$). The Placebo and 1.0 mg/kg groups followed similar patterns: no difference from Day 4 and more presses resulting in 1 pellet. However, only the 1-pellet presses were significantly higher in the Slow strain than the Fast strain with 1.0 mg/kg; the 3-pellet presses with Placebo and 1.0 mg/kg were approaching significance ($p < .107$ and .112, respectively).

The 2.0 mg/kg dose was associated with more complicated changes. In both strains, the number of rewarded presses resulting in 1 pellet decreased from Day 4 to be

significantly lower than Placebo and 1.0 mg/kg. In contrast, with the 3-pellet presses, the opposite occurred: the number increased from Day 4 to be significantly higher than Placebo and 1.0 mg/kg. Interestingly, both strains had the same number of presses resulting in 1 and 3 pellets. See Figure 13.

Proportional (Percent) Rewarded Presses

The presses that triggered a reaction (i.e., a TO or reward) were isolated. From those presses, the proportion (or percent) of presses resulting in a reward was calculated using the following formula: 100*(number of rewarded presses)/[(number of rewarded presses)+(number of TO)]. For Phases 1 to 5, the number of rewarded presses was straightforward. However, for Phase 6 to be included in the Phase comparison analysis, the number of rewarded presses represented the total number of rewarded presses (i.e., combination of the 1- and 3-pellet presses).

Repeated Measures analysis of Days 1 to 4 revealed a significant three-way interaction between Phase, Day and Strain ($F(12,35) = 3.0$, $p < .007$). Analyses were separated into each individual phase. Each phase was examined, first, for strain differences over Days 1 to 4 and, second, for Strain and Drug differences from Day 4 to Day 5. As for Phase 6, there was a slightly different analysis procedure, due to the addition of the Reward variable (more below).

Phase 2: Withholding 2s

Days 1-4

The Slow strain had a higher percent of rewarded presses. There was an increase over Days: Day 2 was higher than Day 1, and Days 3 and 4 were higher than the first two Days. See Figure 14.

Figure 14. Proportion of Rewarded presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 2s and (b) 5s. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine.

Abbrev:
& - effect of Strain
D1, D2, D3, D4 - different from Day 1, 2, 3 and 4, respectively
"__" - approaching significance, $p < .061$

Percent Rewarded +/- SEM
Percent Rewarded +/- SEM
Slow:
D1
D1
D1,D2,D3
Placebo
1.0
Day 5

Days 4 vs. 5

Only the 1.0 mg/kg dose had significant differences from Day 4. Interestingly, though, the strains had opposite changes: the Fast strain increased while the Slow strain decreased the proportion of rewarded presses. See Figure 14.

Phase 3: Withholding 5s

Days 1-4

In the Slow strain, the proportion of rewarded presses started off low then increased to a plateau from Days 2 to 4. In contrast, in the Fast strain, this increase was not evident until the fourth Day and this increase was almost significantly higher than the Slow strain ($p < .061$). See Figure 14.

Days 4 vs. 5

Only the AMPH-dosed groups had significant increases from Day 4, in both strains. See Figure 14.

Phase 4: Withholding 10s

Due to non-normal distribution of this data set, the data was converted with square-root arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed square-root means.

Days 1-4

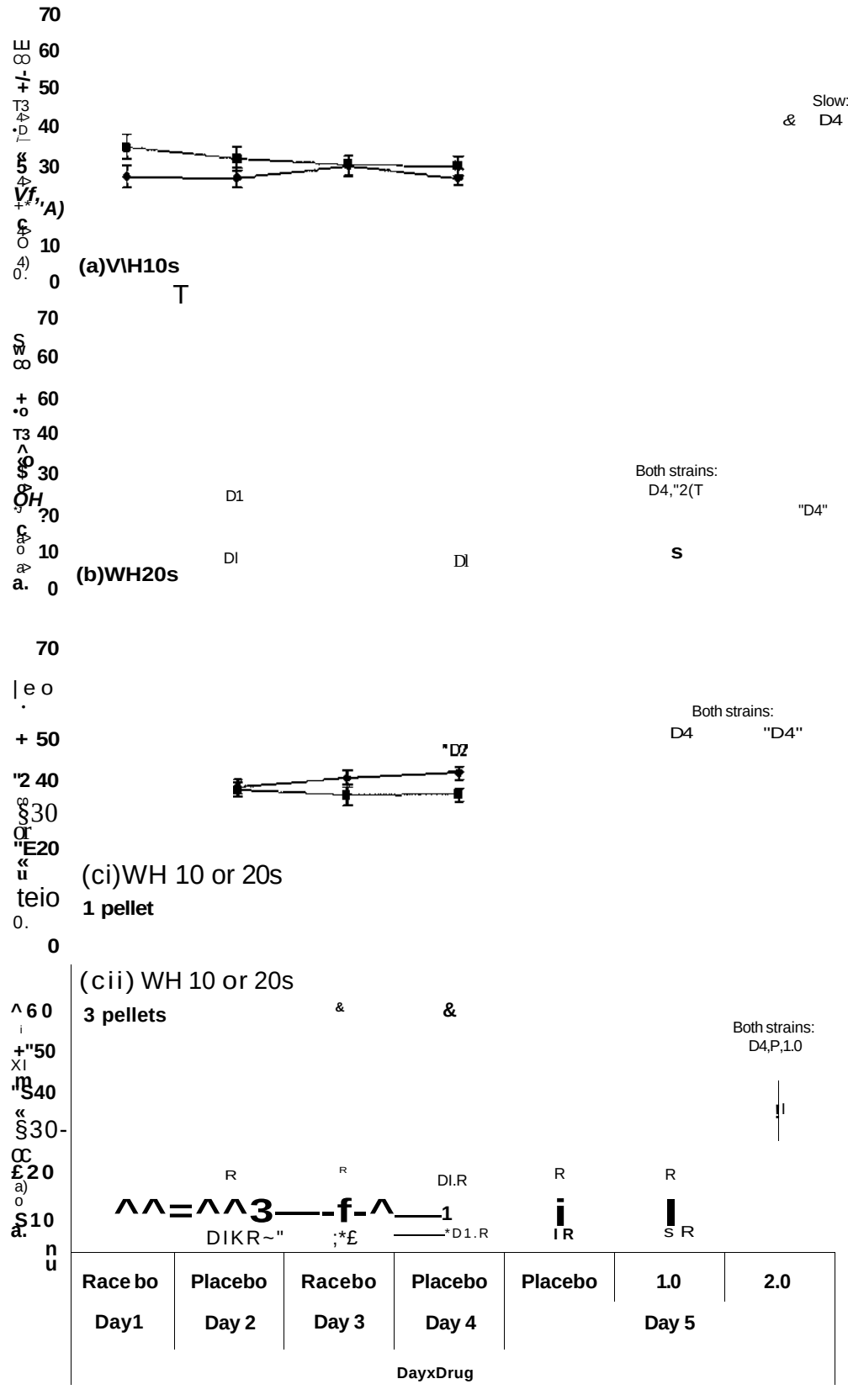
Repeated Measures analysis did not reveal any Day or Strain effect. See Figure 15.

Days 4 vs. 5

The Fast 2.0 mg/kg group had 2 rats, thus, this group was excluded from analysis but the means are provided on Figure 15. The remaining Fast Drug groups (Placebo, 1.0

Figure 15. Proportion of Rewarded presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 10s and (b) 20s. In Phase 6, when the reward size was correlated with withholding time (WH 10 vs. 20s), the graphs are separated into 1-pellet (ci) and 3-pellet presses (cii). For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine. The means for Phase 4 were back-transformed from the square-root while Phases 5 and 6 means were back-transformed from the loglO means. In Phase 4, the Fast 2.0 mg/kg group had 2 rats, thus, this group was excluded from analysis but the means are provided.

Abbrev:
& - effect of Strain
D1, D2, D4 - different from Day 1, 2 and 4, respectively
P - different from Placebo
1.0 - different from 1.0 mg/kg
2.0 - different from 2.0 mg/kg
R - different from 1-pellet
"__" - approaching significance, $p < .097$


70
60
50
40
30
20
10
0
Slow:
& D4
(a)V\H10s
T
Both strains:
D4,"2(T
"D4"
D1
Dl
Dl
s
(b)WH20s
Both strains:
D4 "D4"
"D2
(ci)WH 10 or 20s
1 pellet
(cii) WH 10 or 20s
3 pellets
&
&
Both strains:
D4,P,1.0
R
R
DI.R
R
R
DIKR~"
;*£
*D1.R
I R
s R
Race bo
Day1
Placebo
Day 2
Racebo
Day 3
Placebo
Day 4
Placebo
1.0
2.0
Day 5
DayxDrug
-Slow
-Fast

mg/kg) did not have any significant Day or Drug group effects. As for the Slow strain, only the high dose had a significant increase from Day 4.

Phase 5: Withholding 20s

Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed log 10 means.

Days 1-4

From Day 1 to Day 2, the strains had opposite changes: the Fast decreased while the Slow increased; this was associated with a significant Strain effect. On the third Day, both strains performed the same as Day 1. Then, on the fourth Day, only the Fast strain showed a second drop from Day 1. See Figure 15.

Days 4 vs. 5

Changes from Day 4 occurred only in the AMPH-dosed groups, but in dose- and strain-dependent manner. At the low dose, both strains had a decrease in the proportion of rewarded presses that was also almost significantly lower than the high dose ($p < .061$). With the high dose, the Fast 2.0 mg/kg group had an almost significant ($p < .097$) increase from Day 4. See Figure 15.

Phase 6: Withholding 10 or 20s

To calculate the proportion of 1- and 3-pellet rewarded presses, the number of lever presses delivering 1 pellet and 3 pellets, respectively, was divided by the sum of 1 - pellet, 3-pellet and TO presses. Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed log 10 means. Similar to the total number of rewarded presses, (1) Day 1 was kept separate

from analyses, apart from a comparison with the following three Days to check for differences over time; and (2) Days 2 to 5 were investigated for changes in presses that delivered 1 or 3 pellets.

Days 1-4: 3-pellet

There was a significant interaction between Day and Strain ($F(3,44) = 4.6$, $p < .003$). In Slow strain, the first Day was higher than all four Days while only the Fast strain's fourth Day was lower than the first. Further results are outlined in the Days 2-4 section (below). See Figure 15.

Days 2-4: 1 pellet vs. 3 pellets

There was a significant three-way interaction between Reward size, Day and Strain ($F(2,45) = 4.0$, $p < .027$). The Slow strain showed a decrease in 3-pellet presses (the fourth Day was lower than the second Day) while the 1-pellet presses had a moderate increase (almost significant increase from Day 2 to Day 4, $p < .068$). In contrast, the Fast strain did not have any changes in 1- or 3-pellet presses from Days 2 to 4. These strain-dependent changes resulted in significant Strain effects. On Days 3 and 4, the Fast strain had a higher proportion of rewarded 3-pellet presses than the Slow strain. With the 1-pellet presses, the opposite was found: the Slow strain had significantly higher proportion of rewarded 1-pellet presses than the Fast strain on Day 4. For both strains on Days 2 to 4, a greater proportion of presses resulted in 1 pellet than 3 pellets. See Figure 15.

Days 4 vs. 5

There were significant interactions between Reward size and Strain ($F(1,42) = 8.1$, $p < .008$) and Reward size, Day and Drug ($F(2,42) = 12.4$, $p < .001$). With the 1-pellet presses, both strains had a decrease from Day 4 in the 1.0 mg/kg and (almost significant, $p < .081$) 2.0 mg/kg dose groups; only the 1.0 mg/kg group continued to have

a significant Strain effect. Interestingly, while the Placebo and 1.0 mg/kg drug groups continued to have a higher proportion of 1-pellet rewarded presses, the 2.0 mg/kg did not differ in Reward size. Furthermore, the 2.0 mg/kg drug group, independent of strain, had a significant increase in 3-pellet presses from Day 4, Placebo and 1.0 mg/kg. See Figure 15.

Extraneous Lever Presses: Effort

Extraneous lever presses were the presses that produced no reaction (e.g., the rat continued to press the lever after the previous press was rewarded). Repeated Measures analysis of Days 1 to 4 revealed two significant interactions: Phase by Strain ($F(5,42) = 2.8$, $p < .030$) and Phase by Day ($F(15,32) = 3.6$, $p < .002$). Analyses were separated into each individual phase. Each phase was examined, first, for strain differences over Days 1 to 4 and, second, for Strain and Drug differences from Day 4 to Day 5.

Phase 1: Withholding 0s

Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed loglO means.

Days 1-4

On each of the four days, the Fast strain had significantly more unnecessary lever presses than the Slow strain. The Fast strain had a moderate peak in the extra presses on Day 3 (Day 3 was almost significantly higher than Day 1, $p < .059$). For the Slow strain, Days 3 and 4 were significantly higher than Days 1 and 2. See Figure 16.

Days 4 vs. 5

The Placebo and 1.0 mg/kg continued the first four days' trend: no changes over Days and the Fast strain had more extra presses than the Slow strain. Then, at the highest

Figure 16. The number of extraneous lever presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) Os, (b) 2s and (c) 5 s. Phases 1 and 2 means were back-transformed from the loglO means. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine.

& - effect of Strain
D1, D2, D3, D4 - different from Day 1, 2, 3 and 4, respectively
P - different from Placebo
"__" - almost significantly different ($p < .069$)

Extra Presses +/- SEM
Extra Presses +/- SEM
Extra Presses +/- SEM
WH 2s
Sh-^S
Place
Day
Place
Day
Place
Day
2.0
strains:

dose, the Fast strain had a significant decrease in extraneous presses from Day 4 that was also lower than Placebo; the Slow strain had no differences. See Figure 16.

Phase 2: Withholding 2s

Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed loglO means.

Days 1-4

The Fast strain had more unnecessary lever presses than the Slow strain. Both strains, though, had a parallel decreased from an initial high on Day 1 to the lowest number of extra presses on Day 3. See Figure 16.

Days 4 vs. 5

All AMPH-dosed groups had a decrease from Day 4. See Figure 16.

Phase 3: Withholding 5s

Days 1-4

The Fast strain started with a high number of extra presses followed by a gradual decrease to Day 4. With the Slow strain, the number of extraneous presses also started high but the number quickly decreased to a plateau from Day 2 onwards. On Day 2, the Fast strain had significantly more unnecessary presses than the Slow strain. See Figure 16.

Days 4 vs. 5

All groups had a decrease from Day 4. Interestingly, though, the Placebo group had more extraneous presses than 1.0 mg/kg and (almost significant, $p < .069$) 2.0 mg/kg. See Figure 16.

Phase 4: Withholding 10s

Due to non-normal distribution of this data set, the data was converted with square root arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed square-root means.

Days 1-4

The Fast strain had more unnecessary lever presses than the Slow strain. Neither strain had any significant changes over Days. See Figure 17.

Days 4 vs. 5

The Fast 2.0 mg/kg group had 2 rats, thus, this group was excluded from analysis but the means are provided on Figure 17. With the remaining groups, there were no significant changes from Day 4. In the Placebo group, the Strain effect was significant with the Fast strain having more extraneous presses than the Slow strain.

Phase 5: Withholding 20s

Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed log 10 means.

Days 1-4

Interestingly, the strains had opposite changes over Days: the Fast strain had an increase in extraneous presses from Day 1 to Day 2 while the Slow strain had fewer on Day 3. From Days 2 to 4, the Fast strain had significantly more unnecessary presses than the Slow strain. See Figure 17.

Days 4 vs. 5

The Fast strain had more extra presses than the Slow strain. Neither Day nor Drug effects were significant. See Figure 17.

Figure 17. The number of extraneous lever presses on Days 1 to 5 in the Fast and Slow strains when withholding (WH) time was (a) 10s, (b) 20s and (c) 10 vs. 20s. Phases 1 and 2 means were back-transformed from the log 10 means. For Days 1 to 4, all rats received Placebo. On Day 5, rats received either Placebo, 1.0 mg/kg or 2.0 mg/kg amphetamine. The means for Phase 4 were back-transformed from the square-root while Phases 5 and 6 means were back-transformed from the loglO means. In Phase 4, the Fast 2.0 mg/kg group had 2 rats, thus, this group was excluded from analysis but the means are provided.

& - effect of Strain
D1, D2, D4 - different from Day 1, 2 and 4, respectively

Extra Presses +/- SEM
b) WH 20s
D1,D2,D4
Extra Presses +/- SEM
Extra Presses +/- SEM
Day 1
Day 8
Day
Day

Phase** 6: **Withholding** 10 or **20s

Due to non-normal distribution of this data set, the data was converted with log 10 arithmetic. Upon transformation, the normality assumption was fine. The means reported in the figure are those from the back-transformed loglO means.

Days 1-4

The Fast strain had more unnecessary presses than the Slow strain. No other effect was significant. See Figure 17.

Days 4 vs. 5

There were no significant Day, Strain or Drug effects. See Figure 17.

Discussion

Taste Aversion

None of the Placebo or Slow strain receiving the low dose showed taste aversion. For the AMPH-dose groups, the Fast strain had over 15X greater odds of taste aversion than the Slow strain. Within the Fast strain, the high dose had almost 50X greater odds of taste aversion than the low dose. These results were different from the Lat Maze and DOR experiments. In the former experiment, neither the strains nor doses differed, while taste aversion occurred in the Slow 2.0 mg/kg group. The reasons for this difference are unclear, particularly since the Lat and DOR experiments had repetitive administration and the DRL had four days of Placebo preceding AMPH. Presumably, acute drug administration, relative to chronic administration, should have had minimal chance to develop taste aversion. Alternatively, this group could recognize the effects of AMPH during treat consumption, thereby implying that AMPH could produce effects 'faster' in the male Fast rats and/or a greater ability to learn. Otherwise, the reasons are unclear.

Days 1 to 4 - all Placebo

There were two types of manipulation of the withholding (WH) time variable: it was either fixed (Phases 1 to 5) or depended, to a certain extent, on the rat (Phase 6). In the former manipulation, the WH time went from 0 to 2, 5, 10 then 20s every sixth day. In the latter manipulation, the reward size was correlated with how long the rat WH from pressing the lever: if they waited from 10 to 20s, they received 1 pellet; more than 20s, they received 3 pellets.

Fixed WH time

From Phases 1 to 3 (WH 0, 2 and 5 s), the strains differed primarily in magnitude, but had similar changes over time. In the first Phase, when there was no WH time, the Slow strain pressed the lever more and had more rewarded presses but the Fast strain had a higher number of unnecessary presses. With the addition of the first WH phase (2s), the strains did not differ in the total number of lever presses and time outs, but the Fast and Slow strains continue to have more unnecessary presses and rewarded presses, respectively. With a slightly longer WH time (5s), the strains had the same number of lever presses, but the Slow strain started to exert more effort to receive a reward (e.g., higher TO). Interestingly, since there was an increase in rewarded presses, it appeared as though their strategy worked. The Fast strain, in contrast, continued to exert too much unnecessary effort to obtain a reward (e.g., higher extraneous presses). Thus, while the WH time was either non-existent or very short, the Slow strain appeared to have a greater ability to press the lever when appropriate and, as a result, received more reward for their effort. Furthermore, the strains appeared to be positively modifying their behaviour in response to the longer WH times because both strains had a decrease in total lever presses

and increase in rewarded presses over Days; Phase 2 also showed a decrease in extraneous presses and time outs.

Then, when WH time reached 10s, the performance of each strain changed from that seen at the shorter WH times, suggesting that this intermediate WH time was a turning point, of sorts. For example, the strains were essentially identical (except for extraneous presses) and did not differ on any of the measures over Days. Then, with WH time of 20s, the pattern of performance was either opposite from the WH time of 0 to 5 s and/or strain-dependent changes over Days emerged. For instance, the total number of presses and time outs were higher in the Slow strain when the WH time was WHOs and WH5s, respectively; however, when WH time was 20s, the Fast strain was higher. Interestingly, the Slow strain showed signs of positively modifying their lever pressing in response to the large increase in WH time, as indicated by a general increase in rewarded presses (total number and proportional) and decrease in extraneous presses. The Fast strain, in contrast, appeared to be negatively modifying their lever pressing, as demonstrated by the increase in total presses, time outs and unnecessary presses, and decrease in proportional rewarded presses.

Reward size correlated with withholding time

When the rats were able to choose between (a) a shorter WH time but smaller reward and (b) a longer WH time but larger reward, both strains opted for the former. Granted, the strains did differ in the overall number of presses delivering each reward size (the Slow strain was greater and lower than the Fast strain, respectively). Furthermore, the Slow strain was showing a shift to selecting the smaller reward more

and more as the days progressed while, in contrast, the Fast strain had very little change over days.

The strains did not differ in TO or extraneous presses. The former decreased over days, indicating positive change in their lever pressing. The latter is particularly interesting, though, for two reasons. First, until this phase, the Fast strain had more unnecessary presses than the Slow strain. Second, this was different from Phase 4, even though Phase 6 was almost identical to Phase 4. Essentially, a press before 10s resulted in a time out while a press after 10s produced a reward and any extraneous press had no effect on the outcome. Interestingly, the Fast strain had a decrease in unnecessary presses from Phase 4 to Phase 6; the Slow strain did not. The only other strain-dependent change between the phases was a decrease in total presses in the Fast strain. Consequently, a possible reason for the overlapping extraneous press means between strains in Phase 6 could be an overall decrease in pressing in the Fast strain. While these decreases may be affected by time or learning (and the interjected Phase 5), Phases 5 to 6 had a different pattern of changes (for example, no difference in total lever presses and extraneous presses), suggesting overall lever press modification to the longer WH times had not occurred.

Summary

When the WH time was short (5s or less), the Slow strain performed better and the Fast strain exerted too much unnecessary effort. The latter could signify an inability to maximize reward, another definition of impulsivity. Despite these strain differences, it appeared as though both strains were positively changing their behaviour in response to the increase in withholding time. With the intermediate WH time (10s), there were no

signs that either strain was positively or negatively affected. In fact, the strains were essentially identical, except that the Fast strain continued to over-press the lever. Then, during the last fixed WH time Phase (20s), the Slow strain appeared to be positively modifying their behaviour while the Fast strain showed signs of decreased performance. Thus, when the WH time was very long, the Fast strain appeared to be unable to tolerate delay, fulfilling another definition of impulsivity. During the last Phase, in which the reward size was correlated with WH time, both strains appeared to prefer the smaller reward, with the Slow strain displaying a greater preference. Interestingly, a preference for a smaller, more immediate reward is another definition of impulsivity.

In summary, when the WH time (or reward amount) was fixed, the Fast strain was more impulsive (i.e., was not able to maximum reward during the easy Phases and performed poorly when the WH time was very long). In contrast, when the WH time and reward size was (to a certain extent) under the control of the rat, both strains had an impulsive profile (i.e., preferred the smaller, immediate reward), with the Slow strain showing the more extreme impulsivity profile of the two strains.

Day 5 - Amphetamine Effects

Placebo

For all the measures in all Phases, excluding one exception, the Placebo group did not have any significant changes from Day 4. The exception occurred in Phase 3 because there was a decrease in the number of extraneous presses. However, this measure had been on the decline from Day 1 and remained higher than the AMPH groups, thus, the decrease on Day 5 should be a continuation of this tendency.

Amphetamine: 1.0 or 2.0 mg/kg

The vast majority of AMPH-induced changes were found when the WH time was (a) fixed and short (5 s or less) and (b) correlated with reward size (Phase 6). With the remaining two Phases (WH 10 or 20s), the effects were seen in isolated groups. Consequently, the results will be divided into three main sections: short fixed WH time, long fixed WH time and WH time correlated with reward size. The changes seemed to fall either under the category of performance (i.e., correctly press the lever for a reward, inferring impulsivity) or participation (i.e., willingness to press the lever).

Granted, altered participation could result in altered performance, but it is the overall pattern of changes that was important. During the first Phase (no WH needed), performance and participation were inferred from uncorrelated and correlated changes in the number of rewarded presses and extraneous presses. For example, a decrease in rewarded presses with no change in extraneous presses would indicate a decrease in performance, but decreases in both variables represented a decrease in participation. When the rat had to WH from pressing the lever, a change in performance was reflected in the proportion of rewarded presses, while participation was interpreted from the pattern of changes in the other variables.

Short fixed WH time

Slow

AMPH appeared to have mixed effects on the Slow strain's ability to successfully perform the DRL experiment when the WH time was short and fixed. Interestingly, the doses had similar outcomes, to a certain extent. When there was no need to WH a response (Phase 1), both doses had a decrease in total number of lever presses and rewarded presses, but no effect on extraneous presses. Then, when this strain had to start

withholding a response (Phase 2), both doses had decreases in the number of rewarded presses and extraneous presses. The doses, though, differed in the number of time outs and proportional rewarded presses. With the low dose, there were almost significantly more TO than Placebo and a decrease in proportional rewarded presses. In contrast, the high dose group had a decrease in TO, but no change in proportional rewarded presses. With a slightly longer withholding time (5 s), both doses had decreases in the number of rewarded presses, but also decreases in time outs and extraneous presses, and increases in proportional rewarded presses. Consequently, both doses of AMPH appeared to have an overall negative impact on the Slow strain's performance when the strain was not challenged (WH Os), but a positive impact when a moderate amount of WH was required (WH 5s). The doses differed when a minute amount of self-control was necessary (WH 2s): the low and high doses had a negative effect on performance and participation, respectively.

Fast

In terms of changes from Day 4 in the Fast strain, there were more similarities than dissimilarities between doses, namely a (1) decrease in total number of lever presses, rewarded lever presses, extraneous lever presses, and, when appropriate, time outs and (2) increase in proportional rewarded presses (except for no change in the 2.0 mg/kg during the second Phase), when appropriate. The main dissimilarities, though, centre on the emergence of these day effects. With the low dose, the AMPH-induced changes were seen when withholding was required (Phases 2 and 3); otherwise, AMPH had no effect (e.g., in Phase 1). In contrast to the low dose, the high dose had effects in all of the first three phases.

Additionally, the AMPH doses could be distinguished. For instance, while only day effects were found in the AMPH groups, the low dose did not differ from Placebo; the one exception was fewer extraneous presses during the third phase. The high dose, though, had many more measures lower than Placebo (aside from a couple exceptions, all three phases differed in total number of lever presses, rewarded presses and extraneous presses) and, to a lesser extent, the low dose (Phases 1 and 2 differed in total number of lever presses and rewarded presses). Interestingly, only the high dose had fewer time outs than Placebo when the WH time was 5s. The one measure to buck this trend was the proportional rewarded presses because the Drug groups did not differ. As a result, it appeared as though AMPH either decreased the Fast strain's participation (Phase 1 or 2 with 2.0 mg/kg) or increased performance (Phase 2 with 1.0 mg/kg or Phase 3 with both doses); importantly, it didn't negatively impact on performance.

Long fixed WH time

At the longer withholding times, the low and high doses tended to have opposite effects on performance in a Phase-dependent manner. With the low dose, there was no change in Phase 4 (WH 10s) variables, but an increase in time outs and decreases in the number of rewarded and proportional rewarded presses in both strains in Phase 5 (WH 20s). In fact, during the latter Phase, the 1.0 mg/kg dose's proportional rewarded presses were almost significantly lower than the high dose. With the high dose, the Slow strain had decreases in time outs and an increase in proportional rewarded presses in Phase 4; the Fast strain did not show any differences. At the longest WH time (20s), there was primarily no change except for an almost significant increase in proportional rewarded presses in the Fast strain. Consequently, the low dose had a negative effect on performance in both strains when they were overly challenged, potentially worse than the

high dose. In contrast, the high dose had a positive effect on the Slow and Fast strains' performance when they were moderately (WH 10s) or overly challenged (WH 20s), respectively.

WH time correlated with reward size

When there was a choice between reward size and WH time, AMPH changed both strains' performance, most notably with the 2.0 mg/kg dose. The only instance of the low dose affecting any of the measures was a decrease in the percent of rewarded 1-pellet presses in both strains. Since the number of time outs, number of rewarded 1-pellet presses or 3-pellet measures did not change, this suggested a decrease in participation. With the high dose, on the other hand, there was a concrete pattern of performance alterations in both strains: (1) decreases in time outs and number of rewarded 1-pellet presses that ended up being *lower* than Placebo and 1.0 mg/kg, and (2) increases in the number of rewarded 3-pellet presses and percent of 3-pellet presses that ended up being *higher* than Placebo and 1.0 mg/kg. The percent of 1-pellet presses also decreased (almost significantly) from the previous Day but there were no Drug group differences. Interestingly, the more telling signs of the high AMPH dose's effects may not be the Day or Drug group effects. At this dose, the strains had the same number and percent of rewarded 1- and 3-pellet presses while the other Drug groups maintained a preference for the 1-pellet presses. Consequently, the low dose appeared to decrease the participation of the strains. In contrast, the strains were more successful at waiting for the larger reward with the high AMPH dose.

Summary

For all the measures in all Phases, the Placebo group did not have any unique significant changes from Day 4. In contrast, the changes were solely evident in the

AMPH-dose groups and were a mix of positive and negative effects on DRL performance (i.e., proportional rewarded presses) and/or participation (i.e., willingness to press the lever).

Participation

Both doses had negative impacts on participation; there was never an increase in participation. The low dose did not decrease participation in both strains until there was a correlation between reward size and WH time. The high dose decreased participation in (a) the Fast strain when challenge was non-existent (e.g., WH Os) and (b) both strains when a small amount of self-control was necessary (WH 2s).

Performance

Interestingly, both strains had a positive change in DRL performance following both doses of AMPH when the challenge was moderate (WH 5s). Outside of that phase, either dose had negative and positive effects on performance. For instance, the low dose decreased performance in the Slow strain when withholding was not necessary (WH Os). Then, when a small amount of self-control was needed (WH 2s), this dose had opposite effects on the strains' performance: the Slow strain's performance decreased while the Fast strain increased. Both strains, though, had a decrease in performance when there was a high degree of challenge (WH 20s) with the low dose having a greater negative impact. The high dose (a) decreased the Slow strain's performance when challenge was non-existent (e.g., WH Os), (b) increased performance in the Fast and Slow strains when challenge was high (Phases 4 (WH 10s) and 5 (WH 20s), respectively) and (c) increased both strains' ability to wait for the larger reward. A summary of amphetamine's effects on impulsivity are provided in Table 17.

Table 17. A summary of the positive (green upward arrow) and negative (red downward arrow) changes in DRL performance, indicating a decrease or increase in impulsivity, respectively, in the Fast and Slow strain following either the 1.0 mg/kg or 2.0 mg/kg oral amphetamine doses.

Abbrev:
0 - no effect

WH time	Fast		Slow	
	l.Omg/kg	2.0 mg/kg	1.0 mg/kg	2.0 mg/kg
Os	0	0	£	U
2s	ft	0	&	0
5s	ft	tt	TT	tr
10s	0	0	0	ft
20s	(potentially worse than high dose)	tr	(potentially worse than high dose)	0

Conclusion

In conclusion, both strains met the impulsivity definitions: inability to maximize reward, inability to tolerate delay and preference for smaller/immediate reward. The Fast strain satisfied all three definitions, while the Slow strain met the latter definition. Following oral amphetamine, the pattern of positive and negative changes in impulsivity suggest that a dose should be tailored to the degree of (relative) challenge present in the task; one dose won't have complete advantage over another. Additionally, it is important to note that, while a decrease in overall participation resulted in a decrease in premature responses, premature responses were not the only measure of impulsivity; rewarded effort (or overall performance) needed to be considered.

Overall Conclusion

ADHD Monoamine Circuitry

Breeding the seizure- prone and -resistant strains may have affected the basal catecholamines systems within the prefrontal cortex, but likely did not affect prefrontal monoamine release mechanisms. The behavioural and neurological ramifications are speculative at this point. Since NE can have an anti-epileptic impact (Shin et al., 2004) and increases after stressors (Westerink, 1995), the higher concentrations in the Slow strain than both the Fast strain and published articles suggest that the Slow strain's NE levels are likely related to their seizure-resistant and/or anxious phenotype. Another ADHD model, SHR, has an elevated level of basal NE in the frontal cortex (de Villiers et al., 1995), though, which directly contrasts with the Fast strain results. Granted, altered frontal NE function, in general, has been linked with ADHD, thus, this could be another manifestation of the pathology. In addition, there are several receptor subtypes for NE (beta and three alphas identified so far), some of which have opposite actions (hyperpolarizing versus depolarizing) on the postsynaptic membrane (Mclntyre & Wong, 1986). Much more needs to be known about these subtypes and the neural networks in which they predominate.

It has been previously suggested that the Fast strain's PFC DA neurons under-reacted to environmental stimuli (Anisman et al., 2000). The lower basal DA echoes this suggestion, indicating a hypoactive MCL DA system and matches with ADHD DA theories. To date, no study has been done on the strains' striatum, thus, no conclusion about the NS system is possible. In summary, the strains differ not only in NE and DA activity, but, as discussed on Appendix 4, also likely in the interaction between DA and

5HT. Thus, evidence is pointing to altered PFC monoamine circuitry and, by extension, their behavioural correlates of attention, learning and behavioural inhibition.

ADHD Behaviours

Attention

The strains differ in basal NSA, with the Fast strain appearing to have an NSA profile similar to other ADHD models, relative to the Slow strain. Importantly, these differences initially appear subtle (e.g., evident during the first two minutes) but, with repeated exposure, the strains expressed more concretely differing NSA profiles. Consequently, in addition to having a more ADHD-like NSA profile, the Fast strain had an altered habituation to the Lat Maze.

Impulsivity

Females performed differently from Males on the impulsivity tests. At this point, whether this reflects the 9 boy: 1 girl ratio seen in human ADHD or indicates different impulsivity profiles is unclear. Evidence for the former comes from the fact that the Fast females had an ADHD-like NSA profile relative to the Slow females. On the other hand, the Fast females were indistinguishable from the Slow females on the impulsivity tests, lending support to the latter. More tests are needed to compare the sexes.

Based on the DRL and DOR performances, it appears as though the male Fast strain was showing signs of poor sustained attention and impulsivity, as demonstrated by decreased participation over time, inability to maximize reward, inability to tolerate delay and preference for smaller/immediate reward. By combining those outcomes with the notably high activity level, the Fast strain displayed the hallmark behaviours of the ADHD-Combined subtype: poor sustained attention, impulsivity and hyperactivity.

Interestingly, the male Slow strain had some signs of impulsivity as the Slow strain showed a greater preference for the smaller/immediate reward over the larger/delayed reward than the Fast strain in the DRL paradigm. The reasons for this are unclear. Perhaps sample size (e.g., 11 vs. 5 per strain in DRL and DOR, respectively) or the programming (e.g., approximately 80 trials vs. 12 trials in DRL and DOR, respectively). Both possibilities could account for a lack-of-difference on the first day of DOR only, however; the fifth and tenth days showed a clear preference for the smaller reward in both strains. Aside from experimental-design aspects, it could be due to a previously unidentified difference between the strains, such as poor internal timing in the Slow strain, or the Fast strain's poor sustained attention increased the odds of waiting for the larger reward. Essentially, when the Fast rat opted to do anything but press the lever, the WH time passed and increased the odds that the next lever press resulted in the large reward. Indeed, on many occasions, the Fast strain was observed to be exploring an area away from the lever, not waiting by the lever for the next time to press. In the end, however, the strains had the same number of total lever presses, time outs and extraneous lever presses. Thus, in this case, a 'less is more' strategy technically benefited the Fast strain in the DRL paradigm.

Amphetamine

Attention, as measured by non-selective attention, was negatively affected by acute AMPH at all doses administered. For a quick summary, see Table 18. With subchronic administration (3 days), the lower doses were no longer different from Placebo. Important to note, this does not infer a positive effect, rather a decrease in the negative impact and/or habituation to the drug. Perhaps even longer administration would lead to a positive effect (as happened in the SHR strain with *injected*

Table 18. A summary of amphetamine's effects depending on the amount of self-control needed ('None', 'Moderate' or 'High') in the attentional (Lat Maze) and impulsivity (DOR and DRL) behavioural experiments. The effects are indicated as either positive (e.g., decreased impulsivity; green upward arrow) or negative (e.g., more ADHD-like NSA profile or increased impulsivity; red downward arrow) following 1.0, 2.0 or 5.0 mg/kg oral amphetamine doses in the Fast and Slow strain. If the effect depended on the number of administrations, it was indicated.

Abbrev:
0 - no effect
C - following subchronic, chronic administration
E - earlier than Slow strain

Self-control	Dose (mg/kg)	Experiment	Change in ADHD behaviours	
			Fast	Slow
None	1.0	DOR	0	0
		DRL	0	&
		Lat	&	JJ
	2.0	DOR	0	0
		DRL	0	£
		Lat	£	£
	5.0	Lat	&	ft
Moderate	1.0	DOR	0	0
		DRL	ft(E)	IT
	2.0	DOR	0	0
		DRL	ft	ft
High	1.0	DOR	tto	fto
		DRL	£	11
	2.0	DOR	•Qco	ft(Q
		DRL	ft	ft

methylphenidate; Aspide et al., 2000), but this data set could not answer that question. The highest dose (5.0 mg/kg) continued to have a negative impact. Thus, the ADHD drug did not have any positive effects on this measure of attention; rather, all effects were negative.

The general theme from the oral AMPH administration's effect on impulsivity is that both strains responded similarly to both doses, with slight variations depending on the innate impulsivity, degree of challenge (i.e., amount of self-control needed) and number of administrations; a summary is provided in Table 18. With a moderate amount of challenge, both doses increased the strains' ability to withhold a response, with a positive change evident earlier in the more impulsive Fast strain at the lowest dose, and increased the preference for the larger/delayed reward. With a high degree of challenge, both strains' ability to withhold a response decreased with the 1.0 mg/kg dose but increased with the 2.0 mg/kg dose. Chronic (or, at least subchronic) administration, in contrast, increased both strains' ability to tolerate delay at the 1.0 and 2.0 mg/kg doses.

These patterns raise some interesting points. First, the strains were similarly affected. Not only have ADHD medications been reported to improve attention and calm activity levels of ADHD and non-ADHD individuals, but an ADHD model should respond to AMPH in a similar manner as a non-ADHD model. Granted, the more impulsive strain had a slightly earlier positive response, but the strains tended to overlap. Second, the different administration duration and challenge-dependent effects suggest task-specific impacts, not global improvement or setbacks. Otherwise, these doses tended to decrease performance or participation. Unfortunately, the high dose was not as well tolerated as the low dose in the rats. Consequently, these patterns could suggest that

the dose should be tailored to the (relative) degree of challenge present in the task; one dose may not have complete advantage over another.

Administration method

Following the first exposure to the treat, the rats consumed the chocolate hazelnut treat at faster and faster rates until a plateau of approximately 1 to 1.5 minutes was reached in approximately 10-12 days. This speed was maintained in the lowest AMPH-dosed groups, while the latency increased in the highest-dosed group, indicating that the high dose was not well-tolerated in both strains. There were inconsistencies between the oral and published injected AMPH PFC pharmacokinetics. As a result, any future investigations into the therapeutic effects of stimulants should use oral administration, just like with human ADHD, in order to elucidate results more relevant to the human ADHD.

Side effects

Appetite

The Fast strain's appetite was most likely unaffected, indicated that another factor, such as poor sustained attention, contributed to their decreased participation and the number of uneaten pellets. The Slow strain's high-dosed group may have had a decreased appetite, based on their performance on the appetite tests and the number of uneaten pellets. However, the strain's natural tendency to freeze may have biased their appetite test results. Furthermore, their performance on the DOR experiment indicates that decreased appetite, if present, was not a major contributing factor to the overall outcome.

Addiction

The fact that oral 5.0 mg/kg AMPH produced stereotypy raises a couple interesting points. First, stereotypy is a model for drug addiction. With the Slow, but not the Fast, strain displaying this behaviour, this suggests that the Slow strain could be more sensitive to the addictive properties of this drug class. The other interesting point is the presence of stereotypy itself- even at a supposedly low/therapeutic dose. Previously, this behaviour has been linked with high doses of injected AMPH, particularly when injected chronically. While the 5.0 mg/kg dose could be considered high, this dose was calculated from published plasma AMPH concentrations at therapeutic doses. Consequently, the published values are either too high, where the reported therapeutic concentrations of AMPH can have addiction qualities or the Slow strain has an increased susceptibility to addiction. More tests are recommended to investigate the strains' addiction profile.

Conclusion

In conclusion, the male Fast strain shows signs consistent with ADHD models according to those provided by Sagvolden (2000), namely differences within the catecholamine systems, little to no learning deficits (independent of their impulsivity), sustained hyperactivity with attention deficits and poor inhibitory control, response to a comparable dose of stimulant medications that is the same in control animals (unlike the SHR) and no sensitization. As for no signs of tolerance to or long-term effects of the drug, additional tests are needed because the current experiment designs could not answer these points. Granted, some of these results are relative to the Slow strain; thus, the tests should be rerun with additional controls, such as the Long Evans Hooded rat, to elucidate the full extent of their attention and impulsivity behaviours. Regardless, evidence is

pointing towards the Fast strain's overall profile closely resembling the ADHD-Combined subtype.

Oral AMPH had different effects than injected AMPH, suggesting that any future investigations into the therapeutic use of AMPH should follow an oral AMPH administration method. Oral AMPH had both positive and negative effects. Primarily, if the rat was not challenged, attention and impulsivity was negatively affected. If the rat was challenged, the AMPH could have a positive impact on impulsivity, depending on the (relative) degree of challenge. Thus, the administration should be tailored to the task but it won't have a global improvement in all ADHD behaviours. Furthermore, oral AMPH could be addictive, albeit depending on the underlying neurochemistry.

Future Projects

More studies are recommended, for example: microdialysis of the striatum and nucleus accumbens; compare Fast and Slow strains to the out-bred control (Long Evans Hooded) and directly to the SHRs; elucidate any tolerance and/or long-term effects of the drug; stimulant addiction profiles; more in-depth examination of female ADHD behaviours; examine the sizes/function of the PFC, striatum and nucleus accumbens; investigate GAB A drugs' effects on ADHD behaviours; alter the function of the different monoamine pathways with targeted drug and/or lesion studies; test the strains' response to methylphenidate (Ritalin®); stimulant effects on epileptogenesis; male Fast and Slow NSA profiles; and investigate catecholamine storage and release mechanisms.

Appendix 1: Reasons to administer amphetamine orally

There are three issues that are relevant to ADHD research: extrapolation, pharmaco-kinetics and -dynamics, and long-term consequences. These issues are centered around the administration routes, routes that have a large impact on how the brain reacts to the drug. As a result, the route should be chosen carefully so that it matches the intended human scenario.

Issue 1: Extrapolation Problems

Research on the effects of stimulants in animals has utilized experimental models that are more relevant to the abuse of stimulants (injection of high doses) rather than to their therapeutic use (low doses administered orally) (Diaz Heijtz et al., 2003; Pashko & Vogel, 1980; Vitiello, 2001; Volkow & Insel, 2003). Thus, extrapolation of the animal data to clinical use is difficult.

Issue 2: Different Pharmacokinetics and Pharmacodynamics

The amount of AMPH entering the brain and plasma differs depending on the route of administration. There are three ways to examine these differences: time to maximum concentration (Tmax; see Table 19), half-life *(TVi;* see Table 20) and concentration changes over time (see Tables 21 and 22).

All three routes, regardless of dose, have similar time to peak *plasma* AMPH concentration (see Table 19). The duration, though, is different: the gavage peak lasts for at least one hour (more below). Injections have the same time to peak *brain* AMPH concentration, even though the doses are different. Gavage, in contrast, resulted in a much later peak. Interestingly, similar divergence between injection and oral

Table 19. Time to maximum AMPH concentration (Tmax) in plasma and brain following gavage, i.p. or s.c. administration of different doses.

	Plasma	Brain
Gavage	0.067 mg/kg: within 15 min and lasted for at least 1 hour (Pashko & Vogel, 1980)	5mg/kg: ~ 90 min (Janicke et al., 1989)
i.p.	5mg/kg: 10 min (Kuhn & Schanberg, 1978)	5mg/kg: 20 min (Kuhn & Schanberg, 1978)
s.c.	0.5 mg/kg: 5-15 min (Diaz Heijtz et al., 2003) 8.0 mg/kg: 12 min (Cho et al., 1999)	8.0 mg/kg: 22 min (Kuczenski et al., 1997)

Table 20. Half-life (TV2) of AMPH in plasma and brain following gavage, i.v., i.p., or s.c. administration of different doses.

Abbrev:
NTP - National Toxicology Program

	Plasma	Brain
Gavage	0.067 mg/kg: 63 min; similar as higher doses (Pashko & Vogel, 1980)	< 20 mg/kg: 2-3 h (NTP, 2005) 5 mg/kg: ~1 hr (Janicke et al., 1989)
i.v.	In general: ~ 1 hr (Melega et al., 1995) 0.5 mg/kg: -90 min (NTP, 1991)	In general: ~ 30 min (Melega et al., 1995) 0.5 mg/kg: ~1 hr (NTP, 1991) 5 mg/kg: ~ 1 hr (Janicke et al., 1989) 15 mg/kg: ~ 1 hr (Hutchaleelaha et al., 1994)
i.p.	3 mg/kg: ~1 hr (Groppetti & Costa, 1969) 5.0 mg/kg: 5-9 hr (Kuhn & Schanberg, 1978; NTP, 1991)	2 mg/kg: ~ 1 hr (Lemberger et al., 1970) 3 mg/kg: ~1 hr (Groppetti & Costa, 1969) 5.0 mg/kg: 0.5-0.9 hr (distribution phase)(Kuhn & Schanberg, 1978) and 5-9 hr (elimination phase)(Kuhn & Schanberg, 1978; Lobarinas & Falk, 1999; NTP, 1991) 10 mg/kg: 50 min (Kiely et al., 1987)
s.c.	0.5 mg/kg: ~1 hr (Diaz Heijtz et al., 2003) 8 mg/kg: ~30 min (Cho et al., 1999)	8 mg/kg: -46 min (Cho et al., 1999)

Table 21. Temporal changes in AMPH concentration in plasma (ng/g) following an injection (i.p.) or gavage of 0.067 mg/kg (Pashko & Vogel, 1980). The percent difference (% Diff.) was calculated by comparing the injection to the gavage concentrations.

	Time after administration			
	15 min	30 min	1hr	2hr
Gavage	-2.3	-2.5	-2.5	-1.0
i.p.	-5.5	-4.2	-4.2	-1.2
% Diff.	250%	170%	170%	120%

administration has been seen with methylphenidate in humans (Swanson & Volkow, 2003).

In general, plasma TVi is listed as approximately 1 hour regardless of dose and administration route; some papers have indicated TV2 of 30 minutes, 90 minutes, or 5 to 9 hours (Table 20). A similar TV2 is seen in brain tissue following an injection: the most common value is approximately 1 hour; some papers have indicated 30 minutes or 5 to 9 hours. The *TV2* following gavage ranged from 1 to 3 hours.

As for temporal changes, while the sources and doses were vastly different, the message was the same: the route affects the amount of AMPH entering the system over time (Tables 21 and 22). Very soon after an i.p. injection (15 min) (Table 21), there was more than double the amount of AMPH in the blood compared to a gavage administration of the same dose. Then, within 15 min, the i.p. route showed a drop and remained at that concentration for at least another 30 minutes; these levels were almost double the gavage values. In contrast, the gavage route remained essentially unchanged for the first hour. Thus, both routes reached maximum concentration levels within 15 minutes but the injected AMPH started to decline thereafter while the gavaged AMPH route remained constant. Two hours after administration, both routes were almost similar (Pashko & Vogel, 1980).

One hour after i.v. injection, there was six-fold more AMPH in the rat brain when compared to the same oral dose (Table 22). Over the next three hours, AMPH decreased more rapidly following the i.v. injection but it still remained approximately twice as high as the oral dose (Janicke et al., 1989). Methylphenidate also had divergent profiles following i.v. and oral administration: whereas methylphenidate concentration in the

Table 22. Temporal changes in AMPH concentration in brain (ng/g) following an injection (i.v.) or gavage of 5 mg/kg* (Janicke et al., 1989). The percent difference (% Diff.) was calculated by comparing the injection to the gavage concentrations.

* - Even though the 5.0 mg/kg dose was approximately 75X higher than 0.067 mg/kg, the brain concentrations were approximately 200-times higher than plasma (see Table 21); this discrepancy was expected because the brain always has higher levels of AMPH than plasma (Cho & Kumagai, 1994; Danielson & Boulton, 1976; Melega et al., 1995)

	Time after administration			
	lhr	1.5 hr	2hr	4h
i.v.	-3000		-1500	-400
Gavage	-500	-1000	-700	-200
% Diff.	600%		210%	200%

human brain reached its peak within 10 minutes after an i.v. injection, the same level was not seen until 90 minutes after oral ingestion. Interestingly, even though the same peak concentration was seen in both cases and they had the same half-life, only the injected methylphenidate resulted in a "high" (Swanson & Volkow, 2003). Thus, route of administration may have an effect on how the brain reacts to the drug.

Issue 3: Different long-term consequences

Further complicating matters was the fact that AMPH needed to be administered repeatedly. However, this typically resulted in behavioural sensitization (e.g., stereotypy), a model for drug abuse. While stereotypy was seen following injections of 1.5 mg/kg or greater (Segal & Kuczenski, 1994), there was evidence that even lower doses (0.1 to 1.0 mg/kg s.c.) could produce mild behavioural sensitization (Kuczenski & Segal, 1989, 2001). Gavage may also trigger this phenomenon following nine days of 1.5 mg/kg (Banjaw & Schmidt, 2005). Granted, this was the only article reporting this effect. Indeed, another study did not find sensitization with doses ranging from 0.5 to 8 mg/kg (Lobarinas & Falk, 1999). Thus, repeated administration (primarily injections) will result in an altered behavioural response, something that is not seen in clinical settings.

Summary

To summarize, (a) extrapolation of the animal data from injection studies to clinical use was difficult, (b) route of administration may have an effect on how the brain reacted to the drug and (c) repeated administration (primarily injections) resulted in an altered behavioural response, something that is not seen in clinical settings. The dose, though, remained to be determined. In the following section, the therapeutic properties of AMPH were examined in order to lead to the suggested experimental procedure.

Amphetamine's Therapeutic Use

In humans, the lowest plasma AMPH concentration associated with a decrease in ADHD behaviours was 30-40 ng/ml (Angrist et al., 1987; Greenhill et al., 2003; Kupietz et al, 1985; Perez-Reyes et al., 1992). This level was found following 0.22 to 0.25 mg/kg (Angrist et al., 1987; Greenhill et al., 2003), which was well within the effective dose range of 0.2 to 0.6 mg/kg (Diaz Heijtz et al., 2003; Seeman & Madras, 1998; Solanto, 2000). Doubling the dose (but still within the effective dose range) to 0.5 mg/kg resulted in a doubling of the peak plasma levels to 60-70 ng/ml (Brown et al., 1979, 1980).

In experimental settings, the same plasma levels are seen in rats with the doses and administration routes outlined in Table 23. Based on the values in Table 23, if an oral route was to be used, then doses ranging from 2.0 mg/kg to 5.0 mg/kg could be administered. Importantly, the 2.0-5.0 mg/kg gavage dose is a matter of concern because a lower dose (1.5 mg/kg) for nine days produced behavioural sensitization (Banjaw & Schmidt, 2005). Granted, this was the only article reporting this effect.

To administer orally, it is quite common for Adderall (dl-AMPH in 3:1 isomer ratio) to be sprinkled over applesauce then given to children (e.g., Tulloch et al., 2002) - a similar procedure could be used for the rats. While gavage was an option, adding AMPH to a treat may be more agreeable to the recipients (especially the stress-sensitive Slow strain), and may have the same pharmaco-kinetics and -dynamics as gavage. In a pilot study, rats readily consumed a chocolate hazelnut spread (like Nutella®), not applesauce or peanut butter, though.

Table 23. Amphetamine doses resulting in rat plasma concentration of 30-40 or 60-70 ng/ml according to the administration route.

Abbrev:
a - These values were used to calculate the dose resulting in 30-40 ng/ml. Other studies indicate that both plasma (Angrist et al., 1987; Brown et al., 1979, 1980; Greenhill et al., 2003) and brain (Melega et al., 1995) AMPH concentrations follow a linear relationship, thus, half the dose should produce half the plasma concentration.
** - Administering AMPH through the drinking water resulted in highly variable plasma concentration levels. On top of that, most rats decreased their intake of water when AMPH was administered at those doses. Thus, this method was not ideal and was not considered further.

Administration route	Plasma concentration	
	30-40 ng/ml	60-70 ng/ml
Gavage	~2.0 (National Toxicology Program, 2005) to ~2.5 mg/kg (Janicke & Coper 1984)	5.0 mg/kg (Janicke & Coper 1984)[a]
s.c. (Diaz Heijtz et al., 2003)	-0.25 mg/kg	0.5 mg/kg[a]
Via drinking water (Janicke & Coper 1984)**	4-12 mg/kg	7-12 mg/kg

Conclusion

Since AMPH is readily absorbed when administered orally (McGough et al., 2003; National Toxicology Program, 1991; Patrick & Markowitz, 1997), this route should be use in order to more closely examine the therapeutic effects. It could be administered in a chocolate hazelnut treat in doses ranging from 2.0 mg/kg to 5.0 mg/kg.

Appendix 2: Chocolate Hazelnut Treat Consumption Times

Introduction

Since all of the oral amphetamine (AMPH) administration experiments followed the same exposure/training and administration of the chocolate hazelnut treat methodology, this method was isolated for two reasons. First, minimize repetition. Second, underscore the effectiveness of this administration method by analyzing the treat consumption times. The chocolate hazelnut consumption times were collected from the oral AMPH administration experiments, both throughout training and AMPH administration phases. Various days were examined for changes in consumption times.

Materials and Methods

Animals

Subjects were Slow and Fast rats from the other experiments, approximately 3 months of age at the start of training; males and females were combined for each strain. See Table 24 for sample sizes.

Training

Each rat was given approximately 1 g of chocolate hazelnut spread, making note of the time the bowl was placed on the floor of the cage and the time at which that the treat was consumed. The cut-off was 20 minutes; in those cases, a small amount of the treat was put onto some rat chow and left in the cage. After approximately two days of this routine, food restriction commenced in the DOR and DRL experiments. It is important to note that the food was restricted to maintain an interest in the treats, not weight reduction. Furthermore, severe food deprivation (leading to weight reduction) can increase the response to AMPH (Pothos et al., 1005) thereby affecting the test outcomes. Thus, the ideal situation was to find 1 g of food in the cage the next day; if food

Table 24. The number of Slow and Fast rats included in the Training and Amphetamine Administration phases.

			Slow	Fast
Training			62	62
		Drug Group		
Amphetamine Administration	Day 1	Placebo	24	26
		l.Omg/kg	21	19
		2.0 mg/kg	24	23
		5.0 mg/kg	15	14
	First, Middle and Last Days	Placebo	24	25
		1.0 mg/kg	21	19
		2.0 mg/kg	24	23
		5.0 mg/kg	3	3

remained, it was removed at least 20 minutes before administration. The rats were given their food (approximately 15-30 g depending on their weight) after testing. On weekends, food was also freely available; water was always *ad lib*. After at least 5 days of the training routine, the experiments started. In some cases, the training continued until the AMPH-dosed treats commenced.

Amphetamine Administration

The same procedure during Training was used to administer the AMPH-dosed chocolate hazelnut treats; for exact details, see the individual experiments. The amount of Placebo or dosed-treat was based on the rat's weight so that (a) rats with similar weights received a similar amount of chocolate hazelnut treat, regardless of dose, and (b) the amount of treat increased with the rat. Sample amounts are provided in Table 25.

Analysis

To analyze the data, three days were chosen for the Training and AMPH Administration phases: First, Midway and Last. There were two main reasons for that approach. First, not every experiment had the same number of days (for example, the DOR rats continued to receive plain chocolate hazelnut spread throughout their lever-press training; or, the Microdialysis experiment had 1 AMPH day). Second, each experiment had varying administration days (i.e., not daily). See Table 26 for the maximum range of days for each experiment.

For the Training phases, Repeated Measure analysis was used to analyze the Within-Subject variable, Day (3 levels: First, Midway and Last), and the Between-Subjects variable, Strain (2 levels: Slow, Fast). Additionally, the third Day of Training was compared to the First day to underscore how quickly the rats adjusted to the routine;

Table 25. Based on the rat's weight (in g) and drug group (Placebo, 1.0, 2.0 or 5.0 mg/kg), the rat received an amount of amphetamine (AMPH; in mg) mixed in the chocolate hazelnut treat (CHT; in g).

Drag Group	Weight (g)	AMPH (mg)	CHT (g)
Placebo & 1.0 mg/kg	305-314	0.31	0.61
	405-414	0.41	0.81
	505-514	0.51	1.01
2.0 mg/kg	303 - 307	0.61	0.61
	403 - 407	0.81	0.81
	503 - 507	1.01	1.01
5.0 mg/kg	290 - 309	1.5	1.50
	390-409	2.0	2.00
	490 - 509	2.5	2.50

Table 26. Three days were chosen to analyze chocolate hazelnut treat consumption: First, Midway and Last. The range of days from the start of Training or Amphetamine (AMPH) administration phases to Midway (i.e., half-way between the first and last days) and Last Day varied for each experiment. Additionally, the number of Days in which AMPH was administered varied (see 'Admin. Duration').

	Training		AMPH		
	First Day to			First Day to	
Experiment	Midway	Last	Admin. Duration	Midway	Last
Microdialysis	10	24	1		
Lat Maze	6	12	3	1	2
DOR	15	30	11	5	11
DRL	6	12	6	3	6

Strain remained the Between-Subjects variable, as did Day except with two levels (First, Third).

Since the Microdialysis experiment had one AMPH administration day, two separate analysis methods were chosen for the AMPH phases. First, Univariate ANOVA was used to compare the Drug groups (4 levels: Placebo, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/kg) on the first Day. Second, Repeated Measures analysis was also used to analyze the Within-Subject variable, Day (3 levels, as above), and the Between-Subjects variables, Strain (levels as above) and Drug (levels as above). It is important to note the differing sample sizes, as indicated in Table 24. The level of significance in all tests was set to $p < .05$.

Results

Training

Each Days' consumption times were quicker than the previous (Day 1 vs. Day 3: $F(1,122) = 65.3$, $p < .001$; Day 1 vs. Mid-way vs. Last Day: $F(2,121) = 75.7$, $p < .001$). Strain effect was not significant. See Figure 18.

AMPH Administration

First Day

The First Day of AMPH administration had a significant effect of Drug ($F(3,158) = 9.9$, $p < .001$). Post-hoc analysis found that the highest dose group (5.0 mg/kg) took significantly longer to consume their dosed-treat than the other groups. Strain was not significant. See Figure 19.

First vs. Midway vs. Last Days

There were two significant interactions: Day by Strain ($F(2,133) = 3.1$, $p < .049$) and Day by Drug ($F(6,266) = 2.6$, $p < .021$). Additional testing revealed that the 5.0

Figure 18. The amount of time (in minutes) the Fast and Slow strains took to consume their chocolate hazelnut treat on the first, third, midway and last Days during Training (i.e., prior to AMPH administration).

Abbrev:
D - significantly different from the other Days

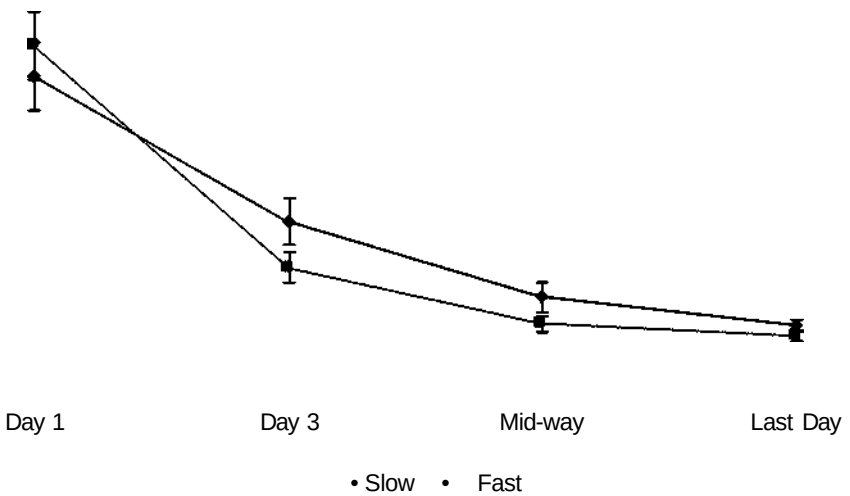
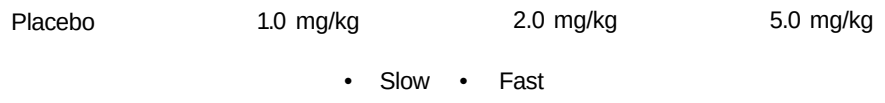

Day 1
Day 3
Mid-way
Last Day
• Slow • Fast

Figure 19. The amount of time (in minutes) the Fast and Slow strains took to consume their chocolate hazelnut treat on the first AMPH Administration Day, depending on if they were in the Placebo, 1.0 mg/kg, 2.0 mg/kg or 5.0 mg/kg AMPH Drug groups.

Abbrev:
* - significantly different from the other Drug Groups


Placebo
1.0 mg/kg
2.0 mg/kg
5.0 mg/kg
• Slow • Fast

mg/kg group took longer to consume the dosed treat as the days progressed. See Figure 20.

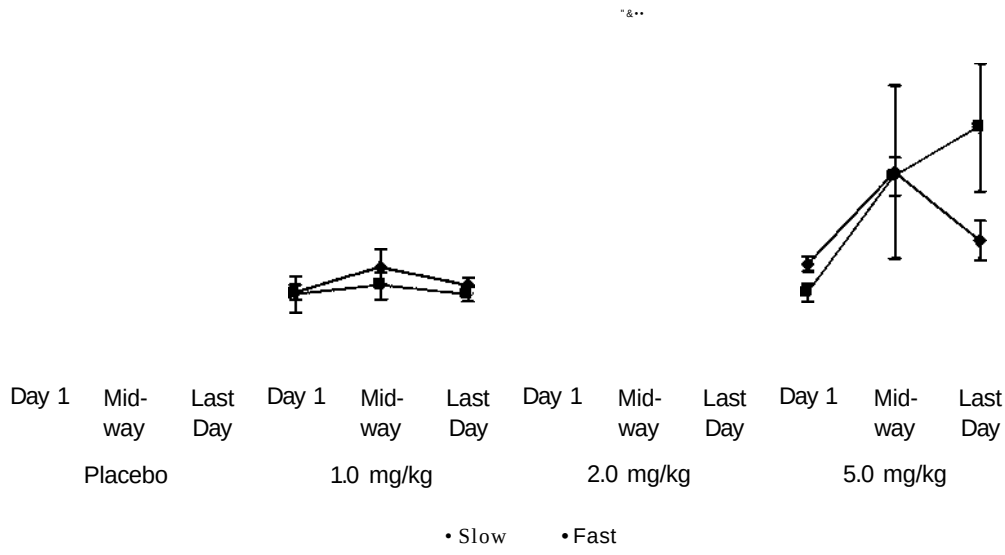
Discussion

From the first to third Day of Training, there was a significant decrease in the amount of time it took the Strains to consume their treat, indicating a rapid adjustment to the treat and the routine in both Strains. Thereafter, the time became faster so that both Strains consumed their treat in approximately one to 1.5 minutes. With AMPH, the low doses were well-tolerated, as indicated by no significant changes over Days or differences from Placebo. Granted, the Slow strain had almost significantly longer consumption times with the 2.0 mg/kg. However, this difference appears to be very small (30 seconds).

The highest dose, 5.0 mg/kg, was not well-tolerated, as demonstrated by an increase in the time it took for both Strains to consume their dosed-treat and an increase relative to the other Drug groups. There is a difference between the First Day analysis and Repeated Measures analysis: the former is higher than the latter. This should be attributable to the administration environment and changes in sample sizes. First, more rats were analyzed in the First Day analysis (6 rats from the Microdialysis experiment and 3 rats from the Lat Maze experiment, totalling 9 per Strain) while only the Lat Maze had repeated administration (n = 3 rats per Strain). Second, the Microdialysis rats received their treat in a novel environment, the microdialysis chamber but the Lat Maze rats had their treat in their home cage. Thus, the novel environment may have skewed the consumption time on that First Day. Regardless, this dose was not easily consumed.

Figure 20. The amount of time (in minutes) the Fast and Slow strains took to consume their chocolate hazelnut treat on the First, Midway and Last AMPH Administration Days, depending on if they were in the Placebo, 1.0 mg/kg, 2.0 mg/kg or 5.0 mg/kg AMPH Drug groups.

Abbrev:
& - effect of Strain ($p < .060$)
D-effect of Day
* - significantly different from the other Drug Groups
"__" - approaching significance ($p < .064$)


Day 1
Mid-way
Last Day
Day 1
Mid-way
Last Day
Day 1
Mid-way
Last Day
Day 1
Mid-way
Last Day
Placebo
1.0 mg/kg
2.0 mg/kg
5.0 mg/kg
• Slow
• Fast

Conclusion

Following the first exposure to the treat, the rats consumed the chocolate hazelnut treat at faster and faster rates until a plateau of approximately 1 to 1.5 minutes was reached in approximately 10-12 days. The low dosed-treats were consumed readily, whereas the high dose was not well-tolerated in either strain.

Appendix 3: Appetite

While a decrease in participation in the behavioural experiments could suggest poor attention, it could also mean a lack of interest in the reward, triggered by amphetamine (AMPH). Indeed, AMPH can suppress appetite, a concern for food reward-based experiments. While this confound could counter-indicate the use of a food reinforcer, using water as a reinforcer (e.g., with water-deprived rats) was not a viable alternative because of increased satiety combined with even lower preference for the larger delayed reinforcer (Chelonis & Logue, 1997). Thus, it is possible that the water reinforcer would increase impulsivity on its own.

Since the Lat Maze indicated that acute AMPH could render both strains more ADHD-like, a methodology should be chosen that could detect an increase *or* decrease in impulsivity, not exacerbate any innate impulsive tendencies, thereby minimizing odds of detecting an increase in impulsivity. Furthermore, the increased satiety with a water reinforcer would prematurely end the test on the rats' terms, leading to missed trials that reflect satiety, not inattention. As a result, to maintain a longer response rate and to not exacerbate the potentially innate impulsive nature of the Fast rat or AMPH-dosed rats, a food reinforcer was viewed as the better option.

Consequently, four procedures were performed after testing. First, the time it took each rat to complete the day of testing was recorded. Second, the number of pellets left in the food trough was counted. This step, however, did not provide any insight as to whether the rat was not interested or hungry; the next two procedures were geared to elucidating either a decrease in appetite or lack of interest in the chosen food reinforcer (chocolate pellets). Third, each rat was given a dish with equal amounts of regular rat

chow and chocolate pellets. Fourth, observe the latency to start consuming their daily food amount.

Presumably, a lack of appetite would be reflected in a rat uninterested in either the freely-offered chocolate pellets or food. Alternatively, selecting the regular food over the chocolate pellets could indicate that the rat was not interested in the reward (or opted for the larger food item). Apart from those outcomes, it is possible to readily consume either option while performing poorly in the behavioural tests, suggesting poor attention rather than appetite. Interestingly, the latter may be occurring in the Fast strain. The Slow strain, in contrast, may have had their appetite suppressed but it may be more likely that they were affected by their innate anxious nature.

Materials and Methods

Animals

The same rats from the Delay of Reward (DOR) and Differential Reinforcement of Low Rates of Responding (DRL) experiments were included in these analyses; females were available only for the DOR uneaten pellets analysis.

Experimental Procedure

Total Time

At the end of DOR testing, the time each rat took to complete the day of testing was recorded. Analysis of the DRL experiment was not necessary because all testing lasted 20 minutes.

Total Time and Uneaten Pellets

At the end of DOR and DRL testing, the number of uneaten pellets in the food trough was recorded.

Chocolate vs. Regular Food

At varying days after the DOR and DRL experiments, a dish was placed in the rat's cage. On this dish was 1 g of regular rat chow and 1 g of chocolate pellets. The rat's first choice (regular food or chocolate pellets) was recorded. After 30 seconds, the dish, and any uneaten food, was removed.

Eat within 30 sec

After the *Chocolate vs. Regular Food* test, the rat was given his daily food and observed for 30 seconds. Whether the rat started to eat within 30 seconds was recorded.

Analysis

The level of statistical significance was set to $p < .05$.

Total Time

Each DOR Phase was analyzed for the time it took the rat to finish the Day's testing. For Phases 1 to 3, both variables had Day (3 levels: Day 1, 5 and 10) as the Within-subject variable, while the Between-subjects variables were Strain (2 levels: Slow, Fast), and, if applicable, Sex (2 levels: Male, Female) and Drug (3 levels: Placebo, 1.0 mg/kg AMPH, 2.0 mg/kg AMPH). For Phase 4, Univariate ANOVA was used to compare the Between-subjects variables of Strain and Drug.

Uneaten Pellets

Repeated Measures analysis was used to examine the number of uneaten pellets for the DOR and DRL experiments. The designs were the same as those experiments. Briefly, the Between-Subjects variables were Strain (2 levels: Slow, Fast) and, when relevant, Sex (2 levels: Female, Male) and Drug (3 levels: Placebo, 1.0 mg/kg, 2.0 mg/kg). The DOR Within-Subjects variable was Day (3 levels: Day 1, 5, 10). The DRL

Within-Subjects variable was also Day but the levels depended on the test: 4 levels to compared Days 1, 2, 3 and 4, 2 levels to compare Days 4 to 5.

***Chocolate vs. Regular Food, Eat within 30** sec*

Each experiment had at least five days of these procedures; the mode response was determined and examined with nominal regression. For *Chocolate vs. Regular Food,* the Dependent Variable was Choice (3 levels: Neither, Regular Food, Chocolate; Chocolate was the reference category) and the Between-Subject variables were Strain (2 levels: Slow, Fast) and Drug (3 levels: Placebo, 1.0 mg/kg, 2.0 mg/kg; Placebo was the reference group). For *Eat within 30 sec,* the Dependent Variable was Eat (2 levels: Yes, No) and the Between-Subject variables were Strain (levels as above) and Drug (levels as above).

Results

Total Time

Each DOR Phase was analyzed for the time it took the rat to finish the Day's testing.

Phase 1 (0ITI, 0 Delay)

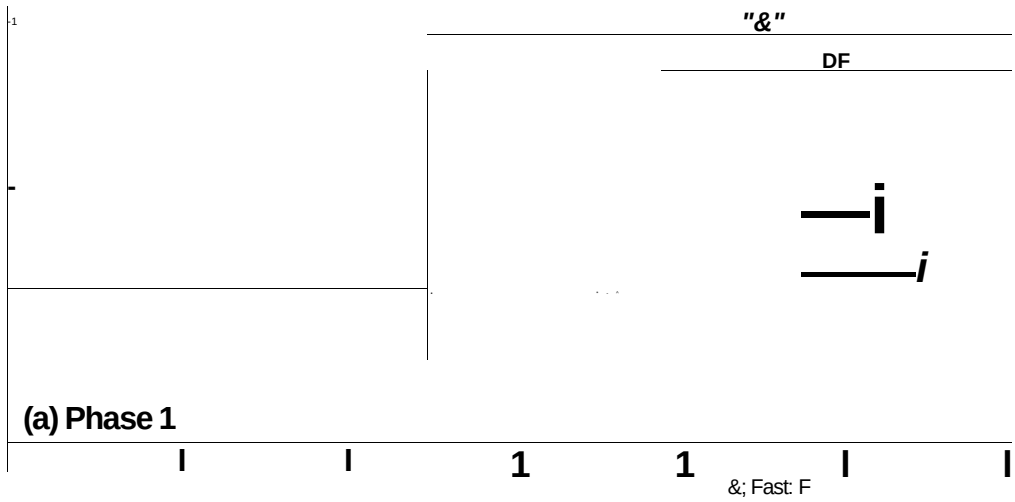
There was a significant interaction between Days and Sex ($F(2,39) = 3.2$, $p < .05$). The males increased the completion time over days, notably in the Fast strain. See Figure 21.

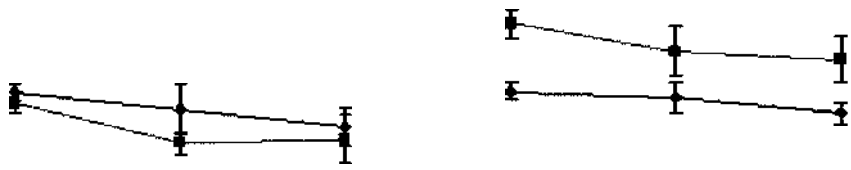
Phase 2 (0-20 ITI, 0 Delay)

There was a significant interaction between Sex and Strain ($F(1,39) = 4.5$, $p < .040$). All groups took less time to complete over days. However, the Fast males took the most time overall. See Figure 21.

Figure 21. The time (in seconds) to complete Day 1, 5 and 10 testing for the Female and Male Fast and Slow strains during (a) Phase 1 and (b) Phase 2.

Abbrev:
D - different from Day 1
F - different from Females
& - different from Slow strain
"__" - almost significant, $p < .089$





(b) Phase 2

Day 1 **Day 5** **Day 10** **Day 1** **Day 5** **Day 10**

Female **Male**

• Slow • Fast

Phase 3 (Os ITI, 0-20s Delay)

There was a significant interaction between Day and Drug ($F(4,46) = 5.2$, $p < .001$) in Phase 3. The Placebo groups took less time to complete over days while the 1.0 mg/kg groups did not change. As for the 2.0 mg/kg groups, the overall trend was an increase in completion time over days and relative to Placebo, notably in the Fast strain. See Figure 22.

Phase 4 (0 ITI, 20-0s Delay)

Univariate ANOVA found a significant Drug effect ($F(2,24) = 9.1$, $p < .002$) with the high dosed groups taking longer to complete than Placebo groups and a tendency ($p < .088$) for this difference compared to the low dose groups. There was no Strain effect. See Figure 23.

Uneaten Pellets

DOR

Phases 1 and 2 - No Delay Trials

Phase 1 and 2 had a trend towards an interaction and a significant interaction, respectively, between the two groups, Sex and Strain (Phase 1: $F(1,38) = 3.9$, $p < .058$; Phase 2: $F(1,40) = 5.3$, $p < .027$). The Male Fast strain appeared to be the major source of the differences with more uneaten pellets than any other group. See Figure 24.

Phase 3 (0 ITI, 0-20 Delay)

While the Strain and Drug interaction was significant ($F(2,23) = 4.6$, $p < .022$), further investigation only trends towards differences between groups. Presumably, the large degree of variability hindered the analysis, even after an outlier was removed. See Figure 25.

Figure 22. The time (in seconds) to complete Day 1, 5 and 10 testing for the Fast and Slow strains following Placebo, 1.0 mg/kg AMPH or 2.0 mg/kg AMPH during Phase 3.

Abbrev:
D - different from Day 1
P - different from Placebo
1 - different from 1.0 mg/kg
& - effect of Strain
"__" - approaching significance ($p < .063$)

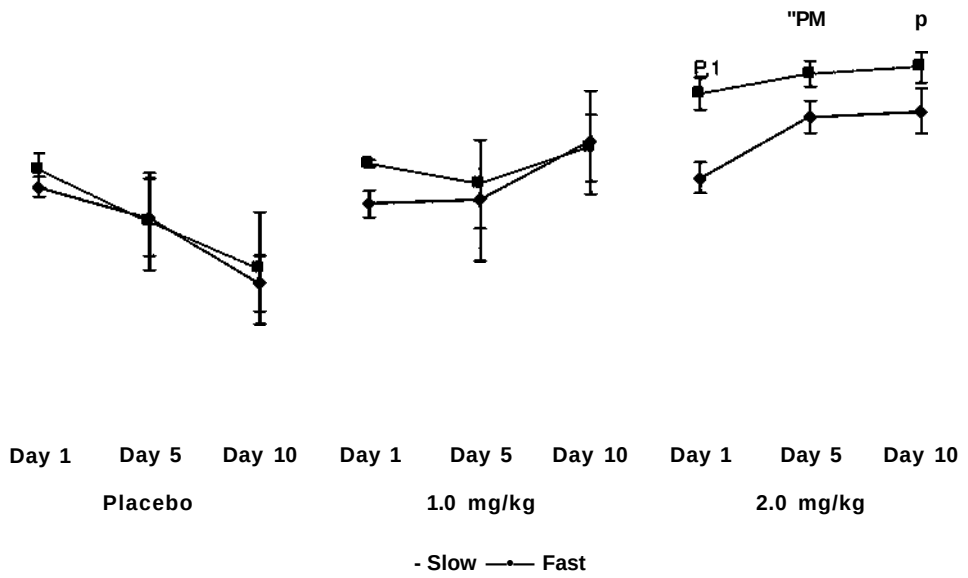

"PM
p
P1
Day 1
Day 5
Day 10
Day 1
Day 5
Day 10
Day 1
Day 5
Day 10
Placebo
1.0 mg/kg
2.0 mg/kg
- Slow —•— Fast

Figure 23. The time (in seconds) to complete Phase 4 testing for the Fast and Slow strains following Placebo, 1.0 mg/kg AMPH or 2.0 mg/kg AMPH.

Abbrev:
P - different from Placebo
" 1 " - almost significantly different from 1.0 mg/kg ($p < .088$)

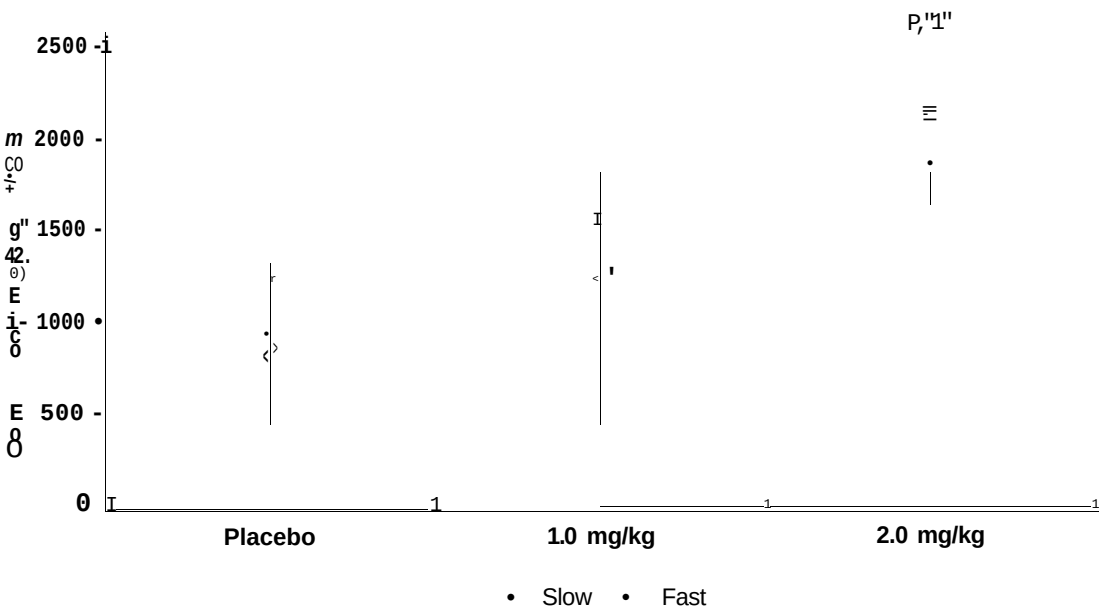

2500
2000
1500
1000
500
0
Placebo
1.0 mg/kg
2.0 mg/kg
Slow
Fast

Figure 24. The number of uneaten pellets at the end of DOR testing on Days 1, 5 and 10 in the female and male Fast and Slow rats, in (a) Phase 1 and (b) Phase 2.

Abbrev:
& - effect of Strain
D - different from Day 1
F - different from Females
"__" - almost significant, $p < .062$

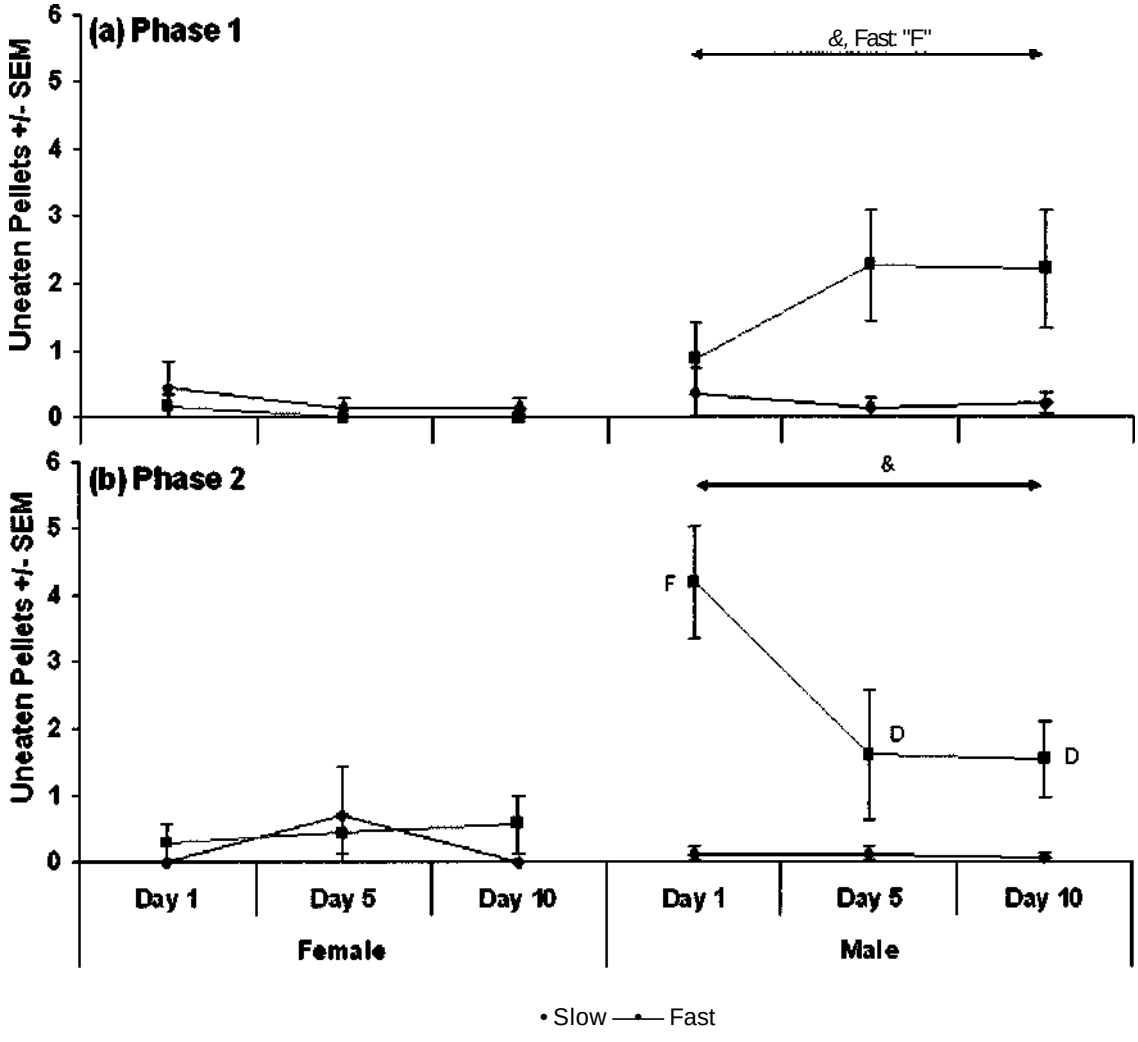
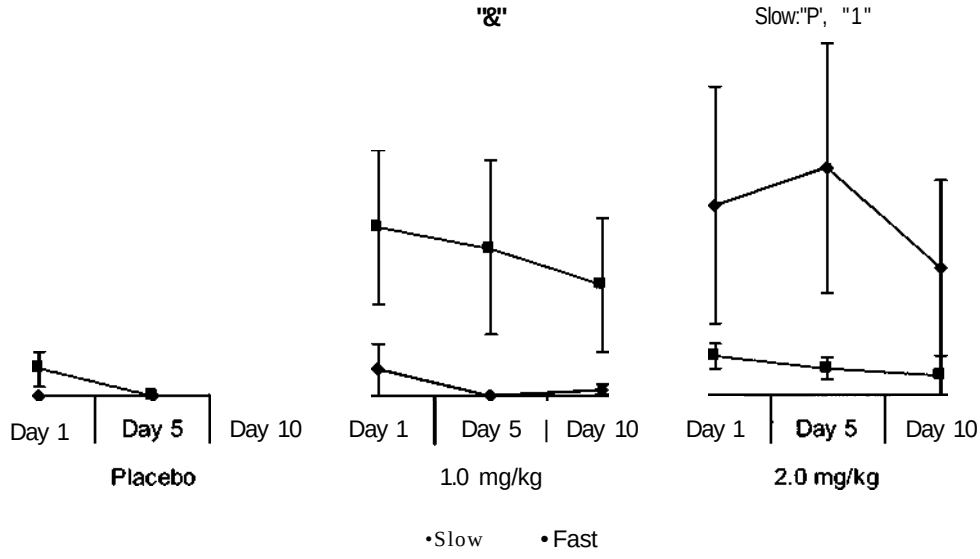

(a) Phase 1
&, Fast "F"
Uneaten Pellets +/- SEM
6
5
4
3
2
1
0
(b) Phase 2
&
F
D
D
Uneaten Pellets +/- SEM
Day 1
Day 5
Day 10
Day 1
Day 5
Day 10
Female
Male
• Slow —•— Fast

Figure 25. The number of uneaten pellets at the end of DOR testing on Days 1, 5 and 10 in the male Fast and Slow strains following Placebo, 1.0 mg/kg AMPH or 2.0 mg/kg AMPH, in Phase 3.

Abbrev:
"&" - almost significant effect of Strain, $p < .091$
"P" - almost significantly different from Placebo, $p < .057$
"1" - almost significantly different from 1.0 mg/kg, $p < .073$


"&"
Slow:"P', "1"
Day 1
Day 5
Day 10
Day 1
Day 5
Day 10
Day 1
Day 5
Day 10
Placebo
1.0 mg/kg
2.0 mg/kg
•Slow
• Fast

Phase 4 (0ITI, 20-0 Delay)

Univariate analysis revealed that the Fast strain left behind significantly more pellets than the Slow strain (Strain effect, $F(1,22) = 6.5$, $p < .019$). See Figure 26.

DRL

Days 1-4

The Fast strain left behind more uneaten pellets than the Slow strain when WH time was Os (Strain effect, $F(1,46) = 6.3$, $p < .017$), 2s (Strain effect, $F(1,46) = 8.4$, $p < .007$), 5s (Strain effect, $F(1,45) = 3.9$, $p < .056$; one Slow outlier was removed) and correlated with reward size (e.g., Phase 6; Strain effect, $F(1,46) = 4.1$, $p < .051$). Granted, the mean difference when the WH time was 5s was less than 1 pellet. See Figure 27. The remaining Phases (WH 10s, WH 20s) had no unconsumed chocolate pellets (data not presented). The one exception was on the third Day of Phase 4: the Fast strain's mean was 0.08 ± .06.

Days 4 vs. 5

Three of the six Phases had significant interactions (WH 0s: Day, Strain and Drug, $F(2,42) = 3.4$, $p < .044$; WH 5s: Day, Strain and Drug, $F(2,40) = 5.6$, $p < .008$; and, WH 10 or 20s: Day and Drug, $F(2,42) = 14.1$, $p < .001$) while a fourth, WH 2s, showed a trend for an interaction between the Day and Strain variables ($F(1,42) = 3.2$, $p < .082$). Additional testing found that the 2.0 mg/kg group accounted for the vast majority of the differences, leaving more unconsumed pellets. See Figure 27. Just like Days 1 to 4, the WH 10s and WH 20s phases had no unconsumed chocolate pellets (data not presented).

Chocolate vs. Regular Food *and* Eat within 30 sec

For Chocolate vs. Regular Food and Eat within 30 sec, there was a significant interaction between Strain and Drug (%io^2 = 60.5, $p < .001$ and *y/* = 11.2, $p < .048$,

Figure 26. The number of uneaten pellets at the end of DOR testing in the male Fast and Slow strains following Placebo, 1.0 mg/kg AMPH or 2.0 mg/kg AMPH, in Phase 4.

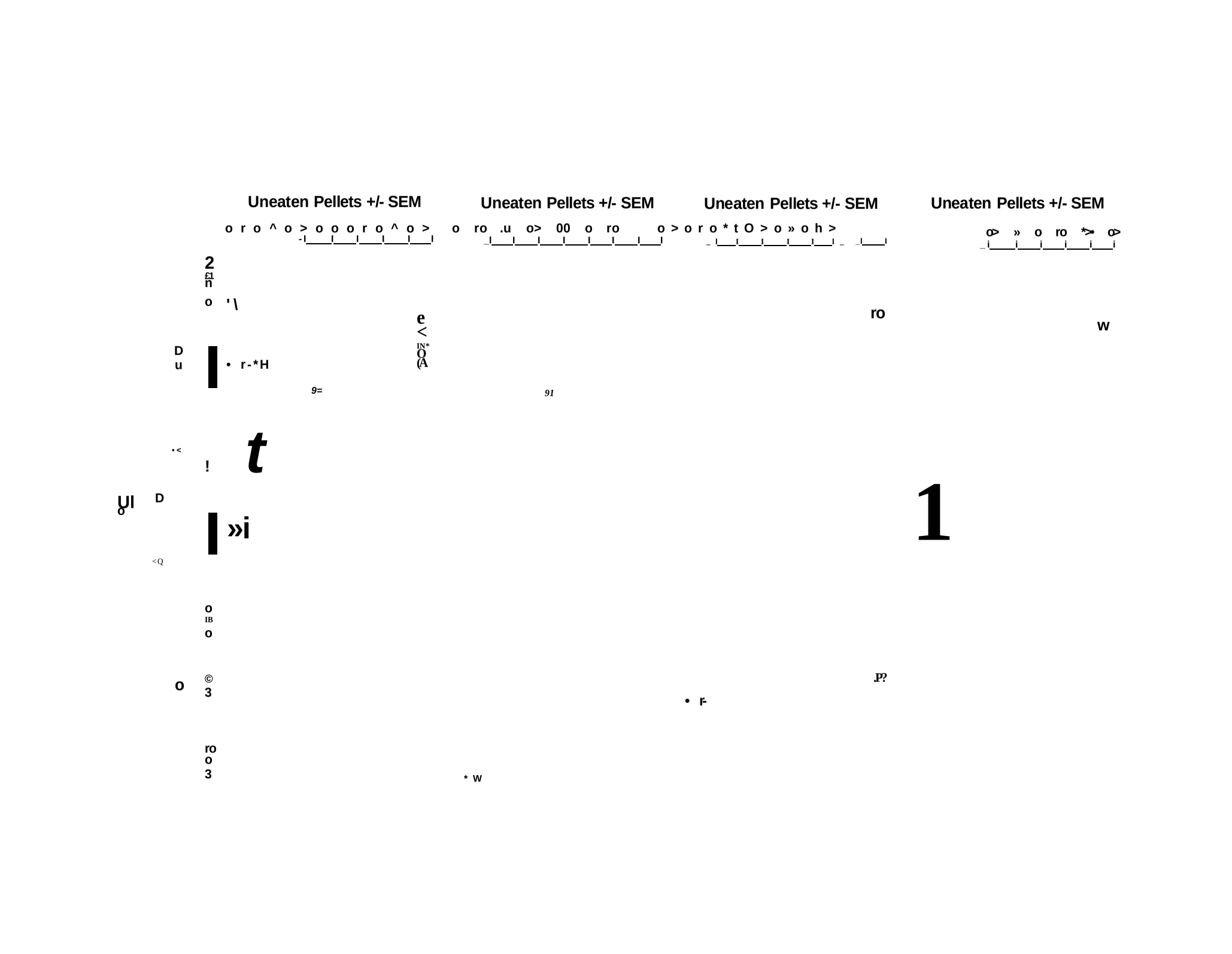
Abbrev:
& - effect of Strain

Placebo 1.0 mg/kg 2.0 mg/kg

- Slow Fast

Figure 27. The number of uneaten pellets at the end of DRL testing in the male Fast and Slow strains following Placebo, 1.0 mg/kg AMPH or 2.0 mg/kg AMPH, in Phases (a) 1, (b) 2, (c) 3 and (d) 6. Phases 4 and 5 were not presented due all rats consuming all of their treats (one exception on Day 3 of Phase 4: the Fast strain's mean was 0.08 ± 0.06).

Abbrev:
& - effect of Strain
P, 1.0 - different from Placebo, 1.0 mg/kg, respectively
D4 - different from Day 4
"__" - approaching significance ($p < .074$)


Uneaten Pellets +/- SEM
Uneaten Pellets +/- SEM
Uneaten Pellets +/- SEM
Uneaten Pellets +/- SEM
1

respectively). In all Drug groups, the Fast strain had greater odds of choosing chocolate compared to the Slow strain. Relative to Placebo, though, both AMPH drug groups and the 2.0 mg/kg had lower odds of choosing chocolate in the Slow and Fast strains, respectively. The Slow 2.0 mg/kg had lower odds of eating their food within 30 seconds. See Table 27.

Discussion

Time to complete

No changing Delays

Males, in Phase 1, had an increase in test duration over Days that ended up being longer than the females. Additionally, the Fast males tended to take longer than the Slow males. Adding longer and longer ITIs (Phase 2) did not seem to negatively impact either the female or male strains since the time to complete actually decreased over days. However, the Fast males took longer to finish each day's testing than the other groups.

Increasing or decreasing Delays

Similar to Phase 2, both Placebo strains became faster at finishing Phase 3 over days. Thus, adding longer and longer Delays did not negatively affect the strains. AMPH did affect the strains, however, as demonstrated by an increase in duration. For the most part, the Fast strain was the most affected by AMPH, particularly with the 2.0 mg/kg dose. This trend continued with Phase 4 in that the high dose group took longer to complete than the other groups.

Presumably, a decrease in appetite would decrease lever pressing; the next trial would not commence until a 'no response' occurred. This, in turn, would result in the maximum time per trial, thereby increasing the overall test's duration. Consequently, the 'Time to complete measure' is suggesting that appetite was decreased in the AMPH-

Table 27. The number of male Slow and Fast rats choosing (a) chocolate, regular rat food or neither within 30 seconds (see 'Chocolate vs. Food') and (b) to eat their regular rat chow within 30 seconds (see 'Eat within 30 sec') depending on if they were in the Placebo, 1.0 mg/kg or 2.0 mg/kg AMPH groups.

Abbrev:
& - difference between Strains
P - difference from Placebo

Test	Outcome	Slow			Fast		
		Placebo	1.0 mg/kg	2.0 mg/kg	Placebo	1.0 mg/kg	2.0 mg/kg
Chocolate vs. Food	Neither	4	10	13	0	0	5
	Food	0	0	0	1	1	0
	Chocolate	9*	3&)P	0 ^	12	12	8 P
Eat within 30 sec	No	1	4	6	1	1	1
	Yes	12	9	7&,P	12	12	12

dosed groups. However, as revealed with the other appetite tests, this interpretation was not that straight-forward.

Uneaten Pellets, Chocolate vs. Regular Food, Eat within 30 sec

Relative to Placebo, both AMPH drug groups and the 2.0 mg/kg had lower odds of choosing chocolate in the Slow and Fast strains, respectively. Interestingly, in all Drug groups, the Fast strain had greater odds of choosing chocolate compared to the Slow strain, despite leaving behind more uneaten pellets at the end of each day of testing. Based on the *Chocolate vs. Regular Food* test, it would appear as though the Fast strain, regardless of dose, maintained a preference for chocolate pellets. Notably, some Fast rats first chose the lg of regular food, suggesting that the Fast strain didn't like the chocolate pellets. However, this could be an impulsive choice in that the rat went for the larger piece of food. Indeed, some rats didn't inspect the whole dish but immediately grabbed the regular (large chunk of) food. On top of that, while they still had the regular food in their mouth, some continued to try to eat chocolate pellets (naturally, they didn't succeed). This finding indicates that the Fast strain's appetite was not affected, they liked the chocolate pellets, and, by extension, the decreased performance of this strain in the behavioural tests could be due to (in)attention.

In contrast, though, the tests are indicating that the AMPH doses may have decreased appetite in the Slow strain. Interestingly, though, the Slow Placebo technically had decreased appetite, relative to the Fast strain. Thus, this test may be confounded by strain-dependent features. For example, most Slows tended to "freeze" during the 30-sec observations. Consequently, the tendency for the Slow AMPH groups to not choose chocolate or to not eat their food may be a combination of appetite and/or heightened

anxiety. Indeed, the uneaten pellets and the *Eat within 30 sec* test underscore this potential: since it was the 2.0 mg/kg group the left behind more pellets and with decreased odds of eating their food, the decreased appetite may only occur at the highest dose while freezing affected the Placebo and 1.0 mg/kg groups. Furthermore, NE is correlated with anxiety; this strain, as revealed in the Microdialysis experiment, had higher basal NE *and* increased NE after AMPH. Additionally, the DOR Phase 3 and 4 indicate that satiety didn't play a key role in performance. Consequently, more facts are pointing towards anxiety/freezing underlying this Strain's apparent lack-of-preference for either option, not appetite.

Conclusion

The Fast strain did not show signs of AMPH-induced decreased appetite and, thus, should not underlie their decreased participation. Rather, an appetite-independent variable, such as inattention or distraction, could contribute to their decreased participation. The Slow strain's natural tendency to freeze, however, may have biased their results.

Appendix 4: Correlations between Neurotransmitters and Metabolites

To add another dimension to ADHD's etiology, evidence points to interactions between NE, DA and 5HT systems. Aside from the reciprocal connections between PFC and monoaminergic systems, all of their transporters have 50% genetic homology (Oades, 2008). Furthermore, 5HT can affect the levels of NE (Boulougouris & Tsaltas, 2008; Millan et al, 1998, 2000) and DA (Boulougouris & Tsaltas, 2008; Hertel et al., 1996; Millan et al, 1998, 2000; Olvera-Cortes et al., 2008).

The vast majority of these interaction studies, however, have focused on a reciprocal (potentially inhibitory) relationship between dopaminergic and serotonergic systems (Dalley et al., 2002; Puumala & Sirvio, 1998), producing a range of anatomical and neurochemical findings. For instance, not only do serotonergic systems make connections with dopaminergic targets, like the PFC and striatum (Olvera-Cortes et al., 2008), but 5HT receptors have been found on dopaminergic neurons (Hertel et al., 1996) and the raphe nuclei have a high density of DA receptors (Millan et al., 2000; Olvera-Cortes et al., 2008). Furthermore, serotonin and DA appear to modulate the increase in impulsivity seen after AMPH injections or serotonergic lesions, respectively (Boulougouris & Tsaltas, 2008). Thus, all of the monoamines should be considered, not only in terms of concentrations but also in the context of the other monoamines (e.g., correlations).

Analysis

Correlations were determined for each sample between all neurotransmitters and metabolites from Experiment 1; significance ($p < .05$) was two-tailed.

Results

The significant (or showing a tendency towards being significant) correlations are provided in Table 28. Some patterns emerged, independent of and after AMPH. Independent of AMPH, (1) NE was negatively correlated with 5HT in both strains and (2) the Slow strain's neurotransmitters were uncorrelated with the metabolites. After AMPH, the Slow strain had a biphasic negative correlation between NE and DA during the first 20 minutes (approaching significance, $p < .068$) and the 40-60-min sample. Otherwise, this strain did not show any AMPH-dependent changes. In contrast, in the Fast strain, there was a greater range of changes: (1) NE was no longer correlated with DOPAC; (2) serotonin's metabolite was inversely correlated with DA during the second sample; and, (3) serotonin was positively correlated with 5HIAA during the 40-60 min sample.

To bring these correlations together, the strains' NE levels were inversely correlated with the 5HT system (neurotransmitter and metabolites). The DA system, though, had a different pattern between the strains: in the Slow and Fast strains, the DA system was (a) inversely and positively correlated with NE, respectively, but (b) uncorrelated and inversely correlated with the 5HT system, respectively. In other words: Slow strain = [NE] a [DA system or 5HT system]"[1]; Fast strain = [NE a DA system] a [5HT system]"[1].

Discussion

The correlations between all five compounds provided further indication for an imbalance in the PFC systems. In general, NE was inversely correlated with the 5HT system. With DA, though, the Slow and Fast strains' DA system was inversely and positively correlated with NE, respectively. The Fast strain's DA was also inversely

Table 28. Significant (or approaching significant) correlations were determined for all neurotransmitters and metabolites in the baseline and three 20-min post-amphetamine samples.

* - negative correlations approaching significance (.060 < .082)

	Baseline	0-20 min	20-40 min	40-60 min
Slow	NE a 5HT"[1]	NE a 5HT"[1] NEaDA[1]*	NE a 5HT"[1]	NE a 5HT"[1] NE a DA"[1]
Fast	[NE a DOPAC] a 5HT"[1] NEa5HIAA"'*	NE a 5HT"'	NE a 5HT[1]* DA a 5HIAA"[1] DOPAC a 5HT[1]	NE a [5HT a 5HIAA*][-1]

correlated with 5HT; the Slow strain did not have any correlations. In other words, in the PFC: Slow strain = [NE] a [DA system or 5HT system]"[1]; Fast strain = [NE a DA system] a [5HT system]"[1]. This differs, though, from a positive correlation between DA and 5HT systems in the striatum (Kuczenski & Segal, 1989).

Before AMPH, the Slow strain's only correlation was between NE and 5HT while, in the Fast strain, there were correlations between the four of five compounds. Following AMPH, neither strain had any correlations between the neurotransmitters and metabolites during the first 20-min sample. In fact, aside from an almost significant negative correlation between DA and NE ($p < .068$) in the Slow strain, this sample only had the ubiquitous inverse correlation between NE and 5HT. Presumably, AMPH affected DA and 5HT's mechanisms in different ways in the Fast strain.

Then, for the 20-40 min sample, the strains' pattern of correlations returned. Additionally, in the Slow strain, there was another negative correlation between NE and DA in the third 20-minute sample. Combining this correlation with an increase in NE concentration could indicate a slight shift to favour (a) intraneuronal NE synthesis from DA and/or (b) NE release. Unique to the Fast strain's post-AMPH samples, though, was, first, an inverse correlation between DA and 5HIAA then, second, a positive correlation between 5HT and 5HIAA. The former correlation could be an indication that either network was trying to decrease the activity of the other; since the last sample had a positive correlation between 5HT and 5HIAA, it would seem more likely that serotonin's activity was preferentially affected.

Interestingly, there is a mutual antagonism between the DA and 5HT neurotransmitters (Dalley et al., 2002; Puumala & Sirvio, 1998). A balanced antagonism

between two systems may or may not be detectable with correlations; it would depend on the sensitivity of the sampling technique and/or the 'speed' of the reciprocal counterbalancing cycle (e.g., neurotransmitter 'A' may increase then decrease and increase back to original levels within one minute). If the complete counterbalancing cycle took, for example, two minutes, then 1-min samples may isolate the different phases in each compounds' levels and they would appear to be negatively correlated in each sample. On the other hand, with 20-min samples, both compounds would have increased and decreased 20 times, the net effect of which would be no correlation.

Provided the mutual antagonism cycle between DA and 5HT was relatively fast, or at least shorter than 20 minutes, then the Slow strain's lack-of correlation between these two systems would indicate a relatively balanced antagonism in all the samples. Conversely, in the Fast strain, 5HT and its metabolite were inversely correlated with DA and its metabolite. Furthermore, the Fast strain had more 5HIAA than DOPAC. Thus, it could be suggested that the mutual antagonism cycle is altered in this strain. One possible reason is that the cycle takes longer to complete in the Fast strain. With 5HT receptors located on GABAergic interneurons (Millan et al., 1998) and the Fast strain showing altered GAB A function (Poulter et al., 1999), this could be one of the underlying triggers to altered monoamine circuitry.

Indeed, the Fast strain has been shown to have a higher expression of the embryonic GABA(A) subunits (a2, a3 and a5) in the amygdala, piriform cortex, perirhinal cortex and hippocampus while the Slow strain had a greater expression of the adult subunit (a1). Since embryonic GABA(A) receptors tend to leave their channel open longer than adult receptors, this could translate to altered communication/timing

between different synaptic networks (Poulter et al., 1999). At the age tested (3 months), a rat's cortex is expected to have reached the adult pattern of GABA(A) subunit levels (e.g., a greater amount of a1 and a2 subunits than a3 and a5; Yu et al., 2006). Provided the GABA(A) subunit pattern extends outside of the areas tested by Poulter et al. (1999), then the Fast strain's cortex may be similarly affected by poor timing between the different monoamine networks due to an overexpression of the embryonic subunits.

Combining the facts that (a) the Fast strain had overall higher 5HT turnover than DA turnover in the PFC, (b) 5HT receptors are located on GABA interneurons, (c) GABA(A) receptors have been shown to inhibit mesocortical DA neurons (Westerink et al., 1998), and (d) the Fast strain may have altered GABA(A)-modulated timing between monoamine in the PFC, it could be suggested that 5HT is ineffective at controlling DA's activity in the PFC. Alternatively, 5HT could be 'over-reacting' to the presence of DA, 5HT could be 'over-suppressing' the activity of DA, and/or DA is ineffective at controlling 5HT's activity. However, the greater 5HT turnover adds weight to the former theory. Regardless, more tests are needed to understand how these monoamines interact.

Conclusion

The basal interaction between the DA and 5HT neurotransmitters could be altered in the Fast strain. Following oral amphetamine, two themes were identified. First, AMPH appeared to primarily affect the potential interaction between DA and 5HT (e.g., the 5HT/DA antagonism) in the Fast strain, starting potentially during the first 20-minute sample after administration. Granted, correlation does not mean causation so this pattern of changes won't be used to determine behavioural test timing (more below); future

investigation is warranted. Second, there was a ubiquitous inverse correlation between NE and 5HT. The reasons for this are unclear. Again, more investigation is needed.

Appendix 5: Comparisons to PFC Microdialysis Literature

The microdialysis results were also compared to the literature on rat PFC microdialysis, both in terms of basal levels and changes after AMPH injection; oral administration experiments were not found. A number of articles have reported basal PFC catecholamines and serotonin but fewer have studied AMPH-induced effects.

Baselines

Various articles have reported basal PFC NE concentration, DA and its metabolite, and 5HT and its metabolite; their values, converted to pg/uL, are provided in Table 29. Similarly, the values from the current microdialysis study were converted to pg/uL in order to make comparisons with the literature, such as 'similar', 'higher', 'lower' or 'unclear'. From these evaluations, the strains' basal DA levels were similar to the literature, even though the strains were significantly different. The only other similarity to these published studies was the Fast strain's basal NE. In contrast, the Slow strain's NE levels and both strain's 5HT levels were higher than other reported values. The DA and 5HT metabolite concentrations in the literature, though, varied widely; thus, the strains' comparison is unclear. Interestingly, both strains tended to have the same relative comparison to the literature (e.g., both were similar, higher, lower or unclear) except with NE.

Post-AMPH changes

All reviewed published articles administered AMPH via injection (e.g., sc, ip, iv) and reported PFC NE, DA, 5HT, DOPAC and/or 5HIAA. In general, the injected AMPH, regardless of dose, produced increases in NE (Table 30) and DA (Table 31). While the articles differed in their sampling time (e.g., 16, 20 or 30 min), all doses produced (a) an increase evident during the first sample (e.g., within 16, 20 or 30 min)

Table 29. The current microdialysis study values of basal right PFC neurotransmitters (NE, DA and 5HT) and metabolite (DOPAC and 5HIAA) concentrations are compared to published literature. All neurotransmitter and metabolite values have been converted to pg/^L in order to make comparisons between the study (see 'Current study' column) and literature (see 'PFC Literature' column) easier. The Slow and Fast strain's basal levels compared to the literature are provided in the 'Comparison' column. If the article targeted a specific area of the PFC, it was indicated in the 'PFC Literature' column.

& - Strain effect

Neuro-transmitter	Strain	Current study Mean ± SEM	Comparison	PFC Literature
NE[r]	Slow	2.0 ±0.2	Slow higher	0.06 (Yoshitake et al., 2004)
	Fast	1.3 ±0.3	Fast similar	0.2 (Shoblock et al., 2004) 0.8 (Berridge & Stalnaker, 2002) 1.0 (Lavicky & Dunn, 1993)
DA[&]	Slow	0.09 ± 0.01	Slow similar	0.02 (Pehek, 1999)
	Fast	0.06 ±0.01	Fast similar	0.04 (Berridge & Stalnaker, 2002) 0.05 (Westerink et al., 1998) 0.06 (Pum et al., 2007) 0.07 (Shoblock et al., 2003, 2004; Yoshitake et a., 2004) 0.09 (Cartmell et al., 2000) 0.4 (cingulate; Hedou et al., 2001) 0.8 (Lavicky & Dunn, 1993) 1.7 (infralimbic; Hedou et al., 2001)
5HT	Slow	0.5 ±0.1	Slow higher	0.02 (Shoblock et al., 2004)
	Fast	0.6 ±0.1	Fast higher	0.06 (Yoshitake et al., 2004) 0.1 (Rueter & Jacobs, 1996) 0.1 (Pum et a1, 2007)
Metabolite *(PS/fd)*				
DOPAC	Slow	2.5 ± 0.3	Unclear	0.3 (cingulate; Hedou et al., 2001)
	Fast	2.1 ±0.6	Unclear	0.8 (Lavicky & Dunn, 1993) 1.0 (infralimbic; Hedou et al., 2001) 7.9 (Yoshitake et a1, 2004) 8.2 (Shoblock et al., 2004) 20.6 (Shoblock et al., 2003) 22.2 (Cartmell et al., 2004)
5HIAA	Slow	5.9 ±1.4	Unclear	1.2 (Lavicky & Dunn, 1993)
	Fast	6.7 ±1.6	Unclear	5.5 (cingulate; Hedou et al., 2001) 6.9 (Yoshitake et a1, 2004) 10.0 (infralimbic; Hedou et al., 2001) 34.3 (Rueter & Jacobs, 1996) 65.0 (Cartmell et al., 2000)

Table 30. Published percent changes from NE baseline in the PFC following different AMPH injected (s.c. or i.p.) doses. For the various doses, the time to the first increase, maximum increase and return to baseline levels (in minutes) were indicated by the upper-most time of the collected sample (e.g., if the sample was 20-40 minutes after administration then 40 minutes was entered). The maximum percent changes were converted to reflect changes from 0% baseline.

Dose	1st Increase	Max		Baseline at	Ref
		When	How much		
0.15 mg/kg sc	16 min	32 min	75%	128 min	Berridge & Stalnaker, 2002
0.25 mg/kg sc	16	32	175%	112	Berridge & Stalnaker, 2002
0.5 mg/kg sc	20	40	-900%	180	Florin et al., 1994
	20	40	-1600%	120	Kuczenski & Segal, 1992
1.75 mg/kg sc	20	40	-2400%	240	Florin etal., 1994
2 mg/kg ip	20	40	300%	> 160	Shoblock et al., 2004
2.5 mg/kg sc	20	40	-3100%	>240	Florin etal., 1994
	20	40	-4200%	>240	Kuczenski & Segal, 1992

Table 31. Published percent changes from DA baseline in the PFC following different AMPH injected (sc, ip or iv) doses. For the various doses, the time to the first increase, maximum increase and return to baseline levels (in minutes) were indicated by the upper-most time of the collected sample (e.g., if the sample was 20-40 minutes after administration then 40 minutes was entered). The maximum percent changes were converted to reflect changes from 0% baseline.

Dose	1st Increase	Max		Baseline at	Ref
		When	How much		
0.15 mg/kg sc	16 min	32 min	25%	128 min	Berridge & Stalnaker, 2002
0.5 mg/kg ip	20	40	179%	80	Pum et al., 2007
1 mg/kg iv	20	40	225%	120	Moghaddam et al., 1990
1 mg/kg ip	20	40	414%	100	During etal., 1992
	20	40	469%	>180	Pum et al., 2007
1.25 mg/kg ip	30	60	200%	120	Pehek, 1999
1.5 mg/kg sc	30	60	275%	210	Hertel etal, 1995
2 mg/kg ip	20	40	500%	120	Shoblock et al., 2004
2.5 mg/kg ip	30	60	300%	180	Pehek, 1999
	20	40	317%	140	Pum et al., 2007
5.0 mg/kg ip	30	60	650%	150	Pehek, 1999

and (b) a peak increase during the second sample (e.g., during the 16-32, 20-40 or 30-60 min collection). The peak amount and return to baseline, though, was somewhat correlated with dose; however, the varying collection times and administration routes have produced overlap. Regardless, overall trends in peak amount and return to baseline have emerged.

With NE, the smallest peaks (around 100-200%) and earliest return to baseline (around 2 hours) were seen with sc injections below 0.5 mg/kg. In contrast, the largest peaks (around 1000% or more) and later return to baseline (3 hours or more) were seen with sc injections 0.5 mg/kg or greater. Injecting 2 mg/kg ip, though, produced a relatively small peak (300%) but the return to baseline was after the 3-hour mark. With oral 5.0 mg/kg AMPH, PFC NE had a *delayed* and short-term *decrease* from baseline only in the Fast strain. However, during the subsequent 20-min sample, PFC NE had an almost significant increase from baseline in both strains. As a result, the oral administration method either did not match up (the Fast strain's initial drop) or slightly matched up (increase during the third sample) with the injected methods. Interestingly, the earliest change was seen in the strain with the lower basal NE, suggesting that the Fast strain's NE system is more sensitive to AMPH.

As for DA, the smallest peak (25%) was after 0.15 mg/kg sc. Increasing the dose to 0.5 to 1.25 mg/kg (ip or iv) had peaks around 200% while doses 1.0 mg/kg or more (ip or sc) had peaks of 300% or more. In general, regardless of dose, DA levels returned to baseline around 2 to 3 hours after administration. Two articles investigated the DA metabolite, DOPAC: after 1.5 or 2.0 mg/kg ip injection, DOPAC had a decrease of 40% below baseline (Pothos et al., 1995; Shoblock et al., 2003). Based on the timeline

provided in Shoblock et al. (2003), the start of this decrease lagged behind DA (40 min), reached a maximum within 80 minutes and returned to baseline 3 hours after administration. With oral 5.0 mg/kg AMPH, though, PFC DA or DOPAC did not change from baseline in either strain. As a result, the oral administration method did not match up with the injected methods.

Only two articles reported AMPH effects on PFC 5HT and they did not concur. At the lowest dose (0.5 mg/kg ip), 5HT had a delayed decrease (40 min) that reached a maximum within 80 minutes (approximately -40%) but did not return to baseline for the duration of the test (3 hours; Pum et al., 2007). The higher doses of 1.0 and 2.5 mg/kg ip had an immediate peak (20 min) to maximum (50% and 150%, respectively) and relatively quick return to baseline levels (40 and 50 minutes, respectively; Pum et al., 2007). In contrast, Shoblock et al (2004) did not find any changes in PFC 5HT after 2.0 mg/kg ip. One article reported no change in PFC 5HIAA after 1.5 mg/kg i.p. (Pothos et al., 1995). The latter 5HT article and 5HIAA article match up with oral 5.0 mg/kg AMPH results.

Appendix 6: Comparisons to Amygdala Microdialysis Literature

These monoamines and their metabolites were previously studied in the amygdala before mass stimulation (Shin et al., 2004). In contrast to this microdialysis study, the strains did not differ in basal NE and DA (see Table 32). However, the NE means don't overlap; thus, the strain difference in basal NE may spread beyond the PFC. The remaining amygdala compounds, 5HT, DOPAC and 5HIAA, showed a similar lack-of-difference between the strains. The levels of most monoamines in the amygdala were very different from the PFC levels. First, all of the amygdala neurotransmitters were much higher than the PFC (NE, DA and 5HT approximately 3, 50 and 7X higher in the amygdala, respectively). A similar cortical/subcortical difference has been seen with DA (Fink-Jensen, 2000; Sharp et al., 1986); thus, this may be a normal structural imbalance. PFC DOPAC, in contrast, was approximately 3X higher than the amygdala while 5HIAA did not differ. This could suggest greater DA turnover in the PFC but similar 5HT turnover relative to the amygdala in both strains.

Table 32. The concentrations of norepinephrine (NE), dopamine (DA) and its metabolite (DOPAC), and serotonin (5HT) and its metabolite (5HIAA) in the prefrontal cortex (PFC; this study) and amygdala (Shin et al., 2004). All concentrations are in pg/40 (al (the amygdala data was converted from |jM).

& - Strain effect (see Microdialysis Experiment)

	Slow		Fast	
	PFC	Amygdala	PFC	Amygdala
NE	80.6 ± 6.3*	278 ±51	52.6 ±12.8	120 ±18
DA	3.7 ± 0.4[&]	160 ±33	2.3 ± 0.5	160 ±35
5HT	21.3 ±2.5	144 ±21	23.7 ±3.8	180 ±24
DOPAC	101.0± 11.7	36 ±2	83.2 ± 23.2	31 ±4
5HIAA	236.9 ±55.5	248 ± 78	265.9 ±63.9	205 ± 74

Appendix 7: Microdialysis results from one Fast Striatum

In one (excluded) Fast rat with a probe in the caudate putamen, oral AMPH increased DA while DOPAC had a delayed decreased (see Table 33), which appeared to concur with injected AMPH studies (Callaway et al., 1989; Carboni et al., 1989; Kuczenski & Segal, 1989, 1992; Zetterstrom et al., 1983, 1986). Oral AMPH decreased 5HT but 5HIAA increased then decreased. Interestingly, these compounds showed an increase (Kuczenski & Segal, 1989) or no change (Callaway et al., 1989; Kuczenski & Segal, 1989) from baseline, respectively, when AMPH was injected. The reasons for these inconsistencies are unclear but it is likely due to a combination of strain and administration method factors. Following oral AMPH, NE increased; however, no reports on NE changes in the striatum were found.

Regardless, the orally administered dose was sufficient to change neurotransmitter and metabolite levels but the PFC changes were notably different. Interestingly, these findings were expected because the frontal cortex and this subcortical structure have been shown to have different pharmacological profiles (Jentsch et al., 1997, 1999) and, thus, should respond to AMPH differently.

Table 33. One rat, from the Fast strain, had a microdialysis probe in the caudate putamen, thus, it could not be included in the analysis. However, the changes in norepinephrine (NE), dopamine (DA) and its metabolite (DOPAC), and serotonin (5HT) and its metabolite (5HIAA) may put the PFC effects into perspective. Due to the dramatic changes from baseline, the values were converted to percent change from baseline.

Sample (min)	NE	DA	5HT	DOPAC	5HIAA
0-20	317%	593%	-34%	46%	194%
20-40	84%	343%	-18%	-29%	-31%
40-60	63%	44%	-3%	-40%	15%

Appendix 8: Training Assistance

Some rats needed assistance with training, for example, by decreasing the force required to press the lever. While many had learned that pushing on the lever delivered a pellet, some simply placed their paw on the lever and/or barely applied force to push the lever down. Despite not applying sufficient force, the rat would immediately check the trough for a pellet. Thus, the rat had learned the sequence of events but, with time, the rats essentially self-extinguished their own training and lost interest. For those rats, the goal was to reduce the amount of force needed to trigger pellet delivery and, thus, retain the relevance of pressing the lever.

There were two common methods to increase the leverage. First, the experimenter waited until the paw was on the lever then 'lifted' the outside portion of the lever, thereby pressing the lever down inside the chamber. In many cases, the rat just needed a few of these assisted presses until they applied enough of their own force; assistance stopped at that point. If that method was not successful, then the next approach was to attach a binder clip to the lever. The binder clip added weight and increased the size of the lever, thereby increasing the odds that any amount of force would trigger a pellet. In most cases, the rat needed just one session with the binder clip.

A third intervention method was used to encourage continued lever pressing. Some rats appeared to lose interest in lever pressing, be it because they self-extinguished their own training, they preferred to explore the chamber or they fell asleep. Consequently, if a lever press had not occurred for 2 minutes, the training procedure stopped and the rat was returned to the home cage. After a brief break, the rat was placed back into the chamber and training started again. For those rats that went through this

procedure, there were three common outcomes: (1) they met criterion; (2) they resumed pressing but did not meet criterion; or, (3) continued to have poor participation. Rats that fell into the latter category repeated the procedure up to 5 times; fortunately, these rats generally needed just one day of this procedure. Regardless, the rats demonstrated an ability to press the lever and, furthermore, were generally consistent. Thus, they were not removed from testing.

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