NEUROBEHAVIORAL CORRELATES OF ACTION CONTROL IN AN ANIMAL MODEL OF ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

by

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ABSTRACT OF THE DISSERTATION

Neurobehavioral Correlates of Action Control in an Animal Model of Attention-Deficit/Hyperactivity Disorder

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Attention/deficit-hyperactivity disorder (ADHD) is a psychiatric illness characterized by symptoms of inattention, impulsivity and hyperactivity. Aside from clinical symptoms, patients with ADHD display reward and motivational impairments. A potential mechanism that might underlie these deficits is an impairment in patients' action control to flexibly adapt their behavior to changing consequences. Studies suggest that brain regions responsible for action control (the corticostriatal pathways) and dopamine signaling within these regions show abnormalities in patients with ADHD. Accordingly, we propose that patients with ADHD exhibit an impairment in action control with biased reliance on the habit system (*reflexive* actions) at the expense of the goal-directed system (*reflective* behavior). We used behavioral, pharmacological and immunohistochemistry techniques to examine action control in spontaneously hypertensive rats (SHR), a rat model of ADHD. In two separate studies, we studied the effects of ADHD, methylphenidate (a psychostimulant used to treat ADHD), and dopamine D1

receptor (D1R) and dopamine D2 receptor (D2R) agonists and antagonists on goal-directed behavior. Further, we characterized the neural activation patterns in the brain regions that are involved in action control by quantifying the expression of the immediate early gene c-fos. Finally, we used a computer-based cognitive analogue to replicate and translate our behavioral findings in patients with ADHD.

Our results show that SHR and patients with ADHD exhibit a selective deficit in goal-directed behavior. This deficit was restored by methylphenidate, stimulation of D1R or inhibition of D2R in SHR. At the neural level, we found that SHR showed dominant activity in the dorsolateral striatum (the habit region), whereas control rats showed a dominant activity in the dorsomedial striatum (the goal-directed region). These patterns of activation flipped when rats received methylphenidate. This novel finding indicates that the core behavioral deficit in ADHD might not be a consequence of dopamine hypofunction, but rather due to a misbalance between activation of D1R and D2R pathways that govern action control. Unraveling these mechanisms can broaden our understanding of the neural circuits underlying cognitive symptoms of ADHD. These findings might elucidate novel potential treatment approaches to create a balance between ADHD symptom relief and remediation of behavioral deficits.

DEDICATION

To *Nouralhuda*.

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CHAPTER 1: INTRODUCTION

Neurobehavioral Correlates of Action Control in Attention-Deficit/Hyperactivity Disorder

1.1. OVERVIEW

Attention-deficit/hyperactivity disorder (ADHD) is a psychiatric disorder that is characterized by age-inappropriate symptoms of inattention, impulsivity and hyperactivity. Patients with ADHD also exhibit symptoms of motivational impairments. The behavioral characteristics and neural bases of these impairments are not well understood. This chapter will review the most commonly accepted neurobiological theories of ADHD in relation to core symptoms. Specifically, it will summarize the behavioral and neural correlates of action control in ADHD as well as the effects of psychostimulants and dopaminergic medications on modulating this behavior. Further, it will discuss the validity of a rat strain, which was used in our studies, as an animal model of ADHD. In each section, we will discuss critical gaps in the literature and potential directions to address them.

1.2. BEHAVIORAL CORRELATES OF ACTION CONTROL IN ADHD

1.2.1. ADHD: Background

Attention-deficit/hyperactivity disorder (ADHD) is one of the most prevalent psychiatric disorders that is typically diagnosed during childhood and can continue to adolescence and adulthood. It is characterized by developmentally inappropriate symptoms of inattention (distraction; difficulty sustaining focus), hyperactivity (constant movement that might disrupt behavior), and impulsivity (hasty actions with no forethought or consideration of consequences) (Barkley, 2005; Castellanos & Tannock, 2002). It is estimated that 5-11% of children and 2.5% of adults have been diagnosed with ADHD in the United States. Diagnosis is more common among boys than girls (APA, 2016; CDC) with most cases being diagnosed in early school-aged children when it leads to difficulties in schoolwork and/or disruption in the classroom. The pharmacological treatment of choice for ADHD is psychostimulant medications. Up to 20% of school children with ADHD receive psychostimulant treatment (LeFever, Dawson, & Morrow, 1999). In particular, methylphenidate is the drug that is most commonly used to treat ADHD. It works primarily by increasing synaptic catecholamine availability in specific brain regions.

The most widely used criteria to diagnose ADHD are the fourth edition of the Diagnosis and Statistical Manual of Mental Disorders (DSM-IV) published by the American Psychiatric Association, 1994 (APA, 1994, 2000). The cardinal symptoms of ADHD in the DSM-IV criteria include inattention on one hand, and hyperactivity and impulsivity on the other hand. Some symptoms of each

component must be present before the age of 7 years old. A clear evidence of maladaptive performance/significant impairment must be present and reported in at least two settings (e.g. family, school, workplace). Further, symptoms have to be persistent for at least 6 months. Accordingly, ADHD can be classified into three subtypes: (1) ADHD-combined; criteria for both core symptoms are met for the past 6 months; (2) ADHD-inattentive; (3) ADHD-hyperactive-impulsive (APA, 1994). ADHD is frequently comorbid with a variety of psychiatric disorders such as oppositional defiant (ODD), conduct disorders (CDs), autistic spectrum disorders (ASDs), anxiety and learning disorders. However, there are no guidelines in the DSM-IV criteria that help differentiate symptoms of ADHD from other comorbid disruptive behaviors. Therefore, other assessment tools that provide clear distinction between patients' symptoms are vital for a precise diagnosis of ADHD (Pliszka, 1998, 2000, 2003).

Studies have shown that ADHD is highly heritable. Family and twin studies have provided strong evidence that genetic factors are responsible for a significant portion of ADHD symptomatology (Thapar, Langley, O'Donovan, & Owen, 2006; Thapar, O'Donovan, & Owen, 2005). In fact, estimates of heritability in ADHD were shown to be at 0.77 (Faraone & Doyle, 2000; Tannock, 1998). Gene-candidates in most ADHD genetic studies are motivated by the dopamine hypothesis of ADHD, which states that patients with ADHD have low dopamine signaling in target brain regions (Swanson et al., 2000; R. D. Todd, 2000). Genes linked to noradrenergic pathways have also been studied and

implicated to be associated with the pathophysiology of ADHD (Kim, Waldman, Blakely, & Kim, 2008; R. D. Todd, 2000).

Although genetic variations and mutations play a major role in the etiology of ADHD, environmental factors and their interplay with genetics have also been proposed as risk factors for ADHD (Banerjee, Middleton, & Faraone, 2007). For example, 15-20% of patients with traumatic brain injury develop ADHD post trauma (Catroppa & Anderson, 1999; Cicerone, 1996) that can persist into the chronic stage of recovery (Max et al., 1998). Stroke (Max et al., 2002), premature delivery, low birth weight (Serati, Barkin, Orsenigo, Altamura, & Buoli, 2017), maternal smoking during pregnancy (Schmitz et al., 2006) and alcohol exposure (Burger et al., 2011) are all examples of environmental factors that have been studied as risk factors for ADHD. Taken together, ADHD, like many other neuropsychiatric disorders, is influenced by a variety of inherited and non-inherited factors and their interplay (Thapar et al., 2006). Exposure to a risk factor does not always result in the development of ADHD and there is no single factor that can be isolated as a specific cause for ADHD.

1.2.2. Cognitive Correlates of ADHD

The majority of research on ADHD has focused on understanding and treating its main three symptoms; inattention, hyperactivity and impulsivity. However, a dysfunction in motivation has also been proposed to have a key role in ADHD symptoms (Carlson & Tamm, 2000; Konrad, Gauggel, Manz, & Scholl, 2000; Luman, Tripp, & Scheres, 2010; McInerney & Kerns, 2003; Slusarek,

Velling, Bunk, & Eggers, 2001; Tripp & Wickens, 2008). For example, children with ADHD show altered sensitivity to positive reinforcement compared to healthy children: they fail to adapt appropriately to changing rates of reinforcement and they require larger incentives to adjust their actions (Tripp & Wickens, 2008; Volkow et al., 2011). Further, behavior in individuals with ADHD tends to be reflexive and elicited by stimuli, instead of being intentional and directed by internal goals. For example, patients with ADHD tend to overestimate their physical abilities and take risky behaviors provoked by stimuli that are associated with positive or no consequences (Bruce, Ungar, & Waschbusch, 2009). Patients' lack of ability to foresee the consequences of their actions is a major concern, particularly in an academic or work setting. If they are less sensitive to the desirability of consequences, patients with ADHD will have difficulties modifying their behavior and maintaining their goals. Instead, they will be forced to rely on habits that can be triggered by stimuli, regardless of the outcome. An inability to maintain goals may contribute to deficits in sustained attention or motor inhibition observed in ADHD. A deficit in action control might be a potential mechanism that underlies these symptoms of inattention and hyperactivity. In particular, patients with ADHD might be impaired at modifying specific behaviors even if the consequences of those behaviors are changing. Here, we attempt to interpret ADHD in terms of action-control theories to further understand motivational impairments in patients with this disorder.

1.2.3. Behavioral Aspects of Action Control

Humans and animals learn to make responses to obtain or avoid particular consequences. Learning behaviors that determine whether a consequence will occur or not is referred to as instrumental conditioning; a process in which subjects develop a contingency between response and consequence, which can be referred to as the outcome. Actions that lead to desirable outcomes increase future response, whereas actions that lead to undesirable outcomes decrease future response. Accordingly, a reinforcer is a behavioral outcome that makes a future behavior more likely; while a punisher is a behavioral outcome that makes a future behavior less likely. This process is called reinforcement learning. Therefore, instrumental conditioning has three components: a discriminative stimulus (S), a response, that is also referred to as an action, and an outcome (O) that may be a reinforcer or a punisher. Behavioral theories suggest that actions depend on learning this three-way association; S → R → O (Thorndike, 1905). Temporal acquisition of R-O associations is referred to as "performance" and it can be measured during training, while long-term retention/retrieval of R-O associations is referred to as "learning" and it reflects the relatively permanent changes in knowledge (Bouton, 1993; S. S. Kantak & Winstein, 2012; Soderstrom & Bjork, 2015; T. P. Todd, Vurbic, & Bouton, 2014).

Eliciting responses independent of discrete trials is a type of instrumental learning that is referred to as free-operant conditioning. In this type of learning, subjects may repeatedly respond "freely" over a specific period of time, where responses can be made without interference from the experimenter. Learners'

trial-independent responses are measured with a cumulative recorder (Skinner, 1938, 1948). The desire to perform an action is referred to as motivation, which could be directed towards a positive stimulus or away from a negative one. Thus, high vs. low motivation corresponds to high vs. low probability of making an action.

Acquiring stimulus-response associations is known as habitual behavior; whereas forming action-outcome associations is known as goal-directed behavior. Together, habitual and goal-directed behaviors constitute the two main systems that guide action control; an instrumental responding process by which voluntary actions are selected and executed based on prior reinforcement learning (Dickinson, 1985; Tolman & Gleitman, 1949).

Healthy subjects should have the ability to flexibly select between stimuli according to the obtained outcomes. For example, if the action of eating dairy products causes intestinal upset, this behavior should be modified to avoid the associated outcome. This flexible change represents goal-directed behavior. Conversely, habitual behavior is displayed when people fail to modify their behaviors according to the outcomes. Thus, intestinal upset that results from eating dairy products would not lead to a change in subject's behavior.

The most widely used test to distinguish between habitual and goal-directed processes is the reinforcer or outcome devaluation paradigm (Adams & Dickinson, 1981; Dickinson, 1985). It is a well-validated paradigm in the animal literature that typically consists of three phases: (1) a free-operant training phase in which rats separately acquire distinct action-outcome contingencies. In this

phase, rats are trained to press a lever and receive a food reward as an outcome. (2) A devaluation phase in which the food outcome is devalued either through specific satiety or through pairing the food reward with illness such as lithium chloride injections, which induces gastric illness. (3) A choice test conducted in extinction. In this phase rats are given the opportunity to press the lever; however, they do not receive a reward for their action. If rats are sensitive to outcome devaluation, their response on the lever that is associated with the devalued outcome will decrease, showing goal-directed behavior (**Table 1.1**). Conversely, if rats lack sensitivity to devaluation and are not influenced by the current value of the outcome, their lever response will not change and their behavior is considered habitual (Jonkman, Kosaki, Everitt, & Dickinson, 2010). Further, if rats receive extensive operant training, they lose sensitivity to outcome devaluation; thus, their response becomes dominantly habitual, even if the rewarding outcome has been devalued (Adams & Dickinson, 1981; Dickinson, 1985).

The outcome devaluation paradigm has been extensively used in animal studies (Balleine & Dickinson, 1998). Recently, it has also been employed in human research (Daw, Gershman, Seymour, Dayan, & Dolan, 2011; de Wit, Niry, Wariyar, Aitken, & Dickinson, 2007; de Wit et al., 2012; Gillan et al., 2011; Klossek, Russell, & Dickinson, 2008; Klossek, Yu, & Dickinson, 2011; E. Tricomi, Balleine, & O'Doherty, 2009; Valentin, Dickinson, & O'Doherty, 2007). Devaluation in human studies is produced by either specific satiety (Tricomi 2009) or by altering a previously rewarding outcome to make it less desirable, for

example superimposing a cross on an image of a specific outcome to indicate that it is no longer worth credits (de Wit et al., 2007; de Wit et al., 2012; Gillan et al., 2011). Similar to animal experiments, participants show goal-directed behavior by responding less on the stimuli that is associated with the devalued outcome. With extensive training on a free-operant task, participants lose their sensitivity to outcome devaluation similar to rats (Balleine & Dickinson, 1998; E. Tricomi et al., 2009).

Another behavioral paradigm that is used to distinguish goal-directed from habitual behavior is contingency degradation (Hammond, 1980). While the outcome devaluation paradigm relies on the value subjects place on the outcome, contingency degradation relies on subjects' expectations of receiving the outcome (Balleine & O'Doherty, 2010; Hammond, 1980; Yin, Ostlund, Knowlton, & Balleine, 2005). That is, rather than delivering outcomes in response to corresponding actions, instrumental contingencies are weakened by delivering outcomes in a non-contingent manner, independent of actions. This training is followed by a choice test carried out in extinction, in which rats are given a choice between a contingent and a non-contingent stimulus. According to this paradigm, rats that are sensitive to goal-directed behavior will choose the contingent stimulus (**Table 1.1**) (Balleine & O'Doherty, 2010). This procedure provides an additional and reliable method for understanding neural systems underlying habitual and goal-directed action control.

Critical Gaps in the Literature and Future Directions

Extensive research has examined behavioral and neural aspects of action control in animals. In humans, some studies have employed this paradigm as a measure of goal-directed behavior in healthy subjects and in patient populations such as Parkinson's disease and obsessive-compulsive disorder (de Wit, Barker, Dickinson, & Cools, 2011; Gillan, Morein-Zamir, Kaser, et al., 2014; Gillan, Morein-Zamir, Urcelay, et al., 2014; Gillan et al., 2011; Gillan & Robbins, 2014; Redgrave et al., 2010; E. Tricomi et al., 2009). Although motivational impairments, such as altered sensitivity to positive reinforcement, are well described *clinically* in ADHD, no studies have directly examined action control over behavior in ADHD patients or animal models.

To address this, we employed an animal model to investigate the behavioral correlates of action control in ADHD. The goal of this project is to investigate the neural pathology underlying behavioral deficits in a widely accepted rodent model of ADHD. By studying goal-directed action control in patients with ADHD and a rodent model of ADHD, we address a critical gap in the literature that heretofore has not been investigated. These studies can yield new insights into ADHD pathophysiology. Studying goal-directed action control in ADHD will broaden our understanding of the networks involved in feedback-based and contingency learning, thereby revealing new behavioral and neural mechanisms of this disorder (Griffiths et al. 2014).

1.3. NEURAL CORRELATES OF ACTION CONTROL IN ADHD

1.3.1. Overview of Neurobiological Theories of ADHD

The neurobiological underpinnings of ADHD are not well established; however, dopaminergic hypofunction is thought to play an important role in the etiology of this disorder (Bush, Valera, & Seidman, 2005; Gill, Daly, Heron, Hawi, & Fitzgerald, 1997; Hynd et al., 1993; Russell, 2003; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005; Sagvolden & Sergeant, 1998; Waldman et al., 1998). Consistent with this notion, imaging studies have shown reduction in the volume of brain areas that contain high density of dopamine receptors in patients with ADHD (Bush, Valera, & Seidman, 2005). Furthermore, familial studies have consistently indicated a genetic contribution displayed as altered expression of different dopamine genes in patients with ADHD (Faraone et al., 2005; Gizer, Ficks, & Waldman, 2009; Wang et al., 2014). For example, several studies have found a strong association of 7-repeat allele of the human dopamine receptor D4 gene with ADHD. Expression of this allele is found to produce reduced response to dopamine; indicating lower levels of dopamine availability (Faraone et al., 1999; Grady et al., 2003; Turic, Swanson, & Sonuga-Barke, 2010).

In addition, ADHD symptoms are reduced in response to dopaminergic medications, such as methylphenidate (Ritalin®), a psychostimulant that preferentially blocks the reuptake of catecholamines, including dopamine and norepinephrine, in both the striatum and the prefrontal cortex (Heal, Cheetham, & Smith, 2009; Mehta, Calloway, & Sahakian, 2000; Mehta, Goodyer, & Sahakian, 2004; Roman et al., 2002; Schiffer et al., 2006). Methylphenidate is one of the

most commonly prescribed medications for ADHD treatment (Heal, Cheetham, & Smith, 2009). It successfully improves cognitive function in children with ADHD such as spatial working memory and response inhibition (Hawk, Yartz, Pelham, & Lock, 2003; Mehta et al., 2000; Mehta et al., 2004). Further, studies have shown that methylphenidate increases motivational sensitivity in children with ADHD by reducing the time it takes a patient to respond and obtain a reinforcer (Chelonis et al., 2011; Rubia et al., 2009; Volkow et al., 2009). Frontal and striatal dopamine availability are enhanced in response to methylphenidate, leading to improvement in cognitive functions that are modulated by dopamine. For example, studies have shown that methylphenidate enhances attention and reduces distractibility in patients with ADHD. Further, it enhances learning from salient stimuli and motivation; thus, improving patients' performance (Volkow, Fowler, Wang, Ding, & Gatley, 2002; Volkow, Wang, Fowler, & Ding, 2005; Volkow et al., 2012). Although there is a strong basis in the literature about the basic biochemical actions of methylphenidate, there is a huge gap in understanding the physiological basis for its effects on patients with ADHD. Therefore, investigating action control in ADHD might provide new insights about the mechanism of action of methylphenidate in this disorder.

The most accepted theories of the pathophysiology of ADHD could be explained using the diagram in **Figure 1.1**. Symptoms of ADHD are thought to result from: (1) hypofunctioning in the dopamine systems including the mesostriatal and the mesocortical pathways (Russell, 2003; Sagvolden & Sergeant, 1998), and/or (2) a pathology in local striatal circuits or reduced

functional connectivity in the corticostriatal pathways (Solanto, 2002). The dopaminergic mesostriatal pathway carries a reward signal that influences different aspects of learning (Frank, Santamaria, O'Reilly, & Willcutt, 2007; Frank, Scheres, & Sherman, 2007). Altered dopamine signaling or dysfunctional corticostriatal pathways might lead to inefficient learning (Mueller & Tomblin, 2012). For example, functional imaging studies have shown that disrupted dopamine pathways are associated with motivational deficits as well as symptoms of inattention in patients with ADHD (Tomasi & Volkow, 2012; Volkow et al., 2009; Volkow et al., 2011; Volkow et al., 2007). Further, patients exhibit altered striatal and frontal neural activity to reward anticipation and reward delivery (Furukawa et al., 2014; von Rhein et al., 2015; Wetterling et al., 2015). Impaired decision-making and learning processes have also been found to correspond to diminished activation in the corticostriatal pathways in ADHD (Hauser et al., 2014). Thus, disrupted dopaminergic/corticostriatal connectivity is well implicated in the pathophysiology of the cognitive symptoms of ADHD (Cubillo et al., 2010).

It is well established that the dorsal striatum is crucial for the selection, initiation, and execution of voluntary movements. Two parallel corticostriatal pathways are involved in this process: the direct pathway and the indirect pathway. These pathways originate from distinct populations of striatal medium spiny neurons, the principal neurons within the striatum, and project to different output structures. The direct pathway, also known as striato-nigral pathway, connects the *striatum* to the substantia *nigra* pars reticulat, one of the nuclei that

constitute the basal ganglia. The indirect pathway, also known as striato-pallidal pathway, connects the *striatum* to the globus *pallidus*, a nucleus located caudomedial to the striatum. These two pathways exert opposite net effects on the cortex via thalamic target structures. Dopamine exerts its effects on these pathways via two sub-populations of striatal medium spiny neurons that carry two subtypes of dopamine receptors; dopamine D1 receptors, which, if activated, stimulate the direct (striato-nigral) pathway, and dopamine D2 receptors, which, if activated, inhibit the indirect (striato-pallidal) pathway. Activation of the direct pathway facilitates the initiation and execution of voluntary movement; whereas activation of the indirect pathway inhibits motor activity (Albin, Young, & Penney, 1989; Gerfen et al., 1990; Wichmann & DeLong, 1996).

Although the corticostriatal pathways are traditionally only associated with motor activity, there is increasing evidence that they are also essential for many aspects of learning (Pennartz et al., 2009; Seger & Cincotta, 2006). Recent studies have shown that activation of the direct (D1R) pathway is critical to control reward-based learning, whereas activation of the indirect (D2R) pathway is key to control avoidance-based learning (Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; Kravitz et al., 2010; Kravitz, Tye, & Kreitzer, 2012; Yawata, Yamaguchi, Danjo, Hikida, & Nakanishi, 2012). Together, these pathways underlie behavioral/action modification according to future outcomes (Macpherson, Morita, & Hikida, 2014; Shan, Ge, Christie, & Balleine, 2014). Additionally, the indirect pathway has been involved in behavioral flexibility through inhibiting actions in reward learning paradigms, leading to flexibly

switching between behaviors (Hikida et al., 2010; Kravitz et al., 2012; Yawata et al., 2012). Evidence suggests that reduced activation in the indirect pathway can lead to loss of inhibitory control, resulting in behavioral deficits such as compulsivity, impulsivity, or excessive habit formation (Bock et al., 2013; Johnson & Kenny, 2010; Seger & Spiering, 2011; Yin, Knowlton, & Balleine, 2004). Accordingly, normal corticostriatal function should represent a balanced activation/inactivation in the direct and the indirect pathways. Excessive dopamine in the striatum results in over activation of the direct pathway and inhibition of the indirect pathway; therefore, excessive motor activity. On the other hand, dopamine depletion in the striatum, such as in Parkinson's disease, results in difficulty initiating movement (hypo-activation of the direct pathway) as well as slowness of movement (disinhibition of the indirect pathway). In ADHD, some studies proposed that the motor hyperactivity may reflect a 'reverse Parkinsonism' that is characterized by either overstimulation of dopaminergic activity in the direct pathway, or excessive dopaminergic inhibition in the indirect pathway (Castellanos, 1997). In Huntington's disease, the indirect pathway deteriorates leading to a bias in the dopamine system toward activation of the direct pathway (Rangel-Barajas & Rebec, 2016; Richfield, Maguire-Zeiss, Cox, Gilmore, & Voorn, 1995; Richfield, Maguire-Zeiss, Vonkeman, & Voorn, 1995; Sapp et al., 1995); therefore, induction of hyperkinetic behaviors, such as chorea (Albin et al., 1992). Dopamine receptor modulation of the corticostriatal pathways also contributes to the cognitive decline seen in Huntington's disease (Covey, Dantrassy, Zlebnik, Gildish, & Cheer, 2016; Giralt et al., 2011; Lawrence

et al., 1998). Further, in Parkinson's disease; hypo-activation/hypo-inhibition of the direct/indirect pathways, respectively, does not only account for motor dysfunction (Galvan & Wichmann, 2008; Magrinelli et al., 2016), but also explains the cognitive deficits in patients with Parkinson's disease such as impaired reinforcement and motivational learning and heightened learning from negative feedback (de Wit et al., 2011; Frank, Seeberger, & O'Reilly R, 2004; Redgrave et al., 2010). Here, we ask if dopamine receptor modulation of the direct and indirect pathways might explain motivational impairments in patients with ADHD, in addition to their motor symptoms**.**

Altogether, theories on the pathophysiology of ADHD are not consistent. Some theories suggest that the dopaminergic system in ADHD is hypofunctioning; while others suggest that striatal dopamine is over activated, thus resulting in excessive motor activity. Therefore, the neural mechanisms underlying ADHD might not be reflected by the excess or lack of dopamine. Rather, symptoms of ADHD might reflect activation discrepancy in the direct and indirect corticostriatal pathways.

1.3.2. Neural Underpinnings of Action Control Processes

Goal-directed behavior (forming action-outcome associations) and habitual behavior (acquiring stimulus-response associations) are the two main systems that underlie instrumental responding, where subject's behavior depends on consequences. The goal-directed system is rooted in the medial prefrontal and the prelimbic cortices (mPFC and PL) and their projections to the

dorsomedial striatum (DMS), whereas the habit system is based in the infralimbic cortex (IL) and its projections to the dorsolateral striatum (DLS) (Shiflett & Balleine, 2011b; Shiflett, Brown, & Balleine, 2010) (see **Figure 1.2**). Imaging studies in humans show that the caudate nucleus corresponds to the rat DMS and exhibit sensitivity to goal-directed behavior, whereas the posterior putamen corresponds to the rat DLS and is consistent with habit formation (E. Tricomi et al., 2009; E. M. Tricomi, Delgado, & Fiez, 2004). Using free-operant paradigms in humans, the posterior putamen shows higher activation in response to stimuli that are associated with specific actions (stimulus-response learning) E. Tricomi et al., 2009), while the caudate nucleus is significantly activated in response to actions that determined valuable outcomes (action-outcome learning) (E. M. Tricomi, Delgado, & Fiez, 2004).

Lesion studies or inactivation of IL and/or DLS brought normal habitual actions under the control of the goal-directed system (Coutureau & Killcross, 2003; Yin et al., 2004; Yin et al., 2005). Conversely, lesions to or inactivation of mPFC, PL and/or DMS disrupt goal-directed behavior and promote habitual responses (Corbit & Balleine, 2003; Ostlund & Balleine, 2005; Yin et al., 2005), in which lesions of mPFC impair the acquisition of goal-directed behavior (Ostlund & Balleine, 2005), whereas lesions to the PL impairs encoding action-outcome association by disruption the ability to select a response based on previously learnt action-outcome associations (Corbit & Balleine, 2003). The same pattern of impairment was found in animals that underwent extensive instrumental training; they show a dominance of habitual response and a deficit in goal-

directed action control (Adams & Dickinson, 1981; Dickinson, 1985). In addition, previous research has shown that exposure to amphetamine (A. Nelson & Killcross, 2006; Nordquist et al., 2007), alcohol (Corbit, Nie, & Janak, 2012), stress (Dias-Ferreira et al., 2009) or binge-like consumption of a palatable food (Furlong, Jayaweera, Balleine, & Corbit, 2014) can lead to accelerated habitual control. Accordingly, behaviors resulting from such exposure enhance habit learning at the expense of goal-directed behavior. Interestingly, this pattern of habitual behavior dominance was reversed by the administration of D1 receptor antagonists (SCH23390) and enhanced by the administration of D2 receptor antagonists (Etclopride) in animals with repeated exposure to amphetamine (A. J. Nelson & Killcross, 2013). Further, infusion of the D1 receptor antagonist, SCH23390, in the DLS (habit system), restored the normal pattern of goal-directed behavior in animals that previously displayed habitual behavior (Furlong et al., 2014). Overtrained rats with lesions to the nigrostriatal dopamine pathway are sensitive to outcome devaluation displaying goal-directed, instead of habitual behavior, evidencing that striatal dopamine is critical in habit formation (Faure, Haberland, Conde, & El Massioui, 2005). Further, under dopamine agonists, these rats show perseverative sensitivity to outcome devaluation with higher response to D2R over D1R agonists, indicating that D2R might more likely be involved in the modulation of the learning process (Faure, Leblanc-Veyrac, & El Massioui, 2010).

Taken together, normal patterns of goal-directed behavior seem to rely on intact underlying brain regions (mPFC, PL and DMS), as well as optimal levels of

D1 and D2 dopamine receptor activation (D1R, D2R) in these regions through the direct and indirect striatal pathways; where activation of these two receptors should be in balance. Hence, over activation of D1R (hyper-activation of the direct pathway) or under activation of D2R (hypo-inhibition of the indirect pathway) could present as disrupted goal-directed behavior and/or dominant habitual behavior (**Figure 1.3**).

As we discussed earlier, brain regions responsible for action control (corticostriatal pathways), as well as dopamine signaling within these regions, show abnormalities in patients with ADHD such as reduced activation and functional connectivity of the corticostriatal networks (Cubillo, A. et al. 2010). In support of this, we hypothesize that patients with ADHD have a deficit in goal-directed behavior that may arise, in part, from misbalanced dopamine signaling within the striatum. In particular, we postulate that patients with ADHD might display over-activation of D1R at the expense of D2R, an imbalance that could lead to impaired action control. In this view, patients with ADHD would be reliant on the habit system, exerting *reflexive* actions to stimuli at the expense of the goal-directed system, which exerts *reflective* behavior modulated by action consequences.

Consistent with this, naturally occurring polymorphisms of the D1R and D2R genes are implicated in ADHD; however, data on the functional significance of specific polymorphisms of these two genes are still inconclusive and warrant further studies of expression and/or activity of D1R and D2R in healthy subjects and patients with ADHD (Bobb et al., 2005; Luca et al., 2007; Ribases et al.,

2012; Rowe et al., 1999; Sery et al., 2006). Given that these polymorphisms present an indirect measure of dopamine, future studies ought to investigate neural markers in animal models of ADHD to study direct measures of dopamine effects on goal-directed behavior in ADHD. To examine activation of specific brain regions, the expression of immediate early genes using immunohistochemistry techniques can be used.

Immediate early genes are activated in a rapid and transient time course in neurons in response to a wide variety of stimuli (S. Davis, Bozon, & Laroche, 2003). For example, the peak expression of c-fos, an immediate early gene, is generally found 30 minutes following the triggering stimulus, and is diminished by 120 minutes (Cullinan, Herman, Battaglia, Akil, & Watson, 1995) (**Figure 1.4**). Immediate early genes allow for examining simultaneous activity of neuronal populations in response to a wide variety of experimental procedures (Dragunow, 1996; Morgan & Curran, 1991; Sheng & Greenberg, 1990). Greater gene expression within a brain region indicates that an increase in neuronal activity in that region has occurred. However, multiple factors can influence the expression of immediate early genes such as stressful events; brief handling, receiving food pellets and food restriction (Carr, 2007; Cullinan et al., 1995; Pan, Siregar, & Carr, 2006). Therefore, immunohistochemistry studies require careful control for these factors.

In chapter four, we measure neural activity in the striatum by comparing the expression of c-fos (**Figure 1.4**), along with specific striatal neuron markers, in the dorsolateral versus the dorsomedial striatum in a rat model of ADHD.

Neural markers allow us to investigate the co-expression of c-fos in striatal project neurons, called the medium spiny neurons, which comprise 95% of the striatal neurons. In-line with our proposed theory; we expect to see an increase in c-fos expression in the brain regions that are responsible for habit learning in a rat model of ADHD.

Critical Gaps in the Literature and Future Directions

Although deficits in sensitivity to motivation have been described, there has been no research conducted to investigate the neural correlates of action control in ADHD patients or animal models. Further, despite the frequent use of psychostimulant medications in the treatment of ADHD, no studies have investigated their behavioral and/or neural effects on action control in this disorder. Similarly, research is lacking on the effects of dopaminergic medications on the modulation of action control in ADHD.

Although dopamine hypoactivity is commonly invoked in models of the pathophysiology of ADHD, it has also been proposed as a potential mechanism that underlies reward deficits in other neuropsychiatric disorders; such as Parkinson's disease and major depressive disorder. Therefore, although this theory can explain the neurobiological underpinnings of the general symptoms and response to psychostimulants in ADHD, it fails to explain the more specific symptoms of this disorder such as motivational impairments and motor symptoms.

To address these gaps, we used an outcome devaluation paradigm and neuropharmacological methods (chapters two and three) as well as immunohistochemistry techniques (chapter four) in an animal model to investigate the neural correlates of action control as well as response to medication in ADHD. We hypothesize that ADHD patients and an animal model of ADHD will exhibit deficits in action control. We expect both to respond to stimuli in a habitual manner as a consequence of misbalanced activation of distinct corticostriatal networks that separately mediate goal-directed and habitual behaviors. With proper validation studies, findings consistent with this hypothesis could change our understanding of neural underpinnings of ADHD, and could also inspire development of novel pharmacotherapies.

1.4. USING THE SPONTANEOUSLY HYPERTENSIVE RAT STRAIN AS AN ANIMAL MODEL OF ADHD

Although neuropsychiatric disorders have extremely disruptive effects on human mental health, progress in uncovering pathophysiological underpinnings and discovering novel therapeutic interventions has been slow. In part, this might be due to (1) the significant ethical and technical limitations in our ability to investigate biomolecular and neural mechanisms of the human brain, and (2) the exceptionally complex neurobiology of higher brain function. Therefore, the development of appropriate animal models of neuropsychiatric disorders is indispensable to help us further understand the neurobehavioral aspects of the human brain and investigate potential therapeutic agents in preclinical settings.

Using animal models of neuropsychiatric disorders offers a way to overcome the limitations inherent in studying patient populations exclusively. For example, patients may have inconsistent medication histories, so it can be difficult to parse disease effects from medication history on brain activity. Animal models are advantageous in that variables, such as medication, can be more properly controlled. They allow for more direct neural interventions than in humans. Further, animals have simpler nervous systems; thus their behaviors are more easily interpreted compared to human behavior. However, animal models are hindered by the challenges of modeling functions or disorders that are uniquely human. For example, it is not feasible to develop an animal model for language, or hallucinations and delusions. It is only reasonable to use correspondence in animals that might help us understand approximations such as vocal communication or abnormal social behaviors. A further complication in using animal models is the lack of proof on the resemblance between what happens in the animal brain as compared to the human brain. Nonetheless, these limitations do not suggest that useful animal models are impossible to develop; rather, they indicate that they are unlikely to reflect the full aspects of a human neuropsychiatric disorder or a human brain function (Nestler & Hyman, 2010; Stewart & Kalueff, 2015).

In our studies, we use the spontaneously hypertensive rat strain (SHR); a rat model bred from progenitor Wistar Kyoto rats (WKY) (Okamoto & Aoki, 1963). SHR is the most widely accepted rodent model of ADHD (Davids et al. 2003, Sagvolden 2000, Sagvolden et al. 1993). SHR rats share many of the core

neurobiological and behavioral abnormalities observed in patients with ADHD. In particular, SHR rats show deficits in sustained attention, motor impulsiveness, and hyperactivity in a novel environment (Knardahl and Sagvolden 1979, Sagvolden 2000, Sagvolden et al. 1992, Sagvolden, Russell, et al. 2005, Wultz and Sagvolden 1992) Further, SHR rats exhibit reduced dopamine signaling and increased dopamine transporter expression (Heal, Smith, Kulkarni, & Rowley, 2008; Roessner et al., 2010; Russell, 2003; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995). Similar to patients with ADHD, dopamine hypofunction in SHR rats impairs dopaminergic pathways that in turn affects dopamine release and influence corticostriatal circuits that are modulated by dopamine. This disruption leads to displaying behavioral symptoms of ADHD in SHR rats and to developing impaired learning and action control (Sagvolden et al. 1992; Sagvolden 2000; Russell, 2003). Using operant conditioning paradigms, instrumental behavior in SHR rats is markedly different from control rats, with more frequent lever responses, extrapolating to hyperactivity and/or impulsivity in children with ADHD (J. C. Hill, K. Herbst, & F. Sanabria, 2012). Likewise, methylphenidate corrects attentional deficits and motor hyperactivity in SHR rats, lending further support for SHR rats as a model of human ADHD (Kantak et al. 2008, Sagvolden, Johansen, et al. 2005, Sagvolden, Russell, Aase, Johansen and Farshbaf 2005). In fact, most of our knowledge of methylphenidate action is derived from adult animal studies (Arnsten, 2006). Consequently, SHR seems to be the one ADHD animal model that follows the criteria that have been traditionally used to design and evaluate models in general: (1) face validity:

animal models should display the fundamental behavioral symptoms of the human disorder, (2) construct validity: they should demonstrate similar mechanisms that underlie a specific disorder, and (3) predictive validity: their symptoms should be efficiently remediated using the human disorder treatment, and they should predict behavioral, genetic, and neurochemical correlates of that disorder (McKinney & Bunney, 1969; Sarter, Hagan, & Dudchenko, 1992a, 1992b; Volkow et al., 2009; Willner, 1986).

Similar to patients with ADHD, SHR rats show selective impairment in motivation (Tripp & Wickens, 2012). For example, SHR rats show higher sensitivity to reinforcement delay in reward by choosing small/immediate, rather than large/delayed rewards, as compared to control rats (Bizot, David, & Trovero, 2011; Hand, Fox, & Reilly, 2006; Pardey, Homewood, Taylor, & Cornish, 2009). Further, comparable to patients with ADHD, treatment with psychostimulants, such as methylphenidate, remediates these deficits in SHR rats (K. M. Kantak et al., 2008; Liu et al., 2008). Previous studies have shown that SHR rats have high density of striatal D1 dopamine receptors and disrupted D2 dopamine receptor activity (Carey et al., 1998; Lim, Yu, Hoskins, Rockhold, & Ho, 1990a, 1990b; Linthorst, De Jong, De Boer, & Versteeg, 1993; Russell et al., 1995; Yu, Lim, Hoskins, Rockhold, & Ho, 1990) a notion that is consistent with our hypothesis of hyper-activation of the direct pathway and hypo-inhibition of the indirect pathway that might underlie a deficit in action control in patients and animal models of ADHD.

Although SHR rats are thought to be the only animal model that demonstrates all of the behavioral symptoms of ADHD (Sagvolden, 2000; Sagvolden, Aase, Zeiner, & Berger, 1998; Sagvolden, Metzger, et al., 1992; Sagvolden, Pettersen, & Larsen, 1993), they can start to develop symptoms of hypertension between the ages of 4 to 10 weeks which can result in neurological and behavioral deficits (Christiansen, Roald, Tenstad, & Iversen, 2002; Marcil, Thibault, & Anand-Srivastava, 1997; Ueno et al., 2002). Therefore, the most appealing approach is to use younger SHR rats to serve as a model for ADHD. Most SHR studies; however, were conducted in adult animals but have been replicated in adolescent/pre-hypertensive rats.

To test our theories, we used SHR rats to conduct most of our studies. We hypothesize that action control in SHR rats is impaired, and is modulated by higher levels of D1R activation as compared to control rats.

1.5. SUMMARY

Through our studies, major critical gaps in the ADHD literature can be defined. Including (1) the lack of understanding the behavioral correlates of action control, (2) the effects of psychostimulants and dopamine medication modulation on this behavior and (3) the neural underpinnings that underlie motivational impairments in ADHD. Addressing these gaps can significantly broaden our understanding of the networks involved in reward processing and contingency learning in ADHD, to delineate behavioral and neural mechanisms as well as new potential treatments for this disorder.

CHAPTER 2

Characterizing Action Control and the Effects of Methylphenidate in a Rat Model of ADHD

2.1. INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is typically diagnosed in childhood and can continue to adolescence and adulthood. It is characterized by developmentally inappropriate symptoms of inattention, impulsivity, and hyperactivity (Barkley, 2005; Castellanos & Tannock, 2002). The neurobiological underpinnings of ADHD are not well established; however, dopaminergic hypofunction is thought to play a central role in the etiology of this disorder (Bush et al., 2005; Gill et al., 1997; Hynd et al., 1993; Russell, 2003; Sagvolden, Russell, et al., 2005; Waldman et al., 1998). Consistent with this notion, dopaminergic medications, such as methylphenidate (MPH) (Ritalin®), remediate ADHD symptoms. MPH is a psychostimulant that preferentially blocks the reuptake of dopamine in both the striatum and prefrontal cortex (Heal et al., 2009; Mehta et al., 2000; Mehta et al., 2004; Schiffer et al., 2006).

The spontaneously hypertensive rat strain (SHR), a rat model bred from progenitor Wistar Kyoto rats (WKY) (Okamoto & Aoki, 1963), is the most widely accepted rodent model of ADHD (Davids, Zhang, Tarazi, & Baldessarini, 2003; Sagvolden, 2000; Sagvolden et al., 1993). SHR rats display the main behavioral, genetics and neurochemical characteristics of ADHD (Knardahl & Sagvolden,

1979; Sagvolden, 2000; Sagvolden, Hendley, & Knardahl, 1992; Sagvolden, Russell, et al., 2005; Wultz & Sagvolden, 1992), (Heal et al., 2008; Roessner et al., 2010; Russell, 2003; Russell et al., 1995). Likewise, MPH corrects attentional deficits and motor hyperactivity in SHR rats, lending further support for the SHR strain as a model for ADHD (K. M. Kantak et al., 2008; Sagvolden, Johansen, Aase, & Russell, 2005; Sagvolden, Russell, et al., 2005).

Although attentional and motor alterations in ADHD have been well characterized, less is known about how this disorder impacts action control, which is the process by which voluntary actions are selected and executed based on prior reinforcement learning. Maze performance of SHR rats suggests they preferentially use response strategies to guide behavior in spatial tasks (Clements, Saunders, Robertson, & Wainwright, 2007; Clements & Wainwright, 2006; K. M. Kantak et al., 2008). Here, we employed operant conditioning procedures to distinguish action-outcome from stimulus-response action control in SHR rats. Most recent theories of action control suggest that two processes guide action control: (1) a goal-directed system based on current knowledge of action-outcome contingencies and (2) a habit system based on acquired stimulus-response associations (Dickinson, 1985). Operant paradigms, such as pressing a lever to receive a food reward, provide means of characterizing goal-directed and habitual action control. Behavior of SHR rats has yet to be assessed using these paradigms. Studying goal-directed behavior in ADHD will advance our understanding of the brain networks involved in reward processing and

contingency learning in ADHD, thereby revealing new mechanisms and potential treatments for this disorder (Griffiths, Morris, & Balleine, 2014).

Although previous research has shown overactive instrumental responding in SHR rats that was corrected by MPH, it is not known whether animals responded in a goal-directed or habitual manner (Sagvolden et al., 1993). In the present study, we examined goal-directed action control in SHR rats using (1) an instrumental learning paradigm; a free-operant training phase in which rats separately acquired two distinct action-outcome associations, (2) an outcome devaluation paradigm; a choice test conducted in extinction prior to which one of the food outcomes was devalued through specific satiety and (3) a contingency degradation paradigm; another choice test conducted in extinction prior to which one of the action-outcome associations was degraded using subject's expectations of outcomes rather than the value they place on those outcomes. We used these paradigms to probe goal-directed behavior in adult and adolescent SHR and WKY rats. We also examined the effects of different acute doses of MPH on choice behavior following outcome devaluation in both rat strains**. Table 2.1** represents a roadmap for chapter 2 experiments.

2.2. METHODS AND RESULTS

2.2.1. General Procedures

2.2.1.1. Operant Chambers

Behavioral training and testing took place in 12 identical rat operant conditioning chambers (Med Associates, St. Albans, VT). Each operant

conditioning chamber measures 30.5×24.1×21 cm (w×h×d) and is constructed of stainless steel and clear plastic walls and a stainless-steel grid floor. A food cup with infrared detectors is centered on one wall of the operant conditioning chamber. Retractable levers are situated to the left and right of the food cup. Responses on these levers deliver one food pellet from a pellet dispenser mounted outside the operant conditioning chamber. Two types of pellets are used in the experimental procedures: 45-mg grain-based pellets and chocolate-flavored purified pellets (Bio-serv, Frenchtown, NJ). Each operant conditioning chamber is housed in a sound attenuating shell and equipped with a ventilation fan that was activated during behavioral procedures. Control over the operant conditioning chambers is enabled by a personal computer operating through an interface. Operant conditioning chamber operation and data collection are carried out with Med Associates proprietary software (Med-PC).

2.2.1.2. Behavioral Procedures

General procedures and Habituation: Behavioral procedures commenced after one week of food restriction. Rats were provided with two 15-minute sessions to habituate to the testing chamber, after which they began behavioral training. **Table 2.2** represents a schematic paradigm for all behavioral procedures that we used in this chapter.

Instrumental training: Rats underwent two training sessions per day; in one session, responses on one lever were associated with delivery of grain pellets and in the other session responses on a different lever were associated

with chocolate pellet delivery (see **Table 2.2**). For each training session, one lever was inserted into the chamber and responses the rats made on the lever delivered a single food pellet associated with that lever. The session terminated when rats earned 20 pellets or 25 minutes elapsed. Rats were trained daily on each lever in separate sessions with a 30-minute interval between sessions. Training lasted for 10 days; on days 1–3, each response on the lever resulted in pellet delivery (continuous reinforcement). On days 4–5, pellets were delivered according to a variable-ratio (VR) 5 schedule, which requires, on average, 5 responses to earn a pellet reward. On days 6–7, pellets were delivered according to a VR-15 schedule. On days 8–10, pellets were delivered according to a VR-20 schedule.

Outcome devaluation: Rats were placed in individual cages identical to their home cage and were provided with 25g of chocolate-flavored pellets. After 30 minutes, rats were given an injection of methylphenidate hydrochloride (Sigma Aldrich, St. Louis MO USA) dissolved in 0.8% saline or, for the control condition, an equal volume of 0.8% saline. Rats were returned to the cages containing chocolate pellets for an additional 30 minutes (see **Table 2.2**).

Contingency degradation training: Rats underwent selective degradation of one of the instrumental contingencies by weakening one of the action-outcome associations that rats learned during instrumental conditioning. During contingency training, responses on each lever continued to deliver the same outcomes as during instrumental training. However, one of the two outcomes was also delivered non-contingently; that is, independent of rat's actions, for

every second in which rats made no lever response, where there was a 5% probability of dispensing one pellet. For half of the animals, the degraded outcome was chocolate pellets, and for the remaining animals it was grain pellets. Non-contingent outcome delivery occurred during all training sessions. Thus, for one lever-training session, the non-contingent outcome was the same as the outcome that rats received during instrumental training, whereas for the other lever-training session, the non-contingent outcome was different from the outcome that rats received during instrumental training. Rats were given two 20-minute training sessions each day, one on each lever with a break of approximately one hour between sessions. Training continued for 4-5 days (see **Table 2.2**).

Choice test: After devaluation or contingency degradation training, rats were placed in the operant conditioning chamber and both levers were inserted. Rats had the opportunity to respond on either lever for 10 minutes. No outcomes were presented in this session (see **Table 2.2**).

Locomotor activity assay: Rats were individually placed in an activity monitoring arena equipped with an automated locomotor activity detection system (Accuscan, Columbus OH, USA). Rats were placed in the arena for a 60-minute habituation session. Immediately after habituation, rats were injected with saline and returned to the arena for 60 minutes, followed by a 60-minute session with 2mg/kg MPH injections. A measure of locomotor activity (HACTV: average horizontal activity) was collected based on the number of photobeam breaks that occurred as animals moved through the arena.

2.2.1.3. Statistical Analysis

For instrumental conditioning tests, the rate of responses was calculated as the number of lever presses per minute during each session. Reinforcer type (chocolate or grain pellet) was collapsed across training sessions since no effect of reinforcer type was observed on measures of response rate. Responses on the two levers were categorized as valued or devalued for the outcome devaluation test, and degraded and non-degraded for the contingency degradation test. The lever that delivered grain pellets was labeled as valued, and the one that delivered chocolate pellets was labeled as devalued. Similarly, the lever associated with the contingent outcome was labeled as non-degraded, and the lever associated with the non-contingent outcome was labeled as degraded (see **Table 2.3**). Data were normalized by dividing responses on the valued or devalued lever by total (valued plus devalued) responses. Normalization was carried out because of strain differences in overall response rates during the tests. MPH and saline injections were intermixed for all experiments; therefore, there was no injection-order effect to influence outcome devaluation responding. For the contingency degradation test (Experiment #1), we lost data for one SHR rat due to a technical error. Additionally, food consumption and locomotor activity tests were conducted on 12 WKY and 12 SHR rats in Experiment #1. Data analysis was conducted using SPSS for Mac Version 20.0. The normality of data distribution was checked using Kolmogorov–Smirnov tests. All data were normally distributed ($p > 0.1$). To analyze instrumental performance, we used mixed-model ANOVA and planned

comparisons using two-tailed t-tests. The level of significance was set at α = 0.05.

2.2.2. Experiment #1

2.2.2.1. Subjects and experiment description

Subjects: We tested a group of 12 male adult spontaneously hypertensive rats (SHR) (ADHD model) from Charles River Laboratories (Wilmington, MA), and a group of 17 male adult Wistar-Kyoto rats (WKY), the normotensive control strain from Harlan Laboratories (Indianapolis, IN). The choice of strains from different vendors is based on previous studies showing that the WKY strain from Harlan is the most similar genetically to Charles River SHR rats (Sagvolden et al. 2012). Rats (P75-P105) weighed approximately 175-250g at the time of testing and were housed in pairs in 47.6×20.3×26 cm (w×h×d) polycarbonate containers with Alpha Chip bedding material (Northeastern Products Corp., Warrensburg, NY) and had free access to water. One week after arrival, all rats were placed on a restricted food diet of approximately 20g of standard rat pellets per day (Purina, St. Louis, MO). Rats were fed after their daily behavioral training session. Food restriction continued for the duration of the experiment. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee.

Experiment description: We trained all rats on instrumental conditioning for 10 days. After rats acquired the instrumental paradigm successfully, we devalued one of the two reward outcomes using selective satiety. Afterwards, we

placed the rats into the operant chambers with levers associated with both outcomes out for a choice test to examine rats' goal-directed behavior. Prior to extinction, we injected the rats with a dose of 2.0mg/kg body weight of MPH or comparable volume of saline to study the effect of MPH on goal-directed action control. To further assess goal-directed behavior, we used the contingency degradation test. We trained the rats on a specific contingency where we delivered one outcome contingently and the other non-contingently. After training, we placed the rats into the operant chambers for extinction with levers associated with both outcomes out to evaluate rats' action control. A timeline of behavioral procedures is depicted in **Table 2.1**.

2.2.2.2. Instrumental training results

All rats acquired an instrumental response; however, SHR rats exhibited greater response rates across training sessions compared to WKY rats. **Figure 2.1** represents the lever-pressing rate in SHR and WKY rats. Mixed-model ANOVA confirmed a significant effect of (1) training block ($F(3,81)=259.3$, $p<0.001$), (2) strain ($F(1,27)=6.7$, $p=0.015$), and (3) block * strain interaction ($F(3,81)=3.2$, $p=0.028$). SHR responses were significantly higher than WKY responses over blocks two and four ($p=0.001$, $p=0.026$ respectively, independent-samples t-test).

To investigate whether reward type (chocolate vs. grain pellets) had any influence on lever presses, we examined each strain's response with the reward

type as a factor. Repeated-measures ANOVA revealed no effect of reward type in both SHR ($F(1,11)=2.88$, $p=0.12$) and WKY ($F(1,16)=0.36$, $p=0.56$) rats.

2.2.2.3. Outcome devaluation results

Because of variability in overall response rates during the choice test, responses on the valued and devalued levers were normalized as a percentage of total responses during the test. **Figure 2.2** illustrates the percentage of responses on the valued vs. the devalued lever in SHR and WKY rats after saline or MPH injections. Repeated-measures ANOVAs were conducted for each of the two groups tested using outcome value and type of injection as within-subject factors. These analyses revealed significant outcome value * injection interactions among WKY rats ($F(1,16)=4.83$, $p=0.043$) as well as SHR ($F(1,11)=12.52$, $p=0.005$) rats. Following saline injections, WKY rats showed a significant goal-directed behavior by responding more on the valued vs. the devalued lever (paired-samples t-test: $t(16)=2.6$, $p=0.02$). In contrast, MPH disrupted goal-directed behavior in these rats, as their responses did not differ significantly between valued and devalued levers following MPH injection (paired-samples t-test: $t(16)=0.24$, $p=0.82$).

The reverse pattern was observed in SHR rats. Following saline injections, SHR rats showed no goal-directed behavior, responding equally on the valued and devalued levers (paired-samples t-test: $t(11)=0.2$, $p=0.84$). MPH restored goal-directed behavior in these rats, as shown by significantly greater responding

on the valued lever compared to the devalued lever after MPH injection (paired-samples t-test: $t(11)=4.65$, $p=0.001$).

We additionally examined whether MPH injection influenced overall response rates during the devaluation test. **Figure 2.3** shows the effect of MPH on overall response rates (the average of response rates on both levers) of both rat strains during the devaluation test after receiving MPH or saline injections. MPH administration suppressed overall response rates. Mixed-model ANOVA showed a significant effect of injection ($F(1,27)=4.3$, $p<0.05$), but no strain effect ($F(1,27)=3.67$, $p=0.07$), or injection * strain interaction ($F(1,27)=0.002$, $p>0.05$). Although there was a significant main effect of injection, group comparisons were not significant (WKY: paired samples t-test, $t(16)=1.88$, $p=0.079$; SHR $t(11)=1.15$, $p>0.05$, respectively). Overall, these data indicate that MPH might decrease rats' instrumental activity during the choice test; however, this result is not significant.

2.2.2.4. Contingency degradation results

Following outcome devaluation, rats underwent contingency degradation training and choice test. **Figure 2.4** shows response rates, normalized as a percentage of total responses, during the choice test conducted in extinction after contingency degradation training for SHR and WKY rats. Mixed-model ANOVA revealed a significant main effect of degradation ($F(1,26)=11.53$, $p=0.002$) and degradation * strain interaction ($F(1,26)=5.12$, $p=0.03$). A paired-samples t-test showed that WKY responses on the non-degraded lever were significantly higher than their responses on the degraded lever ($t(16)=3.98$, $p=0.001$). However, SHR

rats did not show any difference in their responses on the non-degraded versus the degraded lever ($t(10)=0.98$, $p=0.35$), displaying habitual response.

2.2.2.5. Food consumption

To determine whether MPH or rat strain influenced food consumption during the devaluation procedure, we examined the amount of food rats consumed during the first 30 minutes of the devaluation test prior to injections as well as in the 30 minutes after injections. All rats reached satiety; however, the amount of food required to reach satiety differed by rat strain **(Table 2.4)**. In the 30 minutes prior to injection, SHR rats consumed a significantly greater amount of food than WKY rats (independent-samples t-test: $t(22)=3.69$, $p<0.001$).

The majority of food consumption occurred in the first 30 minutes prior to MPH injection (79%). However, MPH altered food consumption in SHR and WKY rats in the remaining 30 minutes. An ANOVA, using type of injection as a within subject factor, confirmed a significant effect of injection ($F(1,22)=25.52$, $p=0.018$). MPH significantly reduced food consumption in WKY rats (paired-samples t-test: $t(11)=2.97$, $p=0.013$) but not in SHR rats (paired-samples t-test, $t(11)=0.923$, $p=0.38$). Overall, these data indicate that SHR rats consumed more food before reaching satiety and that MPH suppressed food consumption selectively in WKY rats.

2.2.2.6. Locomotor activity test

We examined locomotor activity to determine whether strain and MPH injection influenced this behavior. We found SHR rats traveled a greater distance as measured by HACTV (average horizontal activity) and that MPH increased locomotor activity in both strains. HACTV was averaged across 5-minute blocks for one hour after saline and MPH injections **(Figure 2.5)**. Repeated-measures ANOVA on HACTV revealed a significant effect of strain ($F(1,11)=26.4$, $p<0.001$) and injection ($F(2,22)=13.6$, $p<0.01$). However, there was no phase * strain interaction ($F(2,22)=2.36$, $p>0.05$). MPH injections significantly increased HACTV of both strains as compared to saline injection (**Figure 2.6**, paired-samples t-test- SHR: $t(22)=6.45$, $p<0.001$; WKY: $t(22)=7.77$, $p<0.001$). Moreover, HACTV was significantly greater in SHR rats as compared to WKY rats after both saline and MPH injections (**Figure 2.5**, independent-samples t-test- saline injection: $t(22)=11.62$, $p<0.001$; MPH injection: $t(22)=3.85$, $p=0.001$).

In **experiment #1**, we showed that SHR rats have impaired goal-directed behavior with a dominant habitual action control. MPH remediated this deficit in SHR rats, but impaired goal-directed behavior in control, WKY, rats. However, since (1) WKY rat strain is used to model other neuropsychiatric disorders such as depression, and (2) a few studies revealed some biological variability in this rat strain (Kurtz, Montano, Chan, & Kabra, 1989; Kurtz & Morris, 1987), in **experiment #2**, we assessed the reliability of WKY rats as a control strain for SHR in our studies. We tested an outbred rat strain on the outcome devaluation paradigm to compare its performance to WKY rats.

Another limitation of experiment #1 was injecting all rats with the same dose of MPH (2.0mg/kg), while many studies have reported significant variations in the therapeutic doses of this drug. Further, while we tested adult SHR rats (age 11-15 weeks), some studies suggested that SHR serve better as a model for ADHD before the age of 8 weeks. To address these limitations, in **experiment #3**, we tested the effect of different doses of MPH on action control in adolescent SHR and WKY rats (age 4-7 weeks).

2.2.3. Experiment #2

2.2.3.1. Subjects and experiment description

Subjects: To assess WKY rats' performance as a reliable control group, we tested a group of 32 male adult Long Evans rats, an outbred control rat strain from Harlan Laboratories (Indianapolis IN, USA), age P75-P105, weight approximately 175-250g at the time of testing. Rats were housed in pairs in 47.6×20.3×26 cm (w×h×d) polycarbonate containers with Alpha Chip bedding material (Northeastern Products Corp., Warrensburg, NY) and had free access to water. One week after arrival, all rats were placed on a restricted food diet of approximately 20g of standard rat pellets per day (Purina, St. Louis, MO). Rats were fed after their daily behavioral training session. Food restriction continued for the duration of the experiment. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee.

Experiment description: We trained this group of rats on the same instrumental conditioning paradigm for 10 days. After training, we devalued one

outcome and put rats into chambers for the extinction test to assess goal-directed behavior pattern and compare it to that of WKY rats in Experiment #1. A timeline of behavioral procedures is depicted in **Table 2.1**.

2.2.3.2. Instrumental training results

All rats (WKY and Long Evans) acquired an instrumental response at comparable rates across training sessions. **Figure 2.7** represents the lever-pressing rate in WKY and Long Evans rats. Mixed-model ANOVA showed a significant effect of training block ($F(3,141)=210.43$, $p<0.001$) and block * strain interaction ($F(3,141)=79.95$, $p=0.028$). However, there was no significant strain effect ($F(1,47)=0.25$, $p=0.62$). Independent-samples t-test showed that Long Evans responses were significantly higher than WKY responses only over the first training block ($t(47)=6.28$, $p<0.001$). There was no significant difference between the two strains over blocks two, three and four ($t(45.88)=1.43$, $p=0.16$, $t(47)=0.24$, $p=0.81$ and $t(47)=0.81$, $p=0.42$, respectively).

2.2.3.3. Outcome devaluation results

Figure 2.8 shows response rates normalized as a percentage of total responses during the choice test conducted in extinction after devaluation of one of the two outcomes on which rats were trained. Long Evans rats' behavior displayed a significantly higher response rates on the valued lever as compared to the devalued lever (paired-samples t-test: $t(31)=6.06$, $p<0.001$). **Figure 2.9** shows comparable responses of WKY and Long Evans rats on the devaluation

extinction test. Mixed-model ANOVA showed a significant effect of outcome value ($F(1,47)=31.7$, $p<0.001$) and no significant effect of outcome value * strain interaction ($F(1,47)=0.16$, $p=0.69$). Independent-samples t-tests reveal no significant difference between WKY and Long Evans rats' responses on the devalued or the valued lever ($t(47)=0.4$, $p=0.76$ for both valued and devalued comparisons). These results support the use of WKY rats as a control group for the SHR strain as they show the same pattern of goal-directed behavior compared to an outbreed rat strain.

2.2.4. Experiment #3

2.2.4.1. Subjects and experiment description

Subjects: Many studies have suggested that SHR better represents ADHD symptoms before adulthood since they might start to develop symptoms of hypertension between the ages of 4 to 10 weeks (Christiansen et al., 2002; Marcil et al., 1997; Ueno et al., 2002). Therefore, we tested a group of 18 male adolescent spontaneously hypertensive rats (SHR) (ADHD model), and a group of 18 male adolescent Wistar-Kyoto rats (WKY), the normotensive control strain, age of P30-P50, weighing approximately 75-100g at the time of testing. Rats were housed in pairs in 47.6×20.3×26 cm (w×h×d) polycarbonate containers with Alpha Chip bedding material (Northeastern Products Corp., Warrensburg, NY) and had free access to water. One week after arrival, all rats were placed on a restricted food diet of approximately 12g of standard rat pellets per day (Purina, St. Louis, MO). Rats were fed after their daily behavioral training session. Food

restriction continued for the duration of the experiment. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee.

Experiment description: We trained this group of rats on the same behavioral paradigms in Experiment #1 (instrumental training, outcome devaluation and choice test) to examine goal-directed behavior and to replicate our finding in adult rats in a different age group. In addition to injecting a dose of 2.0mg/kg body weight of MPH, we administered three other different doses of MPH (0.5, 1.0, or 4.0 mg/kg) to learn if there is a dose-dependent effect of MPH on goal-directed behavior. A timeline of behavioral procedures is depicted in **Table 2.1**.

2.2.4.2. Instrumental training results

All rats acquired an instrumental response; however, SHR adolescent rats exhibited greater response rates across training sessions compared to WKY adolescent rats. **Figure 2.10** represents the lever-pressing rate in SHR and WKY rats. Mixed-model ANOVA confirmed a significant effect of training block ($F(3,102)=124.58$, $p<0.001$), and block * strain interaction ($F(3,102)=3.11$, $p=0.03$) and no strain effect ($F(1,34)=2.01$, $p=0.17$). SHR responses were significantly higher than WKY responses over block three ($t(34)=2.49$, $p=0.018$, independent-samples t-test).

2.2.4.3. Outcome devaluation results

Our results illustrate that adolescent WKY rats showed a pattern of goal-directed behavior, which replicated our results in adult WKY rats. Their normalized response rate on the valued lever was higher compared to the devalued lever. This pattern was observed under saline injections; however, when adolescent WKY rats received any dose of MPH (0.5mg/kg, 1.0mg/kg, or 4.0mg/kg body weight), this behavioral pattern was disrupted **(Figure 2.11)**. On the other hand, adolescent SHR rats did not show a lever preference under saline injections. Yet, at a dose of 1.0mg/kg body weight MPH, adolescent SHR rats' responses shifted to display a goal-directed behavior **(Figure 2.12)**. Repeated-measures ANOVAs were conducted for each of the two groups tested using outcome value and type of injection (0.5mg/kg MPH, 1.0mg/kg MPH, 4.0mg/kg MPH or 2.0mg/kg saline) as within-subject factors. These analyses revealed that although adolescent WKY rats' response rate on the valued lever was higher than on the devalued lever, there was no significant effect of outcome value ($F(1,52)=0.81$, $p=0.37$), outcome value * injection interaction ($F(3,52)=0.51$, $p=0.68$) or dose ($F(3,52)=0.86$, $p=0.47$). A different pattern was observed in adolescent SHR rats. Repeated-measures ANOVA confirmed a significant effect of outcome value ($F(1,56)=4.56$, $p=0.037$) and dose ($F(3,56)=10.86$, $p<0.001$), with no significant effect of outcome value * injection interaction ($F(3,56)=2.05$, $p=0.12$). Paired-samples t-tests revealed that following saline injections, adolescent SHR rats showed no goal-directed behavior, responding equally on the valued and devalued levers ($t(14)=0.48$, $p=0.64$). MPH restored goal-directed

behavior in these rats, as shown by significantly greater responding on the valued lever compared to the devalued lever after a dose of 1.0mg/kg MPH injections ($t(12)=2.8$, $p=0.016$). However, MPH injections at doses of 0.5mg/kg and 4.0mg/kg did not restore goal-directed behavior in adolescent SHR rats ($t(15)=1.32$, $p=0.21$, $t(15)=0.54$, $p=0.6$, respectively). Together, these results highlight the potential differences among adult and adolescent rats' response to different doses of MPH.

2.3. CONCLUSIONS

Using an instrumental conditioning paradigm, we present the first experimental evidence of disrupted goal-directed behavior using instrumental procedures in a rat model of ADHD. Both control WKY rats and SHR rats were successful at acquiring an instrumental response, with SHR rats showing a significantly greater response rate during training. Further, using an open field test to evaluate rat locomotor activity, SHR rats showed enhanced locomotor activity as compared to WKY rats.

Our results suggest a fundamental impairment in goal-directed action control in SHR rats that was remediated by MPH. We used the outcome devaluation paradigm to assess whether adult and adolescent animals formed action-outcome (goal-directed) or stimulus-response (habit) associations. While WKY rats showed goal-directed action control in both paradigms, SHR rats demonstrated a marked deficit in sensitivity to changes in outcome value and to changes in the action-outcome contingency. Furthermore, we found that deficits

in goal-directed action control following outcome devaluation were remediated in SHR rats with MPH administration in adult and adolescent rats under doses of 2.0mg/kg and 1.0mg/kg body weight, respectively. In contrast, while goal-directed behavior was exhibited by WKY rats receiving saline, it was disrupted by MPH administration

To further fortify our finding of impaired goal-directed behavior in SHR rats, and to exclude any effect of outcome devaluation on this behavior, we used a contingency degradation test in adult rats after training them on a selective degradation of the instrumental contingency. Like the outcome devaluation test, WKY rats displayed intact goal-directed behavior while SHR rats were impaired on this test. SHR rats' performance was comparable on the lever for which the contingency had been degraded compared to the non-degraded lever. This result further supports the notion of impaired goal-directed action control in SHR rats.

Further, to assess WKY rats' performance as a reliable control group, we tested a group of adult Long Evans rats, an outbred control rat strain, using outcome devaluation paradigms. Long Evans rats showed a comparable response to WKY rats during the choice test displaying goal-directed action control.

2.4. LIMITATIONS AND FUTURE DIRECTIONS

Although we tested the effect of different doses of MPH on goal-directed behavior in adolescent rats, one limitation of our study was testing the effect of only one dose of MPH in adult rats. Many studies have reported significant

variations in the therapeutic doses of MPH. Therefore, the use of one drug dose limits the conclusions we can draw from this study. A future study exploring the dose-response relationship between MPH and action control in both adult and adolescent rats is required to fully address this issue.

Further, we only assessed the acute effects of MPH administration on action control with a single dose of MPH prior to choice test. Many studies have shown that MPH differently achieves its behavioral effects when administered acutely vs. chronically. Hence, further studies ought to investigate the effects of chronic MPH administration on action control.

In this chapter, we showed that goal-directed behavior is impaired in adult and adolescent SHR rats. MPH restored this behavior in both age groups in a dose dependent pattern. Further, we showed that goal-directed behavior, under MPH effects in SHR and WKY rats, follows a nonlinear relationship (**Figure 2.13**) where dopamine levels correlate with behavioral performance according to an inverted U-shaped function (Cools, Barker, Sahakian, & Robbins, 2001; Williams & Goldman-Rakic, 1995; Zahrt, Taylor, Mathew, & Arnsten, 1997). Both WKY rats on normal saline and SHR rats on MPH were sensitive to goal-directed behavior, indicating optimal dopamine levels. Conversely, animals with low/high dopamine level (SHR on normal saline/WKY on MPH) showed impairment in goal-directed behavior. However, since MPH is not a specific dopamine agonist, in **chapter 3**, we tested the effects of specific dopamine agonists and antagonists on action control. Previous studies have shown that dopamine D1 receptor (D1R) and dopamine D2 receptor (D2R) activation have different effects on goal-

directed and habitual behaviors in animals. Thus, we used an outcome devaluation paradigm to study dopamine modulation of action control in SHR and WKY rats using the following dopaminergic drugs: D1R agonist, D1R antagonist, D2R agonist and D2R antagonist.

CHAPTER 3

Characterizing the Effects of Dopamine Modulation of Action Control in a Rat Model of ADHD

3.1. INTRODUCTION

Attention-Deficit/ Hyperactivity Disorder (ADHD) is characterized by symptoms of inattention, hyperactivity and impulsivity. Although these symptoms are well described in the literature, motivational impairments and deficits in reward processing are other less studied symptoms of ADHD. A potential mechanism that underlies these symptoms might be an impaired action control; which is the process by which voluntary actions are selected and executed based on prior reinforcement learning. The two main systems that modulate action control are (1) goal-directed system, forming action-outcome associations, and (2) habitual system, acquiring stimulus-response associations. Healthy individuals should be able to flexibly change their selection between stimuli according to the obtained outcomes to adapt to a dynamic environment. This flexible change represents goal-directed behavior. On the other hand, the reflexive response to stimuli, independent of feedback, represents habitual behavior. Dickinson et al. have shown that extensive instrumental training of animals manifested as a disrupted goal-directed behavior and a dominant habitual response (Adams & Dickinson, 1981; Dickinson, 1985). In addition, previous research has shown that exposure to amphetamine (A. Nelson &

Killcross, 2006; Nordquist et al., 2007), alcohol (Corbit et al., 2012), stress (Dias-Ferreira et al., 2009) or binge-like consumption of a palatable food (Furlong et al., 2014) can lead to accelerated habitual control. Accordingly, experience-based behaviors resulting from such exposure enhance habit learning at the expense of goal-directed behavior. Interestingly, this pattern of instrumental behavior was reversed by the administration of D1 receptor antagonists (SCH23390) and enhanced by the administration of D2 receptor antagonists (Eticlopride) in animals with repeated exposure to amphetamine (A. J. Nelson & Killcross, 2013). Further, infusion of the D1 receptor antagonist, SCH23390, in the brain region where habitual behavior is rooted, the dorsolateral striatum, restored the normal pattern of goal-directed behavior in animals that had restricted access (binge-like consumption) of a palatable food (Furlong et al., 2014). Taken together, normal patterns of goal-directed behavior seem to rely on optimal levels of D1 and D2 dopamine receptors (D1R, D2R) where the activation of these two receptors should be in balance. Over activation of D1R or under activation of D2R could exhibit as disrupted goal-directed behavior and/or dominant habitual behavior. In our previous study (Natsheh & Shiflett, 2015), we found that spontaneously hypertensive rats (SHR), a rat model of ADHD, showed a deficit in goal-directed behavior that was restored by administration of methylphenidate (MPH). Here, we hypothesized that this pattern of impaired goal-directed behavior in SHR rats might be a result of a misbalance in D1R and D2R. Previous studies have shown that SHR rats have high density of striatal D1R (Carey et al., 1998; Kirouac & Ganguly, 1993; Watanabe et al., 1997). Conversely, there is contradicting

evidence regarding the density of D2R in SHR rats with down-regulation, up-regulation, or similar levels to control rats all described (Carey et al., 1998; Lim et al., 1990a, 1990b; Linthorst et al., 1993; Russell et al., 1995; Yu et al., 1990). In sum, we tested the hypothesis that action control in SHR rats is modulated by higher levels of D1R, and similar to- or lower levels of D2R as compared to control rats. In **experiment #1**, we assessed the effects of a D1R antagonist (SCH23390) and a D2R agonist (Quinpirole) on goal-directed behavior in SHR rats. To examine the inverse relationship, in **experiment #2**, we assessed the effects of D1R agonist (SKF38393) and D2R antagonist (Raclopride) on goal-directed behavior in SHR rats. **Table 3.1** represents a roadmap for chapter 3 experiments.

We predicted that SHR would show a deficit in goal-directed behavior, whereas WKY rats would show no impairment, replicating our findings in chapter 2. Based on our hypothesis that action control deficits arise from over-activation of D1R and/or under-activation of D2R, we expected that the D1R antagonist (SCH23390) and the D2R agonist (Quinpirole) would restore goal-directed behavior in SHR rats and impair this behavior in control rats. The opposite set of agonist/antagonist (SKF38393/Raclopride) would further impair performance in SHR rats as well as in control rats. See **Table 3.2**.

3.2. METHODS AND RESULTS

3.2.1. Experiment #1: Methods and experiment description

3.2.1.1. Subjects and apparatus

Forty-eight male adult (P49-P80) rats were used in this study; 24 of which were SHR (ADHD rat model) from Charles River Laboratories (Wilmington, MA), and 24 were Wistar-Kyoto rats (WKY), the normotensive control strain, from ENVIGO (UK). The choice of strains from different vendors is based on previous studies showing that the WKY strain from Harlan is most similar genetically to Charles River SHR rats (Sagvolden & Johansen, 2012). Rats weighed approximately 110-175g at the time of testing. Rats were housed in pairs in 47.6×20.3×26 cm (w×h×d) polycarbonate containers with Alpha Chip bedding material (Northeastern Products Corp., Warrensburg, NY) and had free access to water. One week after arrival, all rats were placed on a restricted food diet of approximately 15g of standard rat pellets per day (Purina, St. Louis, MO). Rats were fed after their daily behavioral training session. Food restriction continued for the duration of the experiment. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee.

Behavioral training and testing took place in 12 identical rat operant conditioning chambers (Med Associates, St. Albans, VT). Each operant conditioning chamber measured 30.5×24.1×21 cm (w×h×d) and was constructed of stainless steel and clear plastic walls and a stainless-steel grid floor. A food cup with infrared detectors was centered on one wall of the operant conditioning chamber. Retractable levers were situated to the left and right of the food cup. Responses on these levers delivered one food pellet from a pellet dispenser mounted outside the operant conditioning chamber. Two types of pellets were used in the experimental procedures: 45-mg grain-based pellets and chocolate-

flavored purified pellets (Bio-serv, Frenchtown, NJ). Each operant conditioning chamber was housed in a sound attenuating shell and equipped with a ventilation fan that was activated during behavioral procedures. Control over the operant conditioning chambers was enabled by a personal computer operating through an interface. Operant conditioning chamber operation and data collection were carried out with Med Associates proprietary software (Med-PC).

3.2.1.2. Behavioral procedures

General procedures: A timeline of behavioral procedures is depicted in **Table 3.1**. Behavioral procedures commenced after one week of food restriction. Rats were provided with two 15-minute sessions to habituate to the testing chamber, after which they began behavioral training. **Table 3.3** represents a schematic paradigm for all behavioral procedures that we used in this chapter.

Instrumental conditioning: Rats underwent two training sessions per day; in one session, responses on one lever were associated with delivery of grain pellets and in the other session responses on a different lever were associated with chocolate pellet delivery (see **Table 3.3**). For each training session, one lever was inserted into the chamber and responses the rats made on the lever delivered a single food pellet associated with that lever. The session terminated when rats earned 20 pellets or 25 minutes had elapsed. Rats were trained daily on each lever in separate sessions with a 30-minute interval between sessions. Training lasted for 10 days (see **Table 3.1**); on days 1–3, each response on the lever resulted in pellet delivery (continuous reinforcement). On days 4–5, pellets

were delivered according to a variable-ratio (VR) 5 schedule, which required, on average, 5 responses to earn a pellet reward. On days 6–8, pellets were delivered according to a VR-15 schedule. On days 9–10, pellets were delivered according to a VR-20 schedule.

Outcome devaluation test and drug injection: Rats were placed in individual cages identical to their home cage and provided with 25g of food pellets. After 40 minutes, rats were given an intraperitoneal injection of normal saline (as a control), SCH23390 (D1 antagonist) or Quinpirole (D2 agonist). Rats were returned to the cages containing food pellets for an additional 15 minutes. They were then placed in the operant conditioning chamber and both levers were inserted. Rats had the opportunity to respond on either lever for 5 minutes. No outcomes were presented in this session. Devaluation test was repeated six times. Under each medication condition, rats underwent chocolate and grain devaluation to control for pellet preference. Medication status (normal saline; SCH23390; Quinpirole) and pellet devaluation (chocolate; grain) were counterbalanced across devaluation sessions. Rats received reminder instrumental training sessions at least once a week to ascertain that their sensitivity to choice test was not affected by consecutive extinction sessions (see **Table 3.3**).

Locomotor activity assay: Rats were individually placed in an activity-monitoring arena equipped with an automated locomotor activity detection system (Accuscan, Columbus OH, USA). Rats were placed in the arena for a 30-minute habituation session. Immediately after habituation, rats were injected with

normal saline and returned to the arena for 30 minutes, followed by a 30-minute session with SCH23390 injections at a dose of 0.0025mg/kg or Quinpirole injections at doses of 0.01mg/kg; 0.001mg/kg. A measure of locomotor activity (HACTV) was collected based on the number of photobeam breaks that occurred as animals moved through the arena.

3.2.1.3. Dopamine medications

We assessed the following doses per drug using locomotor activity test as follows: SCH23390 (Dopamine D1-antagonist): 0.0025mg/kg, Quinpirole (dopamine D2-agonist): 0.01mg/kg; 0.001mg/kg. Based on locomotor activity test results as well as previous studies that used these drugs, we chose a dose of 0.0025mg/kg for SCH23390 and a dose of 0.001mg/kg for Quinpirole. Both drugs were purchased from Sigma Aldrich, St. Louis MO USA and dissolved in 0.8% normal saline.

3.2.1.4. Statistics and data analysis

For instrumental conditioning tests, the rate of response was calculated as the number of lever presses per minute during each session. Reinforcer type (chocolate or grain pellet) was collapsed across training sessions, as no effect of reinforcer type was observed on measures of response rate. Responses on the two levers were categorized as devalued or valued for the outcome devaluation test. In chocolate devaluation sessions, the lever that delivers chocolate in the choice test pellets was labeled as devalued, and the one that delivers grain

pellets was labeled as valued. In grain devaluation sessions, the lever that delivers grain in the choice test pellets was labeled as devalued, and the one that delivers chocolate pellets was labeled as valued. Data were normalized by dividing responses on the valued or devalued lever by total (valued plus devalued) responses. Normalization was carried out because of strain differences in overall response rates during the tests. Drug and saline injections were intermixed for all experiments; therefore, there was no injection-order effect to influence outcome devaluation responding. Similarly, chocolate and grain devaluation was counterbalanced across devaluation sessions to control for devaluation flavor-order effect. Additionally, food consumption and locomotor activity tests were conducted on 12 SHR and 12 WKY rats. Data analysis was conducted using SPSS for Mac Version 20.0. The normality of data distribution was checked using Kolmogorov–Smirnov tests. All data were normally distributed ($p > 0.1$). To analyze instrumental performance, we used 2-factor ANOVA and planned comparisons using two-tailed *t*-tests. The level of significance was set at $\alpha = 0.05$ throughout our analyses.

To analyze outcome devaluation data, we used mixed-model ANOVA and planned comparisons using two-tailed t-tests. We ran 6 devaluation sessions; however, with each devaluation test, the total response rate dropped; thus, rats' sensitivity to outcome devaluation decreased **(Figure 3.1)**.

To control for this, we conducted our analyses using the first devaluation test under each medication status. Exclusion criteria for analysis included: (1) Outcome preference: we excluded rats that had a preference to chocolate or

grain pellets during the last block of instrumental training with a response rate of > 1.5x responses per minute on the lever that is associated with the preferred outcome. Out of 48 rats, 6 SHR rats and 4 WKY rats were excluded from the outcome devaluation test. (2) Lever preference: we excluded rats that had a preference to the right or left lever during the extinction test with >95% response rate on the preferred lever. Two devaluation sessions of SHR rats were excluded. Although both rats did not have pellet preference during instrumental training, during extinction, they were choosing the valued lever over the devalued lever at a frequency of 96:4 and 98:2. However, since the typical response rate of rats that show goal-directed behavior is 70:30, we argue that this extreme tendency to choosing one lever over the other is due to lever preference rather than goal-directed behavior. (3) Low response rate: we excluded rats that had a low response rate during extinction with a total response of <1 per minute on both levers. Three devaluation sessions of WKY rats were excluded. One WKY rat was not responding during instrumental training and outcome devaluation and was dropped from the experiment. See **Table 3.4** for reference.

3.2.2. Experiment #1: Results

3.2.2.1. Instrumental training data

All rats acquired an instrumental response; however, SHR rats exhibited greater response rates across training sessions compared to WKY rats. **Figure 3.2** represents the lever-pressing rate in SHR and WKY rats. A mixed-model ANOVA confirmed (1) a significant effect of training block ($F(3,135)=366.76$,

p<0.001), (2) a significant effect of strain (F(1,45)=56, p<0.001), and (3) a significant block * strain interaction (F(3,135)=19.26, p<0.001). Independent-samples t-test showed that SHR responses were significantly higher than WKY responses over the four blocks of training with a p-value of <0.001 for blocks 1, 2, 3 and 4.

To investigate whether outcome type (chocolate vs. grain pellets) had any influence on lever presses, we examined each strain's responses with the outcome type as a factor. A mixed-model ANOVA revealed no effect of outcome type (F(1,135)=0.744, p=0.4) or outcome * strain interaction (F(1,135)=0.182, p=0.67).

3.2.2.2. Outcome devaluation results

Because of variability in overall response rates during the devaluation test, responses on the valued and devalued levers were normalized as a percentage of total responses during the test. **Figure 3.3** illustrates the percentage of responses on the valued versus the devalued lever in SHR and WKY rats after normal saline, Quinpirole or SCH23390 injections. Mixed-model ANOVA was conducted using outcome value as within-subject factor and strain and type of injection as between-subject factors. These analyses revealed a significant effect of outcome value (F(1,61)=16.39, p<0.001) as well as outcome value * injection * strain interactions (F(2,61)=3.33, p=0.042). Following saline injections, WKY rats showed goal-directed behavior by responding at a significantly higher rate on the valued versus the devalued lever (paired-samples t-test: t(17)= 3.49, p=0.003). In

contrast, both Quinpirole and SCH23390 injections disrupted goal-directed behavior in these rats as their responses were not significantly different between valued and devalued levers following drug injections (paired-samples t-test; Quinpirole: t(9)=1.08, p=0.31, SCH23390: t(5)=0.1, p=0.93).

The reverse pattern was observed in SHR rats. Following saline injections, SHR rats showed an impaired goal-directed behavior, responding equally on the valued and devalued levers (paired-samples t-test: t(15)=1.25, p=0.23). Quinpirole restored goal-directed behavior in these rats, as shown by significantly greater responding on the valued lever compared to the devalued lever after Quinpirole injections (paired-samples t-test: t(7)=2.71, p=0.03). SCH23390 did not restore this behavior in SHR rats; however, compared to rats' response under saline injections, SCH23390 increased the number of responses on the valued versus the devalued lever showing a higher reliability on goal-directed behavior. However, this effect did not reach statistical significance with the relatively small sample available after exclusions (paired-samples t-test: t(8)=2.16, p=0.063).

Total response rate under different drug status during devaluation test

We examined whether Quinpirole and SCH23390 injections influenced total responses per minute during the devaluation test. **Figure 3.4** shows the effect of Quinpirole and SCH23390 compared to normal saline injections on total responses per minute (the average of responses on both levers per minute) for both rat strains during the devaluation test. A 2x3 multifactorial ANOVA with total responses per minute as dependent variable and strain and medication status as

fixed factors showed no significant effects of drug ($F(2,61)=1.76$, $p=0.18$) or strain * drug interaction ($F(2,61)=0.65$, $p=0.52$). However, there was a significant effect of Strain ($F(1,61)=34$, $p<0.001$). Overall, these results indicate that drug injections did not affect total response rate during the devaluation test.

Goal-directed score

For this analysis, rats were categorized as Goal-directed or Habitual based on the percentage of responses on the valued vs. the devalued lever during devaluation test under normal saline injections. To calculate goal-directed score, we used the following formula: [(% of valued responses - % of devalued responses)/(% of valued responses + % of devalued responses)]. Rats with a goal-directed score less than 0.2 were considered habitual, those above or equal to 0.2 were considered goal-directed. This cutoff number is based on the assumption that goal-directed rats should have at least 60% responses on the valued lever, compared to 40% on the devalued lever. This analysis included rats that underwent devaluation under normal saline injections on one devaluation session and at least one drug (SCH23390 or Quinpirole) on another devaluation session to characterize the effect of drugs on goal-directed score in reference to the control state. Most SHR rats started as Habitual and most WKY rats started as Goal-directed under normal saline injections. However, there were individual differences among rats in both strains. **Figure 3.5** shows average goal-directed score in SHR and WKY rats under normal saline, SCH23390 and Quinpirole injections. Independent-samples t-test was conducted using goal-directed score

under different drug conditions as test variables and strain as grouping variable. This analysis revealed a significant difference between the two strains under Quinpirole ($t(14)=2.7$, $p=0.017$). However, although goal-directed score was higher in WKY rats under normal saline and lower under SCH23390, the difference between the two strains was not significant ($t(22)=0.91$, $p=0.38$).and SCH23390: $t(12)=1.62$, $p=0.13$, respectively).

3.2.2.3. Food consumption

To determine whether drug or rat strain influenced food consumption during the devaluation procedure, we examined the amount of food rats consumed during the first 40 minutes of the devaluation test prior to injections as well as in the 20 minutes after injections. All rats reached satiety with a comparable amount of food required to reach satiety for both strains (**Table 3.5**). Normal saline and drug injections did not alter food consumption in SHR nor in WKY rats in the remaining 20 minutes. A mixed-model ANOVA, using consumption at 40 minutes and 60 minutes as within subject factor and type of injection and strain as between subject factors revealed no effect of Strain ($F(1,31)=0.72$, $p=0.4$), drug ($F(2,31)=0.16$, $p=0.85$) or strain * drug interaction ($F(1,31)=0.29$, $p=0.59$) and a significant effect of Consumption during the first 40 minutes vs. the last 20 minutes ($F(1,31)=177.8$, $p>0.001$). The majority of food consumption occurred during the first 40 minutes prior to injections (84%). Overall, these data indicate that SHR and WKY rats consumed similar amount of food and there was no effect of drugs on food consumption post injections.

3.2.2.4. Locomotor activity test

We examined locomotor activity to determine whether strain and drug injections influenced this behavior, and to choose drug doses that do not affect rats' motor activity. Rats were placed in the arena for a 30-min habituation session. Immediately after habituation, rats were injected with normal saline and returned to the arena for 30 minutes, followed by a 30-min session with SCH23390 0.0025mg/kg, Quinpirole 0.001mg/kg or Quinpirole 0.01mg/kg. Horizontal activity was averaged across 5-minute blocks for each session (blocks 1-6).

Differences in HACTV between SHR and WKY

We found SHR rats traveled a greater distance as measured by HACTV (average horizontal activity) during habituation and under saline and drug injections. For SCH23390, a mixed-model ANOVA on HACTV revealed a significant effect of strain ($F(1,10)=82.31$, $p=0.025$) and phase *(habituation; saline injections; SCH23390 0.0025mg/kg injections)* ($F(2,20)=21.22$, $p<0.001$). However, there was no phase * strain interaction ($F(2,20)=0.07$, $p=0.94$). Under Quinpirole injections, a mixed-model ANOVA on HACTV revealed a significant effect of strain ($F(1,10)=21.72$, $p=0.001$), phase *(habituation; saline injections; Quinpirole 0.001mg/kg injections; Quinpirole 0.01mg/kg injections)* ($F(3,30)=56.76$, $p<0.001$) and phase * strain interaction ($F(3,30)=3.86$, $p=0.019$).

Independent-samples t-tests showed that HACTV was significantly greater in SHR rats as compared to WKY rats during habituation ($t(22)=3.36$, $p=0.003$);

under saline ($t(15.72)=4.81$, $p<0.001$), SCH23390 0.0025mg/kg ($t(10)=4.31$, $p=0.002$), Quinpirole 0.001mg/kg ($t(10)=2.33$, $p=0.042$) and Quinpirole 0.01mg/kg ($t(6.2)=7.92$, $p<0.001$) injections.

The effect of drug injections on HACTV in WKY rats

In WKY rats, SCH23390 0.0025mg/kg injections did not affect HACTV significantly compared to saline injection (**Figure 3.6, A)**, paired-samples t-test: $t(5)=2.24$, $p=0.076$). Similarly, Quinpirole 0.001mg/kg injections did not affect HACTV as compared to saline injections (**Figure 3.6, B)**, paired-samples t-test: $t(5)=0.17$, $p=0.83$). However, Quinpirole 0.01mg/kg did significantly decrease HACTV in WKY rats as compared to saline injections (**Figure 3.6, B)**, paired-samples t-test: $t(5)=5.99$, $p=0.002$).

The effect of drug injections on HACTV in SHR rats

In SHR rats, SCH23390 0.0025mg/kg injections did not significantly affect HACTV as compared to saline injection (**Figure 3.7, A)**, paired-samples t-test: $t(5)=2.24$, $p=0.075$). Similarly, Quinpirole 0.001mg/kg injections did not significantly affect HACTV as compared to saline injections (**Figure 3.7, B)**, paired-samples t-test: $t(5)=2.18$, $p=0.08$). However, Quinpirole 0.01mg/kg did significantly decrease HACTV in SHR rats as compared to saline injections (**Figure 3.7, B)**, paired-samples t-test: $t(5)=2.61$, $p=0.047$).

3.2.3. Experiment #2: Methods and experiment description

3.2.3.1. Subjects and apparatus

Twenty-four male adult (P49-P80) rats were used in this study; 12 of which were SHR from Charles River Laboratories (Wilmington, MA), and 12 were WKY, the normotensive control strain, from ENVIGO (UK). Rats weighed approximately 110-175g at the time of testing. Housing conditions and food restriction were the same as those in **experiment #1**. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee. Behavioral training and testing took place in 12 identical rat operant conditioning chambers (Med Associates, St. Albans, VT). See ***Subjects and apparatus 2.3.1.1.*** for chambers description.

3.2.3.2. Behavioral procedures

Behavioral experiments followed the same schedule as in **experiment #1** (see **Table 3.2**). Rats were provided with two 15-minute sessions to habituate to the testing chamber, after which they underwent instrumental conditioning, outcome devaluation test and locomotor activity test. During outcome devaluation test, rats were given an intraperitoneal injection of normal saline (as a control state), SKF38393 (D1 agonist) or Raclopride (D2 antagonist). Pellet devaluation was counterbalanced across devaluation sessions. Normal saline injections were carried out during the first two devaluation sessions while drug injections were counterbalanced across the last four devaluation sessions. Drug doses during

locomotor activity test were as follows: SKF38393 injections at doses of 3.0mg/kg; 1.0mg/kg, Raclopride injections at doses of 0.1mg/kg; 0.05mg/kg.

3.2.3.3. Dopamine medications

To generate a dose-response curve, we tested the effect of two doses per drug using locomotor activity test as follows: SKF38393 (Dopamine D1-agonist): 3.0mg/kg; 1.0mg/kg, Raclopride (dopamine D2-antagonist): 0.1mg/kg; 0.05mg/kg. Based on locomotor activity test results as well as previous studies that used these drugs, we chose a dose of 3.0mg/kg for SKF38393 and a dose of 0.1mg/kg for Raclopride. Both drugs were purchased from Sigma Aldrich, St. Louis MO USA and dissolved in 0.8% normal saline.

3.2.3.4. Statistics and data analysis

We used the same statistical analysis methods that were used in **experiment #1**. Exclusion criteria for analysis included: (1) Outcome preference: Out of 24 rats, two SHR rats and one WKY rat were excluded from the outcome devaluation test. (2) Lever preference: Four devaluation sessions of WKY rats were excluded. Two rats had preference for the valued lever, and two rats had preference for the devalued lever. (3) Low response rate: Eight devaluation sessions of one SHR and seven WKY rats were excluded. One SHR rat was not responding during instrumental training and outcome devaluation and was dropped from the experiment. See **Table 3.6** for reference.

3.2.4. Experiment #2: Results

3.2.4.1. Instrumental training data

All rats acquired an instrumental response; however, SHR rats exhibited greater response rates across training sessions compared to WKY rats. **Figure 3.8** represents the lever-pressing rate in SHR and WKY rats. A mixed-model ANOVA confirmed (1) a significant effect of training block ($F(1,63)=460.9$, $p<0.001$), (2) a significant effect of strain ($F(1,21)=5.2$, $p=0.03$). Independent-samples t-test showed that SHR responses were significantly higher than WKY responses over blocks two ($t(21)=2.24$, $p=0.036$) and three ($t(21)=2.51$, $p=0.02$).

To investigate whether outcome type (chocolate vs. grain pellets) had any influence on lever presses, we examined each strain's responses with the outcome type as a factor. A mixed-model ANOVA revealed no effect of outcome type ($F(1,63)=2.24$, $p=0.15$) or outcome * strain interaction ($F(1,63)=0.01$, $p=0.91$).

3.2.4.2. Outcome devaluation results

Because of variability in overall response rates during the devaluation test, responses on the valued and devalued levers were normalized as a percentage of total responses during the test. **Figure 3.9** illustrates the percentage of responses on the valued versus the devalued lever in SHR and WKY rats after normal saline, Raclopride or SKF38393 injections. Mixed-model ANOVA was conducted using outcome value as within-subject factor and strain and type of injection as between-subject factors. These analyses revealed a significant effect

of Outcome value (F(1,42)=4.52, p=0.039) with a non-significant outcome value * injection * strain interactions (F(2,42)=3.03, p=0.059). However, given this trend (p=0.059), we followed this analysis with paired samples t-test across medication status in each rat strain. Following saline injections, WKY rats showed significant goal-directed behavior by responding more on the valued versus the devalued lever (paired-samples t-test: t(8)= 2.56, p=0.034). In contrast, both Raclopride and SKF38393 injections disrupted goal-directed behavior in these rats as their responses were not significantly different between valued and devalued levers following drug injections (paired-samples t-test; Raclopride: t(4)=1.49, p=0.21, SKF38393: t(7)=0.88, p=0.41).

On the contrary, following saline injections, SHR rats showed impaired goal-directed behavior, responding equally on the valued and devalued levers (paired-samples t-test: t(8)=1.54, p=0.16). Neither Raclopride or SKF38393 improved goal-directed behavior in these rats (paired-samples t-test; Raclopride: t(8)=0.81, p=0.45, SKF38393: t(7)=1.33, p=0.23).

Total response rate under different drug status during devaluation test

We examined whether Raclopride and SKF38393 injections influenced total responses per minute during the devaluation test. **Figure 3.10** shows the effect of Raclopride and SKF38393 compared to normal saline injections on the total responses per minute (the average of responses on both levers per minute) for both rat strains during the devaluation test. A 2x3 multifactorial ANOVA with total responses per minute as dependent variable and strain and medication

status as fixed factors showed significant effects of drug ($F(2,42)=25.65$, $p>0.001$) and strain* drug interaction ($F(2,42)=4.08$, $p=0.024$) with strain effect approaching significance ($F(1,42)=4.04$, $p=0.051$). Independent-samples t-test revealed a significant difference in total response per minute between SHR and WKY rats under normal saline ($t(16)=2.4$, $p=0.029$), and SKF38393 ($t(14)=2.85$, $p=0.013$) but no significant difference between the two strains under Raclopride ($t(4.87)=0.9$, $p=0.41$). Further, independent-samples t-test showed a significant difference in total response rate per minute between rats under medication vs. normal saline injections in SHR rats (normal saline vs. Raclopride: $t(9.82)=6.41$, $p>0.001$, normal saline vs. SKF38393: $t(10.93)=5.07$, $p>0.001$), and WKY rats comparing normal saline vs. SKF38393 ($t(8.41)=3.4$, $p=0.006$) but not normal saline vs. Raclopride ($t(12)=1.52$, $p=0.15$). Overall, these results indicate that drug injections affected total response rate during the devaluation test in the two strains as compared to responses under normal saline injections. However, in this experiment, unlike **experiment #1**, the first two devaluation sessions were carried out under normal saline injections, while drug injections were carried out during the last four devaluation sessions. Therefore, since the decrease in response rate was observed under injections of both drugs (Raclopride [D2-*antagonist*] and SKF38393 [D1-*agonist*]), lower sensitivity to devaluation test, which can ensue with repetitive devaluation sessions, could account for these results. In line with this, locomotor activity test results showed that both drug injections did not affect HACTV at doses of 0.1mg/kg Raclopride or 3.0mg/kg SKF38393 *(see Locomotor activity test results)*.

Goal-directed score

For this analysis, rats were categorized as Goal-directed or Habitual based on the percentage of responses on the valued vs. the devalued lever during devaluation test under normal saline injections. To calculate goal-directed score, we used the following formula: [(% of valued responses - % of devalued responses)/(% of valued responses + % of devalued responses)]. Rats with a goal-directed score less than 0.2 were considered habitual, those above or equal to 0.2 were considered goal-directed. This cutoff number is based on the assumption that goal-directed rats should have at least 60% responses on the valued lever, compared to 40% on the devalued lever. This analysis included rats that underwent devaluation under normal saline injections on one devaluation session and at least one drug (SKF38393 or Raclopride) on another devaluation session to characterize the effect of drugs on goal-directed score in reference to the control state. When we looked at individual goal-directed scores, we found that, under normal saline injections, SHR rats were distributed into two groups (Goal-directed and Habitual). All rats were included in this analysis. On the other hand, most WKY rats started as Goal-directed. **Figure 3.11** shows average goal-directed score in SHR and WKY rats under normal saline, SKF38393 and Raclopride injections. Independent-samples t-test was conducted using goal-directed score under different drug conditions as test variables and strain as grouping variable. This analysis showed no significant difference between the two strains under any type of injection (Raclopride: $t(12)=1.8$, $p=0.1$; normal saline: $t(13)=0.77$, $p=0.46$); SKF38393: $t(11)=0.72$, $p=0.49$).

3.2.4.3. Food consumption

To determine whether drug or rat strain influenced food consumption during the devaluation procedure, we examined the amount of food rats consumed during the first 40 minutes of the devaluation test prior to injections as well as in the 20 minutes after injections. All rats reached satiety with a comparable amount of food required to reach satiety for both strains (**Table 3.7**). Normal saline and drug injections did not alter food consumption in SHR nor in WKY rats in the remaining 20 minutes. A mixed-model ANOVA, using consumption at 40 minutes and in the last 20 minutes as within subject factor and type of injection and strain as between subject factors revealed no effect of strain ($F(1,138)=2.26$, $p=0.14$) or strain * drug interaction ($F(2,138)=3.34$, $p=0.86$) and significant effects of consumption during the first 40 minutes vs. the last 20 minutes ($F(1,138)=601.84$, $p>0.001$) and drug ($F(2,138)=3.41$, $p=0.036$). However, since the majority of food consumption occurred during the first 40 minutes prior to injections (92%), only 8% of food consumption (average of 1.1g) occurred under the effect of drugs, which implicates that even though there was a significant effect of drugs on food consumption, it was minimal, with a low effect size ($\eta=0.047$).

3.2.4.4. Locomotor activity test

We examined locomotor activity to determine whether strain and drug injections influenced this behavior, and to choose drug doses that do not affect rats' motor activity. Rats were placed in the arena for a 30-minute habituation

session. Immediately after habituation, rats were injected with normal saline and returned to the arena for 30 minutes, followed by a 30-minute session with Raclopride 0.05mg/kg, Raclopride 0.1mg/kg, SKF38393 1.0mg/kg or SKF38393 3.0mg/kg injections. Horizontal activity was averaged across 5-minute blocks for each session (blocks 1-6).

Differences in HACTV between SHR and WKY

We found SHR rats traveled a greater distance as measured by HACTV (average horizontal activity) during habituation and under saline and drug injections. For SKF38393, a mixed-model ANOVA on HACTV revealed a significant effect of Strain ($F(1,10)=11.33$, $p=0.007$) and phase *(habituation; saline injections; SKF38393 1.0mg//kg injections; SKF38393 3.0mg.kg injections)* ($F(3,30)=10.18$, $p<0.001$). However, there was no phase * strain interaction ($F(3,30)=1.68$, $p=0.19$). Under Raclopride injections, a mixed-model ANOVA on HACTV revealed a significant effect of strain ($F(1,10)=5.79$, $p=0.037$), phase *(habituation; saline injections; Raclopride 0.05mg/kg injections; Raclopride 0. 1mg/kg injections)* ($F(3,30)=10.84$, $p<0.001$) and phase * strain interaction ($F(3,30)=3.3$, $p=0.034$).

Independent-samples t-tests showed that HACTV was significantly greater in SHR rats as compared to WKY rats during habituation ($t(10)=2.28$, $p=0.046$); under saline ($t(10)=2.94$, $p=0.015$), SKF38393 3.0mg/kg ($t(10)=3.89$, $p=0.003$) and Raclopride 0.1mg/kg ($t(10)=2.3$, $p<0.045$) injections. However, HACTV was

comparable in both strains under SKF38393 1.0mg/kg (t(10)=1.68, p=0.12) and Raclopride 0.05mg/kg (t(10)=0.46, p=0.66) injections.

The effect of drug injections on HACTV in WKY rats

In WKY rats, 1.0mg/kg and 3.0mg/kg doses of SKF38393 did not affect HACTV as compared to saline injection (**Figure 3.12 (A)**, paired-samples t-test: t(5)=2.08, p=0.93, t(5)=0.11, p=0.92, respectively). Similarly, under Raclopride injections, doses of 0.05mg/kg and 0.1 did not affect HACTV as compared to saline injections (**Figure 3.12 (B)**, paired-samples t-test: t(5)=0.54, p=0.61, t(5)=0.83, p=0.45, respectively).

The effect of drug injections on HACTV in SHR rats

In SHR rats, 1.0mg/kg and 3.0mg/kg doses of SKF38393 did not affect HACTV as compared to saline injection (**Figure 3.13 (A)**, paired-samples t-test: t(5)=0.094, p=0.93, t(5)=1.08, p=0.329, respectively). Under Raclopride injections, a dose of 0.1mg/kg did not significantly affect HACTV compared to saline injections (**Figure 3.13 (B)**, paired-samples t-test: t(5)=2.08, p=0.093). However, a dose of 0.05mg/kg significantly decreased HACTV in SHR rats as compared to saline injections (**Figure 3.13 (B)**, paired-samples t-test: t(5)=2.66, p=0.045).

3.3. CONCLUSIONS

In this study, in line with our previous findings, we showed that SHR rats exhibited impaired goal-directed behavior when tested using an outcome devaluation paradigm. In experiment 1, we found that stimulation of D2R using a D2R agonist (Quinpirole) or inhibition of D1R using D1R antagonist (SCH23390) restored goal-directed behavior in SHR rats. On the other hand, these two drugs impaired goal-directed behavior in WKY rats that previously showed intact goal-directed behavior following saline injection. In experiment 2, we showed that stimulation of D1R using a D1R agonist (SKF38393) or inhibition of D2R using a D2R antagonist (Raclopride) did not improve the already disrupted goal-directed behavior in SHR rats. In WKY rats, these two drugs impaired goal-directed behavior.

In both experiments, control WKY rats and SHR rats successfully acquired an instrumental response, with SHR rats showing a significantly greater response rate during training. Using an open field test to evaluate rat locomotor activity, SHR rats showed enhanced locomotor activity as compared to WKY rats during habituation and under the following injections: normal saline, Quinpirole at a dose of 0.001mg/kg, SCH23390 at a dose of 0.0025mg/kg, Raclopride at a dose of 0.1mg/kg and SKF38393 at a dose of 3.0mg/kg.

In this study, we show for the first time that (1) action control is modulated by a balanced activation of D1R and D2R, and (2) dominant habitual response in SHR rats might be due to an over-activation of D1R and/or under-activation of

D2R. Our results illustrate that modulating dopamine receptor activity has a clear effect on mediating action control behavior in SHR and WKY rats.

3.4. LIMITATIONS AND FUTURE DIRECTIONS

Our experiments examined the effects of acute (one dose) administration of D1R and D2R agonists and antagonists. Further studies ought to explore the effects of chronic administration of dopamine medications on action control.

In this study, we tested the effects of the following drugs on goal-directed behavior: D1R antagonist: SCH23390, D2R agonist: Quinpirole, D1R agonist: SKF38393 and D2R antagonist: Raclopride. Although these drugs are well known as specific dopamine receptor agonists/antagonists, additional research is needed to study how other dopaminergic medications modulate action control in SHR rats.

One limitation of our study is running multiple outcome devaluation tests for each rat. To use within subject comparisons, each rat was tested under injections of normal saline and at least one drug. Each drug condition was tested twice; using chocolate and grain devaluation, to control for outcome preference. Therefore, each rat was tested at least four times on outcome devaluation tests. Consecutive extinction sessions might affect sensitivity to lever presses. To minimize this effect, rats received reminder instrumental training after two sessions of outcome devaluation.

In this chapter, we showed that SHR rats have a deficit in goal-directed behavior, replicating our findings in **chapter 2**. In response to dopaminergic

drugs, SHR rats displayed goal-directed behavior when they received either D1R antagonist or D2R agonist. Conversely, D1R agonist and D2R antagonist did not restore goal-directed behavior in SHR rats. In WKY rats, all dopaminergic drugs impaired goal-directed response. These findings support our theory that goal-directed behavior requires a balanced activation of D1R and D2R. Further, the SHR pattern of response to dopaminergic agonists/antagonists corroborates the notion that they have high baseline D1R activation and low baseline D2R activation.

In the first two experimental chapters, we examined the behavioral correlates of action control in SHR rats. In **chapter 3**, we investigated the neural activity during instrumental performance, and the effects of MPH, in the brain regions that are involved in goal-directed and habitual behaviors. These experiments will further our understanding of the neural mechanisms underlying altered action control in SHR.

CHAPTER 4

Characterizing Dorsal Striatal Activity during Instrumental Training in a Rat Model of ADHD

4.1. INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is a psychiatric illness characterized by (1) inattention, (2) hyperactivity, and (3) impulsivity. A potential behavioral mechanism that underlies all three symptoms might be impairment in patients' ability to determine the consequences of their actions. Specifically, patients with ADHD may have a fundamental deficit in goal-directed action control, which can lead to failure to adapt their behavior to changes in action-outcome associations. Goal-directed behavior (forming action-outcome associations) and habitual behavior (acquiring stimulus-response associations) are the two main systems that underlie instrumental responding, where subject's behavior depends on consequences. The goal-directed system is rooted in the medial prefrontal and the prelimbic cortices (mPFC and PL) and its projections to the dorsomedial striatum (DMS), whereas the habit system is based in the infralimbic cortex (IL) and its projections to the dorsolateral striatum (DLS) (Shiflett & Balleine, 2011b; Shiflett et al., 2010) (see **Figure 4.1**). Lesion studies or inactivation of IL and/or DLS brought normal habitual actions under the control of the goal-directed system (Coutureau & Killcross, 2003; Yin et al., 2004; Yin et al., 2005). Conversely, lesions to or inactivation of mPFC, PL and/or DMS disrupt

goal-directed behavior and promote habitual responses (Corbit & Balleine, 2003; Ostlund & Balleine, 2005; Yin et al., 2005).

We hypothesized that SHR rats will show greater neural activation in the habitual DLS and/or reduced activity in the goal-directed DMS as compared to control rats. Further, we predicted that this pattern of dorsal striatal activity will flip with methylphenidate (MPH) injections in SHR as compared to WKY rats. Using immunohistochemistry, in combination with instrumental training, we measured neural activity in the striatum by comparing the expression of c-fos, an immediate early gene product, along with specific striatal neuron markers, in the dorsolateral versus the dorsomedial striatum in SHR and control rats. Neural markers allow us to investigate the co-expression of c-fos in striatal medium spiny projection neurons, which comprise 95% of striatal neurons. Further, we examined how MPH, versus normal saline, injections modulate c-fos expression in striatal neurons. The neural activation illustrated by c-fos allows for examining simultaneous activity of neuronal populations in response to a wide variety of experimental procedures (Dragunow, 1996; Morgan & Curran, 1991; Sheng & Greenberg, 1990). Peak expression of c-fos is generally found 30 minutes following the triggering stimulus, and is diminished by 120 minutes (Cullinan et al., 1995). Greater c-fos staining within a brain region indicates that an increase in neuronal activity in that region occurred. In control rats, we expected to see an increase in c-fos expression in the DMS compared to the DLS during instrumental conditioning, as an indication of goal-directed behavior. On the other hand, in SHR rats, we expected to see less expression in the DMS versus the

DLS, indicating dominant habitual behavior. Under MPH, we expected to see the opposite pattern of activity in correlation with our previous behavioral experiment results, which indicated that MPH impaired goal-directed behavior in WKY rats and restored this behavior in SHR rats (Natsheh & Shiflett, 2015). We predicted that c-fos activation in SHR rats would be dominant in the DMS; whereas in WKY rats, neural activity would be higher in the DLS.

In rats, many factors can influence the expression of immediate early genes, including c-fos, such as undergoing stressful events, brief handling, receiving food pellets and food restriction (Carr, 2007; Cullinan et al., 1995; Pan et al., 2006). Further, studies have shown that MPH alone exerts its stimulant effects by regulating c-fos expression in adult and developing rat striatum. For example, a single dose of MPH increases c-fos activation, while a chronic administration results in attenuation of c-fos expression in the striatum (Chase, Brown, Carrey, & Wilkinson, 2003; Chase, Carrey, Brown, & Wilkinson, 2005a, 2005b). To control for these effects, we pre-exposed the rats to the food pellets and to the behavioral chambers before running the experiment. Further, we also included two types of control rats: (1) yoked controls; a group of rats that were coupled with experimental rats during instrumental conditioning and received food pellets independent of their actions, and (2) quiet controls; a group of rats that remained in the chambers without access to the lever or to outcomes (see the Behavioral Procedures section). A timeline for behavioral and immunostaining procedures is described in **Table 4.1**.

4.2. MATERIAL AND METHODS

4.2.1. Subjects and apparatus

Subjects: Sixteen male adult (P49-P80) rats were used in this study; 7 of which were spontaneously hypertensive rats (SHR) (ADHD model) from Charles River Laboratories (Wilmington, MA), and 9 were Wistar-Kyoto rats (WKY), the normotensive control strain, from ENVIGO (UK). Rats weighed approximately 110-140g at the time of testing. Rats were housed in pairs in 47.6×20.3×26 cm (w×h×d) polycarbonate containers with Alpha Chip bedding material (Northeastern Products Corp., Warrensburg, NY) and had free access to water. One week after arrival, all rats were placed on a restricted food diet of approximately 15g of standard rat pellets per day (Purina, St. Louis, MO). Rats were fed after their daily behavioral training session. Food restriction continued for the duration of the experiment. All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee.

Operant chambers: Behavioral training and testing took place in 12 identical rat operant conditioning chambers (Med Associates, St. Albans, VT). Each operant conditioning chamber measured 30.5×24.1×21 cm (w×h×d) and was constructed of stainless-steel and clear plastic walls and a stainless-steel grid floor. A food cup with infrared detectors was centered on one wall of the operant conditioning chamber. Retractable levers were situated to the left and right of the food cup. Responses on these levers delivered one food pellet from a pellet dispenser mounted outside the operant conditioning chamber. Two types of pellets were used in the experimental procedures: 45-mg grain-based pellets

and chocolate-flavored purified pellets (Bio-serv, Frenchtown, NJ). Each operant conditioning chamber was housed in a sound attenuating shell and equipped with a ventilation fan that was activated during behavioral procedures. Control over the operant conditioning chambers was enabled by a personal computer operating through an interface. Operant conditioning chamber operation and data collection were carried out with Med Associates proprietary software (Med-PC).

4.2.2. Behavioral Procedures

General procedures and Habituation: Behavioral procedures commenced after one week of food restriction. Rats were provided with two 15-minute sessions to habituate to the testing chamber, after which they began behavioral training.

Rat groups: (1) **Experimental rats (N=12, Table 4.2)**: We trained SHR and WKY rats on an instrumental conditioning paradigm in which animals made responses in the operant chamber to earn food pellets according to a variable ratio of 1, 5, 15 and 20. (2) **Yoked controls (N=2, Table 4.2)**: Each rat in this group was paired with an experimental rat. Every time an experimental rat received a food pellet; its yoked control also received a pellet. Yoked rats had access to the lever but responses on the lever had no consequences. (3) **Quiet controls (N=2, Table 4.2)**: These rats underwent habituation where they were provided with 15-minute sessions with both levers retracted and no opportunity to earn food pellets. This group served as a baseline for c-fos expression under normal saline and MPH injections. All rats underwent daily training sessions for 8

days according to their group assignment. Prior to the last training session (on day 8), rats received MPH or normal saline injections. **Table 4.2** shows rat groups and injection assignment.

Instrumental training: Experimental rats underwent one training session per day. Rats were trained on the left lever that was associated with delivery of chocolate pellets. For each training session, the left lever was inserted into the chamber and responses the rats made on that lever delivered a single food pellet. The session terminated when rats earned 20 pellets or 25 minutes elapsed. Rats were trained daily for 8 days; on days 1–3, each response on the lever resulted in pellet delivery (continuous reinforcement). On days 4–5, pellets were delivered according to a variable-ratio (VR) 5 schedule, which requires, on average, 5 responses to earn a pellet reward. On days 6–7, pellets were delivered according to a VR-15 schedule. On day 8, pellets were delivered according to a VR-20 schedule. A new variable ratio represents a novel learning element.

4.2.3. Immunohistochemistry Procedures

Perfusion: Within one hour of completing the final training session, rats were anesthetized and transcardially perfused with 1x phosphate-buffered saline (PBS) solution, followed by 4% Paraformaldehyde diluted in PBS (PFA). Brains were extracted and post-fixed in 4% PFA overnight at 4°C. Brains were cryoprotected in a 30% sucrose solution and then saved at -20° C for future analysis. Sectioning: Coronal sections were collected at 40 µm intervals from the

whole striatum at the anterior, middle and posterior levels (+2.16 to -0.72 mm relative to Bregma). Sections were distributed in three 12-well cell-culture plates (VWR) for each rat. Four sections at each level were chosen for staining. Staining: On day-1 staining, sections were washed in 1xPBS twice for 5 minutes each, permeabilized with 0.8% Triton for 10 minutes, incubated in blocking solution for 1 hour at room temperature and then labeled overnight with the following primary antibodies: rabbit polyclonal antibodies against c-fos (1:1000, Santa Cruz) and mouse monoclonal antibodies against DARPP-32 (1:250, BD Biosciences). On the following day, sections were washed in 1xPBS twice for 10 minutes each, and were incubated in the following secondary antibodies for 2 hours, covered from light, at room temperature: donkey anti-rabbit Alexa Fluor® 488 conjugate (1:800, Invitrogen Life Technologies) and donkey anti-mouse Alexa Fluor® 594 conjugate (1:800 or 1:500, Invitrogen Life Technologie). Sections were washed in 1xPBS three times for 10 minutes each and coverslipped using DAPI mounting solution. Finally, sections were stored at 4°C covered from light. Imaging and cell counting: We used Olympus FluoView™ Confocal Microscope for imaging. For each rat brain, 5 image stacks/Z series (10 µm each) were acquired across the rostrocaudal extent of the striatum. Six sections were imaged at 10x magnification for each rat brain. All images were acquired from the left hemisphere. We used ImageJ for data quantification. For each subject, counts were made manually for both the DMS and the DLS. Because the DLS extends more caudally than the DMS, we collected and imaged anterior and middle DMS sections and anterior, middle and posterior

DMS sections. Regions of interest were delineated using reference structures (e.g., decussation of the anterior commissure and lateral ventricles). Imaging and counting areas are shown in **Figure 4.2**. For each image, Z stacks of all channels were projected into a single plane according to average intensity. Grids of 10,000 μm^2 squares were used to count areas of 500*500 μm in DARPP-32, c-fos and DARPP-32-c-fos merged channels **(Figure 4.3)**. DARPP-32 labeled cells and c-fos and DARPP-32 double-labeled cells were counted within the DMS and the DLS.

4.2.4. Statistical Analysis

For instrumental conditioning tests, the rate of responses was calculated as the number of lever presses per minute during each session. One WKY rat did not press the lever and was not included in analyses. Immunostaining data were represented as an average of c-fos activity dividing the number of cells that were double-labeled for c-fos and DARPP-32 by the total number of DARPP-32 expressing cells. Middle sections for one experimental SHR rat on MPH were lost during staining. Data analysis was conducted using SPSS for Mac Version 20.0. The normality of data distribution was checked using Kolmogorov–Smirnov tests. All data were normally distributed ($p > 0.1$). To analyze instrumental performance and immunostaining data, we used mixed-model ANOVA and planned comparisons using two-tailed t-tests. Because of the limited number of subjects, we reported the post-hoc power (β) as well as the effect size (η^2) for all tests. The level of significance was set at $\alpha = 0.05$.

4.3. RESULTS

4.3.1. Instrumental training data

All rats acquired an instrumental response; however, SHR rats exhibited greater response rates across training sessions compared to WKY rats. **Figure 4.3** represents the lever-pressing rate in SHR and WKY rats. A mixed-model ANOVA confirmed a significant effect of training block ($F(3,27)=95.81$, $p<0.001$, $\beta=1.0$, $\eta^2=0.92$) and strain ($F(1,9)=369.2$, $p<0.001$, $\beta=0.92$, $\eta^2=0.61$) with no significant block * strain interaction ($F(3,27)=2.84$, $p=0.057$, $\beta=0.62$, $\eta^2=0.24$). SHR responses were significantly higher than WKY responses over blocks two and three ($t(9)=5.42$, $p<0.001$, $t(9)=3.01$, $p=0.015$ respectively, independent-sample t-test).

To investigate whether MPH injections had any influence on the rate of lever presses, we examined each strain's instrumental activity with the response rate as a dependent variable. One-way ANOVA revealed no effect of medication status in both WKY ($F(1,4)=0.006$, $p=0.95$) and SHR ($F(1,5)=1.84$, $p=0.25$) rats.

4.3.2. Regional c-fos activity in experimental rats

Figure 4.4 shows the patterns of the average c-fos activity across anterior, middle and posterior DMS and DLS in SHR and WKY experimental rats. Under normal saline injections, WKY rats showed higher c-fos activity in medium spiny neurons in the DMS compared to the DLS. However, SHR rats showed a slightly higher c-fos activity in the DLS compared to the DMS. The opposite pattern of c-fos activity was observed under MPH injections; WKY rats showed a higher activity in the DLS, and SHR rats showed a higher activity in the DMS

(Figure 4.4). A mixed-model ANOVA, using regional c-fos activity in DMS versus DLS (regional activity) as a within subject factor and strain and medication status as between subject factors, confirmed a significant regional activity * medication ($F(1, 7)=19.00$, $p=0.003$, $\beta=0.69$, $\eta^2=0.73$) and regional activity * medication * strain ($F(1, 7)=64.34$, $p<0.001$, $\beta=1.0$, $\eta^2=0.90$) interactions. Paired sample t-tests confirmed a significantly higher c-fos activity in DMS versus DLS in WKY rats under saline injections ($t(2)=10.93$, $p=0.008$) but no significant difference in activity under MPH injections ($t(2)=3.53$, $p=0.18$). In SHR rats, there was no significant difference between c-fos activity in DMS versus DLS under saline ($t(2)=1.24$, $p=0.34$) or MPH ($t(2)=0.92$, $p=0.41$) injections. The same pattern of c-fos activity was observed across the anterior, middle and posterior levels separately (**Figures 4.5** and **4.6**).

4.3.3. Regional c-fos activity in experimental versus yoked controls

To set a baseline for c-fos signal in response to instrumental training versus food reward, we included one WKY rat and one SHR rat as yoked controls. Both rats received saline injections prior to the last training session, on day 8, where they received food pellets independent of their lever responses. Both WKY and SHR yoked controls showed a slightly higher activity in the DMS compared to the DLS. Further, WKY and SHR rats showed lower c-fos activity when compared to experimental WKY and SHR rats on normal saline injections, respectively (**Figures 4.7**).

Overall, c-fos activity in the DMS did not differ from that in the DLS in yoked control rats. Further, c-fos activity in yoked control rats was lower than in experimental rats.

4.3.4. Regional c-fos activity in experimental versus quiet controls

To set a baseline for the c-fos signal in response to MPH injections, we included two WKY quiet controls. During behavioral training, both rats underwent habituation sessions with no choice of lever pressing or food reward. Prior to the last session, on day 8, one rat received normal saline injection, and the other rat received MPH injection. **Figure 4.8** shows that c-fos activity was similar in the DMS versus the DLS for the WKY quiet control that received normal saline injections, and slightly higher for the WKY quiet control that received MPH injection. Activity in both rats was low compared to experimental WKY rats on normal saline and MPH injections.

Overall, quiet control rats did not show a difference in activity between DMS versus DLS. Further, they showed lower dorsal striatal activity compared to experimental WKY rats.

4.3.5. Regional c-fos activity in experimental rats in correlation with lever pressing

To eliminate any motor confound in the observed patterns of c-fos activity, we correlated c-fos expression to lever pressing during the last instrumental training session immediately prior to perfusion. Rats were injected with a dose of

MPH or normal saline 10 minutes before this session. **Figure 4.9** shows no correlation between c-fos activity in the DMS or in the DLS with lever response rate measured as number of lever presses per minute. Using linear regression, we found no significant correlation between lever press and c-fos activity in the DMS $F(1,10)=0.37$, $p=0.56$, $r^2=0.039$) or the DLS $F(1,10)<0.001$, $p=0.997$, $r^2<0.001$).

4.4. CONCLUSIONS

In this study, we found that SHR and WKY rats displayed different patterns of c-fos expression following instrumental conditioning. This fits with our previous findings that WKY rats exhibited normal goal-directed behavior whereas SHR rats exhibited impaired goal-directed behavior when tested using an outcome devaluation paradigm. Additionally, MPH restored the behavioral deficit in SHR rats and impaired goal-directed behavior in WKY rats. In line with these findings, WKY rats showed a dominant c-fos activity in the goal-directed region (DMS) while SHR rats showed a dominant c-fos activity in the habit-learning region (DLS) across the rostrocaudal extent of the striatum. Further, corresponding to our behavioral results, these patterns of activation flipped when rats received MPH; SHR rats showed higher activity in the DMS (compared to the DLS), whereas WKY rats showed higher activity in the DLS (compared to the DMS). These patterns of c-fos activity support our hypothesis that action control is impaired in SHR rats at both the behavioral and neural levels.

4.5. LIMITATIONS AND FUTURE DIRECTIONS

We tested a limited number of rats in this experiment. Most of our results did not reach statistical significance. Thus, the number of subjects in each group would need to be increased to further support our findings.

In this study, we characterized the neural activity in the dorsomedial and dorsolateral striatum. Although this brain region is evidently implicated in action control, future studies ought to explore the involvement of other frontal and limbic brain regions in action control, such as the prelimbic prefrontal cortex, the infralimbic cortex, and the nucleus accumbens.

Finally, as described earlier, c-fos can be influenced by many factors. Here, we included two types of controls. However, there are other factors that we could not control for, such as motor activity in SHR rats as compared to WKY rats as well acute versus chronic effects of MPH on c-fos expression. Further studies are required to examine regional c-fos expression under careful control for these factors.

In this chapter, using the expression of the immediate early gene product c-fos, we show that SHR and WKY rats have different patterns of neural activity in the dorsolateral versus the dorsomedial striatum during instrumental training. These results correspond to our findings in the behavioral experiments in chapters 2 and 3. To our knowledge, this is the first study that examines neural correlates of action control in a rat model of ADHD.

In the next chapter, we used a human analogue of the outcome devaluation paradigm to test the prediction that goal-directed behavior is impaired in patients with ADHD as compared to healthy controls. If successful, this study would be clinically significant because it would introduce an action-control deficit in ADHD as a potential part of the disorder.

CHAPTER 5

Characterizing Action Control in Patients with Attention-Deficit/Hyperactivity Disorder

5.1. INTRODUCTION

Attention-deficit/hyperactivity disorder is a behaviorally defined disorder that is typically diagnosed in children. It is characterized by maladaptive, high levels of inattention, hyperactivity and impulsivity. Evidence suggests that patients with ADHD show altered sensitivity to positive reinforcement (Tripp and Wickens 2008, Volkow et al. 2011). A deficit in learning from rewarding stimuli might impair patients' ability to flexibly adapt their behavior to modulation in action consequences. Instead, they rely on reflexive behaviors independent of outcome. The ability to modify one's behavior in response to changing outcome is referred to as goal-directed action control; whereas, reliance on actions regardless of their consequences refers to as habitual response.

Behavioral and imaging studies have shown that healthy children and adults display intact goal-directed behavior (de Wit et al., 2007; de Wit et al., 2012; Gillan et al., 2011). Other studies have reported impaired goal-directed action control in patients with various neuropsychiatric disorders such as obsessive-compulsive disorder (Gillan, Morein-Zamir, Kaser, et al., 2014; Gillan, Morein-Zamir, Urcelay, et al., 2014; Gillan et al., 2011; Gillan & Robbins, 2014) and Parkinson's disease (de Wit et al., 2011; Frank et al., 2004; Redgrave et al.,

2010). However, no studies have examined the behavioral patterns of action control in patients with ADHD. Here, we hypothesize that patients with ADHD have impaired goal-directed behavior. This might represent a fundamental characteristic of ADHD that, if explored, might advance our understanding of the behavioral and neural underpinnings of this disorder.

In a previous study, we described a deficit in goal-directed behavior in a rat model for ADHD using an outcome devaluation paradigm, a well-validated behavioral paradigm that examines the patterns of action control in animals (Dickinson, 1985; Natsheh & Shiflett, 2015). In this study, we tested the pattern of action control in children with ADHD and healthy controls using a computer-based human-analogue of the outcome devaluation paradigm. Our behavioral paradigm consists of three phases; a training phase, a devaluation phase and a choice test. Participants were 6-10 years old and were recruited from neuropediatric clinics (patients) and public schools (health controls) of the same community. We predicted that patients with ADHD would show a deficit in goal-directed behavior and a dominant habitual response during the test phase of the computer-based task.

5.2. MATERIAL AND METHODS

5.2.1. Participants

We recruited off-medication patients with ADHD (N = 19) from various pediatric neurology clinics, mental health care centers and primary healthcare centers throughout the West Bank, Palestine. Two collaborating pediatric

neurologists oversaw the appropriate selection, screening and care of research participants. Healthy control subjects (N = 21) were recruited from the same communities as the ADHD patients to control for socioeconomic and environmental factors. All subjects were caucasians, ranging from 6-10 years of age. Participants were group matched for age (*Mean*±*SD* ADHD: 8.74±1.42, HC: 8.62±1.6), and sex (*Male* ADHD: 79%, HC: 71.4%). All subjects underwent screening evaluations that included a medical history and a physical examination. All patients underwent a structured interview by a pediatric neurologist and were assessed for absence of neurological or psychiatric disorders other than ADHD. Diagnosis with ADHD was confirmed by the Arabic version of the SNAP-IV Rating Scale (Osman, Omer, Mohammed, & Abdalla, 2015). Exclusion criteria were medical illness, including cardiovascular, hepatic, renal, respiratory, endocrine, hematological, neurological or psychiatric disorders; subjects with DSM-IV-TR diagnoses other than ADHD in the patient group; use of psychotropic drugs, except methylphenidate in patients with ADHD; having any visual, physical, major medical, or neurological illness that might affect cognition or subject's ability to properly complete the computer tasks; exclusion for any reason not yet listed herein that is determined by the medical personnel or research staff to be required by good clinical practice. After receiving a complete description of the study, parents provided written informed consent forms. All research was conducted according to the Declaration of Helsinki and approved by Al-Quds University Ethics Committee.

5.2.2. SNAP-IV Rating Scale

The SNAP-IV Rating Scale (Swanson et al., 2012) is a revision of the Swanson, Nolan, and Pelham (SNAP) Questionnaire (Swanson, Sandman, Deutsch, & Baren, 1983). The first 26 items of the SNAP-IV include the 18 ADHD symptoms (nine for inattentive, nine for hyperactive/impulsive) and eight ODD symptoms specified in the DSM-IV. The SNAP-IV is based on a 0 to 3 rating scale: Not at All = 0, Just A Little = 1, Quite A Bit = 2, and Very Much = 3. Subscale scores on the SNAP-IV are calculated by summing the scores on the items in the subset and dividing by the number of items in the subset. In addition to the DSM-IV items for ADHD and ODD, the SNAP-IV contains items from the Conners Index Questionnaire (Conners, Sitarenios, Parker, & Epstein, 1998) and the IOWA Conners Questionnaire (Landau & Milich, 1985; Milich & Fitzgerald, 1985).

Items #41-#80 of the SNAP-IV Rating Scale are from other DSM-IV disorders which may overlap with or masquerade as symptoms of ADHD. In some cases, these may be comorbid disorders, but in other cases the presence of one or more of these disorders may be sufficient to exclude a diagnosis of ADHD. If symptoms of these disorders receive a high (“Quite A Bit” or “Very Much” = “2” or “3”) rating, then an assessment of the implicated non-ADHD disorders may be warranted.

Finally, the SNAP-IV includes the 10 items of the Swanson, Kotkin, Agler, MyInn, and Pelham (SKAMP) Rating Scale. These items are classroom

manifestations of inattention, hyperactivity, and impulsivity. The SKAMP may be used to estimate severity of impairment in the classroom.

5.2.3. Computer-based cognitive task

As a measure of goal-directed behavior using instrumental learning associations, we developed a computer-based cognitive analogue of the behavioral paradigm we used in outcome devaluation test in rats (Natsheh & Shiflett, 2015). The outcome devaluation paradigm is a well-validated paradigm in the animal literature. It usually consists of three phases: (1) a free-operant training phase in which rats separately acquire two distinct action-outcome contingencies, (2) a devaluation phase in which one of the food outcomes is devalued through specific satiety and (3) a choice test conducted in extinction. In our human version of this paradigm, we included (1) a training phase in which participants acquire action-outcome associations, (2) a devaluation phase, and (3) a choice test. To avoid food preference as a confounding variable, we did not use specific satiety for devaluation. Instead, we changed the value of the outcome that subjects received during the training phase: rather than receiving a picture of a food box, subjects received a picture of an empty box in the devaluation phase. The task runs on an Amazon Kindle tablet and requires minimal instructions. It has four phases; acquisition-1, acquisition-2, devaluation and choice test (**Table 5.1**). In the two acquisition phases, subjects learn action-outcome associations for six different stimuli (drawings of bunnies) divided into two groups: three bunnies belong to group A and three bunnies belong to group

B, assigned according to associated outcomes. Group A stimuli are associated with outcome #1; a carrot lunch box, while group B stimuli are associated with outcome #2; a lettuce lunch box. Subjects see one bunny per trial on the screen (stimulus). They learn to touch the face of the bunny (action) to receive its lunch box (outcome) (**Figure 5.1.a**). However, touching anywhere on the screen, other than the bunny's face, represents an error and is associated with the appearance of outcome #3; an empty lunch box. In ***acquisition-1,*** subjects learn stimulus-outcome associations (**Figure 5.2.a**). In this phase, trials are blocked according to stimuli where subjects move to the next stimulus after they make four consecutive correct responses on the previous stimulus. Once they learn these associations for the six stimuli, they move to the next phase; ***acquisition-2***. In this phase, 24 randomized trials are presented where each stimulus appears four times. In the ***devaluation phase***, touching the face of the three bunnies that are associated with outcome #2 (a lettuce lunch box), leads to receiving outcome #3 instead (an empty box) (**Figure 5.2.b**). As in Acquisition phases, touching anywhere else, other than the bunny's face, leads to receiving an empty box and counts as an Error. After 36 trials, subjects move on to the ***choice test***. In this phase, subjects are asked to collect as much food as they can for the bunnies (**Figure 5.2.c**). Two bunnies appear on the screen at the same time; one is associated with outcome #1, and one is associated with outcome #3 (that is used to be associated with outcome #2 in acquisition phases) (**Figure 5.1.b**). To collect more food, subjects should choose the bunny that is associated with outcome #1 (carrot lunch box) rather than the bunny that is associated with

outcome #3 (empty lunch box). Bunnies that are associated with outcome #1 are referred to as “valued” stimuli, whereas bunnies that are associated with outcome #3 are referred to as ”devalued” stimuli. For each trial subjects choose a valued stimulus, their score increases by 1 point. This phase consists of 60 trials. The total points subjects collect represent their goal-directed score out of 60. As in previous phases, touching anywhere on the screen, other than the bunny’s face represents an error. Subjects received an outcome as a consequence of their actions in this phase. A comparison between the instrumental components of the animal vs. human outcome devaluation paradigms is represented in **Table 5.2.**

5.2.4. Statistics and data analysis

The normality of data distribution was checked using the Kolmogorov-Smirnov tests. All data were normally distributed ($p>0.1$). We used mixed-model ANOVA with learning phase in the cognitive task as the within-subject variable, and diagnostic status as between-subject variables, followed by planned comparisons using two-tailed *t*-tests. The level of significance was set at $\alpha = 0.05$. We used SPSS 20.0 for Mac for statistical analysis.

5.3. RESULTS

5.3.1. SNAP-IV Rating Scale

5.3.1.1. ADHD symptoms

Parent cutoff scores for ADHD-Inattention, ADHD-Hyperactivity/Impulsivity and ADHD-Combined are 1.78, 1.44 and 1.67 respectively. We tested 19

patients with ADHD; among which 16 patients were ADHD-C, and 3 patients were ADHD-In. Mean scores ± SD for patients vs. healthy subjects on ADHD-In, ADHD-H/Im and ADHD-C are shown in **Table 5.3**. Independent-sample-t-test showed that patients' scores are significantly higher than HC scores on ADHD-In, ADHD-H/Im and ADHD-C items as follows: $t(29)=14.91$, $p>0.001$, $t(29)=6.91$, $p>0.001$ and $t(26)=15.16$, $p>0.001$ respectively.

5.3.1.2. Other Comorbidities

The SNAP-IV Rating Scale screens for other DSM-IV disorders which may overlap with or masquerade as symptoms of ADHD. An assessment of the implicated non-ADHD disorders may be warranted if symptoms of these disorders receive a high (≥2) rating. Our results show that patients with ADHD as well as healthy subjects scored low (<2) on all screened disorders, including: conduct disorder, intermittent, explosive disorder, Tourette's disorder, stereotypic movement disorder, obsessive compulsive disorder, generalized anxiety disorder, narcolepsy, histrionic personality disorder, narcissistic, personality disorder, borderline personality disorder, manic episode, major depressive episode, dysthymic disorder, PTSD and adjustment disorder. However, scores of oppositional defiant disorder were significantly higher in ADHD as compared to healthy controls. Mean SNAP-IV scores ± SD for patients and HC subjects are shown in **Table 5.4**.

5.3.1.3. Classroom performance

SNAP-IV includes the 10 items of the SKAMP rating scale, which assess for classroom manifestations of inattention, hyperactivity, and impulsivity. Specifically, it estimates severity of academic (orienting, maintaining, directing) and deportment (attention to others, attention to rules) impairments in the classroom. **Tables 5.5 and 5.6** show mean scores ± SD for patients and HC subjects for academic and deportment items respectively. Our results show that patients with ADHD scored higher than healthy subjects on all items; however, only “Maintaining” score was >2 in patients with ADHD.

5.3.2. Computer-based cognitive task

5.3.2.1. Learning action-outcome associations

During the aqcuisiton-1 phase, subjects acquired action (touching the bunny’s face)-outcome (lunch box) associations for 6 stimuli. They learned that touching the face (not anywhere else) of each bunny resulted in the appearance of a lunch box on the screen. Trials in this phase were blocked according to stimuli. Subjects were presented with one stimulus at a time. After making four successful consecutive responses, they were presented with the next stimulus. **Figure 5.3** shows that patients with ADHD needed a higher number of trials to learn action-outcome associations in this phase compared to HC subjects. An independent-sample-t-test confirmed that this difference was significant: $t(38)=2.72$, $p=0.01$).

After learning action-outcome associations in this phase, subjects should maintain this information across the next three phases of the task; Aqcuisition-2, Devaluation and choice test. Anytime subjects touch the screen anywhere other than a bunny's face, the trial counts as an Error, and an empty box appears on the screen. **Figure 5.4** shows the number of Errors subjects made during each phase of the task. Mixed-model ANOVA with task phase as the within-subject factor, and diagnostic group as the between-subject factor, confirmed a significant effect of (1) task phase ($F(3,114)=7.07$, $p<0.001$), (2) phase * group interaction ($F(3,114)=5.42$, $p=0.002$), and (3) group ($F(1,38)=128.57$, $p<0.001$). Independent-samples t-tests showed that patients with ADHD made significantly greater number of errors compared to HC subjects over aqcuisition-1 ($t(38)=2.98$, $p=0.005$), aqcuisition-2 ($t(38)=2.25$, $p=0.03$), devaluation ($t(38)=2.04$, $p=0.048$), and choice test ($t(38)=3.76$, $p=0.001$).

5.3.2.2. Choice test results

During the choice test, subjects were asked to collect as much food as they could, choosing between two bunnies (valued vs. devalued) at each trial. They were expected to choose the bunnies associated with the valued carrot box more frequently compared to the bunnies associated with the devalued empty box (lettuce box in acquisition phases, and empty box in Devaluation) **(Figure 5.5)**. Including only correct responses, mixed-model ANOVA with valuation (valued vs. devalued outcome) as the within-subject factor and diagnostic group as the between-subject factor, showed a significant effect of (1) valuation

($F(1,38)=131$, $p<0.001$), (2) valuation * group interaction ($F(1,38)=55.34$, $p<0.001$), and (3) group ($F(1,38)=4.14$, $p=0.001$). Paired-sample-t-tests showed that both patients with ADHD and HC subjects responded at a higher level on the valued outcome vs. the devalued outcome ($t(18)=2.35$, $p=0.031$), $t(20)=17.00$, $p<0.001$), respectively); however, independent-sample t-tests showed that healthy subjects made a significantly higher number of responses on the valued outcome compared to patients with ADHD ($t(38)=7.66$, $p<0.001$), while patients with ADHD made a significantly higher number of responses on the devalued outcome compared to healthy subjects ($t(38)=6.02$, $p<0.001$). Overall, these results show that reliability of patients with ADHD on goal-directed behavior was significantly lower than that of healthy subjects.

5.3.2.3. Reaction time

Reaction time (RT) was measured for all responses subjects made throughout the task, except when they made an error; that is, when they touched anywhere on the screen other than the bunny's face. For each trial, RT measures as the time between the appearance and disappearance of the stimulus **(Figure 5.6)**. Disappearance of the stimulus happens when subjects respond by touching the screen. Mixed-model ANOVA with task phase as the within-subject factor and diagnostic group as the between-subject factor, showed a significant effect of (1) phase ($F(3,114)=4.97$, $p=0.003$), (2) phase * group interaction ($F(3,114)=3.64$, $p=0.015$) and (3) group ($F(1,38)=6.22$, $p=0.017$). Independent-samples t-tests

showed that patients with ADHD displayed a lower RT over devaluation phase ($t(38)=2.39$, $p=0.02$) and choice test phase ($t(38)=4.24$, $p<0.001$).

5.4. CONCLUSIONS

In this study, using an outcome devaluation computer-based task, we found the first experimental evidence for a selective deficit in goal-directed behavior in children with ADHD compared to healthy subjects. This deficit reflects reliance on the habitual system that can be triggered by stimuli regardless of their consequences.

Further, we found that patients with ADHD required a higher number of trials to acquire action-outcome associations. Patients with ADHD made more errors during the acquisition, devaluation and choice test phases of the computer-based task. This might reflect patients' inability to sustain their attention on the task or their impulsivity in choosing actions without *a priori* goal-directed consideration.

Finally, patients with ADHD showed faster response times during the last two phases of the task; devaluation and choice test. This finding suggests that patients with ADHD executed their actions more impulsively than controls.

5.5. LIMITATIONS AND FUTURE DIRECTIONS

An important limitation of the current study is the inconsistency in patients' medication status. Although all our patients were unmediated at the time of testing, some of them were medication naïve while others were off medication.

Further, this study did not examine the effects of methylphenidate (MPH) on goal-directed behavior in ADHD. Future research should address this by testing patients with ADHD on and off MPH.

In our computer-based task, subjects received an outcome during the choice test phase while most validated outcome devaluation paradigms present stimuli in the absence of outcome during extinction. However, since our task design has never been tested in patients, there is no way to know if patients' responses during the choice test were dependent on their memory or on their action control. Therefore, we included outcome presentation in the choice test to eliminate the possibility that patients' responses were due to a memory deficit, rather than a dominance of habitual response. Thus, our results represent strong evidence of an action control deficit in ADHD because patients were impaired on goal-directed behavior despite receiving feedback on each trial. A future study should be conducted using the same paradigm without providing subjects with outcome in the choice test to examine if patients will show a habitual response without being provided feedback (which is expected) and whether they are habitual during "extinction". If we get the same results, we would show that our human analogue of outcome devaluation shows comparable results with or without feedback during the choice test.

In this chapter, we show that children with ADHD are less reliant on goal-directed behavior compared to healthy controls. This finding is consistent with our previous results in chapters 2, 3 and 4, which indicate that a rat model of

ADHD displays a behavioral deficit in goal-directed action control. To our knowledge, this is the first study to show a selective deficit in goal-directed behavior in patients with ADHD.

CHAPTER 6: DISCUSSION

6.1. OVERVIEW

Using outcome devaluation and contingency degradation paradigms, we show that SHR rats (ADHD model) exhibited a deficit in goal-directed behavior compared to control WKY rats. Further, we found that methylphenidate administration remediated deficits in goal-directed action control following outcome devaluation in SHR rats. In contrast, while goal-directed behavior is well displayed in WKY rats, methylphenidate administration disrupted this behavior. These results suggest that the behavioral phenotype of SHR rats, as well as methylphenidate treatment, play key roles in determining goal-directed action control. Therefore, goal-directed behavior is not only affected by the disease trait (phenotype) but also by the treatment state (methylphenidate). Further, goal-directed behavior, under methylphenidate effects, in SHR and WKY rats, follows a nonlinear relationship (**Figure 6.1**), where dopamine levels correlate with behavioral performance according to an inverted U-shaped function (Cools et al., 2001; Williams & Goldman-Rakic, 1995; Zahrt et al., 1997). Both WKY rats on normal saline and SHR rats on methylphenidate were sensitive to goal-directed behavior, indicating optimal dopamine levels. Conversely, animals with low dopamine level (SHR on normal saline) as well as animals with high dopamine level (WKY on methylphenidate) showed impairment in goal-directed behavior. However, since methylphenidate is not a specific dopamine agonist, we tested the effects of selective dopamine agonists and

antagonists on action control to further investigate these findings. We found that (1) stimulation of dopamine D2 receptor (D2R) using a D2R agonist (Quinpirole) or (2) inhibition of dopamine D1 receptor (D1R) using a D1R antagonist (SCH23390) restored goal-directed behavior in SHR rats. However, stimulation of D1R using a D1R agonist (SKF38393) or inhibition of D2R using a D2R antagonist (Raclopride) did not improve goal-directed behavior in SHR rats. On the other hand, these four drugs impaired goal-directed behavior in WKY rats that previously showed intact goal-directed behavior following saline injection. Aside from the deficit in goal-directed behavior in SHR or methylphenidate-treated WKY rats, these results show for the first time that (1) action control is modulated by a balanced activation of D1R and D2R, and (2) dominant habitual response in SHR rats might be due to an over-activation of D1R and/or under-activation of D2R. Therefore, modulating dopamine receptor activity has a clear effect on mediating action control behavior in SHR and WKY rats.

Consistent with these findings, at the neural level, we describe different patterns of c-fos (an immediate early gene) expression in SHR and WKY rats following instrumental conditioning in brain regions that are involved in action control. WKY rats showed a dominant activity in the goal-directed region (dorsomedial striatum (DMS)) while SHR rats showed a dominant activity in the habit-learning region (dorsolateral striatum (DLS)) across the rostrocaudal extent of the striatum. Further corroborating our behavioral results, these patterns of activation flipped when rats received methylphenidate injections; SHR rats showed higher activity in the DMS (compared to the DLS), whereas WKY rats

showed higher activity in the DLS (compared to the DMS). These patterns of activity support our hypothesis that action control is impaired in SHR rats at both the behavioral and neural levels.

Using a computer-based analogue of the outcome devaluation paradigm, we also observed the first experimental evidence for a selective deficit in goal-directed behavior in children with ADHD compared to healthy subjects. This deficit leads to dominant reliance on the habitual system that can be triggered by stimuli regardless of consequences.

Taken together, our findings suggest that patients and animal models with ADHD failed to engage the goal-directed (action-outcome) system in controlling their actions. We propose that this impairment, along with the consequent reliance on the habit (stimulus-response) system, might explain motivational deficits as well as the observed inflexible action control in ADHD.

6.2. BEHAVIORAL CORRELATES OF ACTION CONTROL IN ADHD

6.2.1. Different patterns of goal-directed behavior in SHR versus WKY rats and distinct effects of methylphenidate

Using an instrumental conditioning paradigm, we present the first experimental evidence of disrupted goal-directed behavior in a rat model of ADHD. Both control WKY rats and SHR rats were successful at acquiring an instrumental response, with SHR rats showing a significantly greater response rate during training. Further, using an open field test to evaluate rat locomotor activity, SHR rats showed enhanced locomotor activity compared to WKY rats.

These findings of SHR operant and motor hyperactivity replicate previous research evaluating the use of the SHR strain as a model of ADHD (J. Hill, K. Herbst, & F. Sanabria, 2012; Wultz, Sagvolden, Moser, & Moser, 1990).

Our results suggest a fundamental impairment in goal-directed action control in SHR rats that was remediated by methylphenidate. We used outcome devaluation and contingency degradation paradigms to assess whether animals had formed action-outcome (goal-directed) or stimulus-response (habit) associations. While WKY rats showed goal-directed action control in both paradigms, SHR rats demonstrated a marked deficit in sensitivity to changes in outcome value and to changes in the action-outcome contingency. Lack of sensitivity to these manipulations may reflect either (1) an inability to use action-outcome information to guide choice behavior (i.e., performance deficit) or (2) an inability of SHR rats to learn and/or retrieve action-outcome associations (i.e., learning/memory deficit). We found that treating SHR rats with methylphenidate prior to the choice test following outcome devaluation enhanced value-sensitive responding in these animals. SHR rats did encode action-outcome associations during instrumental learning; however, they were only able to use these associations to guide behavior when tested under the effects of methylphenidate. Therefore, the deficits we observed in tests of goal-directed behavior in non-medicated SHR rats likely reflect a deficit in performance and not learning of goal-directed actions. In principle, this bears significant similarity to impulsivity, which is one of the cardinal symptoms of ADHD.

Impulsivity is a multidimensional behavior including the urge to perform actions without forethought and the inability to inhibit others despite negative consequences as well as the preference of small immediate over larger delayed rewards (Bari & Robbins, 2013). Previous studies have proposed that a disruption in the balance between goal-directed and habitual action control might underlie impulsivity by demonstrating overreliance on habits (stimulus-response system) at the expense of goal-directed behavior (Everitt et al., 2008; Hogarth, Chase, & Baess, 2012). The mechanisms underlying impulsivity and habits may well overlap, as both are associated with abnormalities in the corticostriatal pathways (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Dalley, Mar, Economidou, & Robbins, 2008; F. C. Davis et al., 2013; Forbes et al., 2009; Jentsch & Taylor, 1999; McClure, Ericson, Laibson, Loewenstein, & Cohen, 2007; Torregrossa, Quinn, & Taylor, 2008; Volkow & Fowler, 2000). Excessive activity in the dorsolateral striatum, a brain region that is specifically associated with habitual behavior, might induce impulsivity as appearance of stimuli repeatedly triggers actions (Shiflett & Balleine, 2011a). Extensive training and/or a deficit in goal-directed behavior can produce a dominant habitual response. Using the outcome devaluation paradigm, our results show that the dominance of habitual response in SHR was elicited by impairment in goal-directed behavior rather than an exaggerated habitual response, given that methylphenidate administration restored goal-directed behavior in SHR. Traditionally, one can interpret the results of this paradigm as either the expression of habitual behavior, or use the concept of impulsivity. Yet, there is no evidence from our

experimental results to distinguish whether rats' responses during extinction were elicited through habitual or impulsive behavior. Previous studies have shown that methylphenidate fails to reduce (Bizot et al., 2007; Wooters & Bardo, 2011) or further increases (Navarra et al., 2008) impulsivity in adult SHR rats using premature responses as a measure of impulsivity. SHR rats respond with shorter or similar inter-trial intervals when tested on methylphenidate as compared to their response when tested under the control condition. In our studies, however, methylphenidate remediated the deficit in goal-directed behavior during extinction. Therefore, we argue that although impulsivity and habitual responses overlap behaviorally and neurally, impaired action control can better be explained by dominant habitual behavior in SHR versus WKY rats during the outcome devaluation test.

The performance of SHR rats following outcome devaluation is not likely mediated by strain differences in the selective satiety process itself. We did find that SHR rats consumed more pellets compared to WKY animals during the satiety process. Nevertheless, many pellets remained after the 1-hour session, by which time all animals had significantly curtailed food consumption, suggesting that all animals had reached a state of satiety. Likewise, although methylphenidate reduced food consumption, it is unlikely that the anorexic effect of methylphenidate altered the devaluation process itself since the majority of food consumption occurred in the 30 minutes prior to methylphenidate injection. Based on these considerations, we are confident that the selective satiety procedure was equally effective in both SHR and WKY rats, and that the effects

of methylphenidate on choice performance were not a consequence of changing the satiety procedure.

Further, we showed that the impaired action control observed during extinction was not due to a motivational deficit in SHR rats. Satiety might reduce rats' motivation to respond during extinction demonstrating habitual behavior (Dickinson & Balleine, 1994); however, given that methylphenidate-treated SHR rats did respond in a goal-directed manner during extinction, motivational deficits are unlikely to explain the impairment in goal-directed behavior.

To further emphasize our finding of impaired goal-directed behavior in SHR rats, and to exclude any effect of outcome devaluation on this behavior, we used a contingency degradation test after training the rats on a selective degradation of the instrumental contingency. Like the outcome devaluation test, SHR rats were impaired on this test. Their performance was comparable on the lever for which the contingency had been degraded compared to the non-degraded lever. This result further supports the notion of impaired goal-directed action control in SHR rats. Moreover, the absence of food/satiety in this paradigm as a factor that might affect rats' drive toward responding strengthens our argument that the action control deficit in SHR is not triggered by motivational impairments.

Overall, our results suggest that SHR rats' impaired goal-directed behavior is neither due to a lack of knowledge of causal consequences or to a failure of the devaluation process prior to the extinction test, nor it is due to behavioral impulsivity or motivational deficit. This impairment is likely due to a

predominance of habitual action control in SHR rats. In contrast, others have reported impaired habit formation and preserved goal-directed behavior in SHR rats (Gauthier, Tassin, Dwoskin, & Kantak, 2014). Differences in experimental design and interpretation may explain some of these discrepant results. Finally, one limitation of our study was injecting all rats with the same dose of methylphenidate, while many studies have reported significant variations in methylphenidate response curves. A future study with different methylphenidate doses is required to address this issue.

6.2.2. Goal-directed behavior is impaired in patients with ADHD

Using a cognitive analogue of the outcome-devaluation paradigm, we showed that goal-directed behavior is impaired in patients with ADHD. Healthy subjects and patients with ADHD were successful at acquiring action-outcome associations, demonstrating that patients with ADHD had an intact instrumental response. Further, compared to healthy subjects, patients with ADHD showed faster reaction times, implicating motor hyperactivity and/or behavioral impulsivity in response to stimuli. To examine the mechanism underlying instrumental response, we used the outcome-devaluation test to investigate action control in ADHD. Patients failed to employ the goal-directed (action-outcome) system to control their actions. Instead, they demonstrated a predominance of habitual behavior (stimulus-response).

To investigate the mechanism underlying this pattern of response, we provided subjects with an outcome during the choice test after devaluation.

While most validated outcome devaluation paradigms present stimuli in the absence of outcome during extinction; it was necessary to include feedback in our task since (1) our task design has never been tested in patients before, and (2) we did not include a medicated group to compare patients' response during extinction and decide whether they were able to retrieve learned action-outcome associations from previous training. Therefore, we included outcome presentation in the choice test to eliminate the possibility that patients' responses were due to a memory deficit rather than a dominance of habitual response. Thus, our results present strong evidence of action-control deficit in ADHD demonstrated in patients' impairment on goal-directed behavior despite receiving feedback during the choice test. A future study including a group of patients on medication is necessary to (1) test the pattern of action control during extinction (absence of outcome during the choice test) and (2) dissociate the effect of disease versus medication on action control in patients with ADHD.

6.3. NEURAL CORRELATES OF ACTION CONTROL IN ADHD

The involvement of corticostriatal circuits in mediating goal-directed behavior and habitual learning is well established in the literature. Functional neuroimaging studies have shown that the ventromedial prefrontal (de Wit, Ostlund, Balleine, & Dickinson, 2009; Glascher, Hampton, & O'Doherty, 2009) and the orbitofrontal (Valentin et al., 2007) cortices as well as dorsal striatum (de Wit et al., 2012; E. Tricomi et al., 2009) are engaged in the execution of action control in humans. In animals, electrophysiological studies using monkeys

(Matsumoto, Suzuki, & Tanaka, 2003; Matsumoto & Tanaka, 2004) and rats (Mulder, Nordquist, Orgut, & Pennartz, 2003) have found neural activity in the prefrontal cortex (PFC) to be related to engagement of specific action-outcome associations. Likewise, lesions of the medial PFC result in behavior that is insensitive to changes in outcome value with a stimulus-elicited, rather than goal-anticipated, instrumental responding (Hitchcott, Quinn, & Taylor, 2007). Furthermore, using lesion and inactivation studies, Yin et al. have shown that the dorsomedial striatum plays a critical role in the acquisition and performance of goal-directed actions (Yin et al., 2005), and that the dorsolateral striatum mediates habitual instrumental performance (Yin et al., 2004). Lesions of the dorsolateral striatum brought normal habitual actions under the control of the goal-directed system.

Consistent with this, dysfunction in the corticostriatal pathways has been implicated in the cognitive and motor symptoms associated with ADHD (Brennan & Arnsten, 2008; Volkow et al., 2009). Accordingly, our findings of insensitivity to outcome devaluation in patients and animal models of ADHD were consistent with findings implicating a dysfunction in the goal-directed corticostriatal pathway in ADHD. This dysfunction led patients with ADHD as well as SHR rats to rely instead on the habit system to control their responding.

6.3.1. Methylphenidate restores goal-directed behavior in SHR rats

The effects of methylphenidate on goal-directed behavior likely occur through its modulation of catecholamine availability in the prefrontal cortex and

the striatum. The therapeutic dose of methylphenidate works primarily via increasing dopamine signaling through multiple actions, including dopamine transporter blockade and significantly enhancing extracellular dopamine release in the striatum (Volkow et al., 2002; Wilens, 2008). Increasing dopamine signaling in the PFC enhances goal-directed behavior (Hitchcott et al., 2007). Further, striatal dopamine signaling is essential in maintaining and using action-outcome associations (Faure et al., 2005; Faure et al., 2010; Lex & Hauber, 2010). Both diminished and excessive dopamine stimulation might cause corticostriatal dysfunction leading to impaired inhibition of undesirable behavior, and a deficit in action control, as these two behaviors are highly regulated by dopaminergic release in the PFC and the striatum (Arnsten, 2011; Brennan & Arnsten, 2008; Russell, 2003). Thus, in SHR rats, dopaminergic availability in the PFC and the striatum might be critical in restoring goal-directed actions by enhancing mechanisms imperative for carrying out goal-directed behavior (Ostlund & Balleine, 2005; Ostlund, Winterbauer, & Balleine, 2009).

In addition to modulating universal dopamine release in the striatum and the PFC, methylphenidate may restore goal-directed behavior through reinforcement-based mechanisms. In reinforcement learning, dopamine neuronal firing is elicited by unexpected reward. Later in learning, dopamine neuronal firing transfers from the actual reward to the cue that predicts this reward (Schultz, 1997). However, in ADHD, dopamine transfer deficit theory suggests that dopamine neuronal firing fails to transfer from reward to predictive cues. Therefore, if a rewarding outcome is delayed or discontinued, the delayed

dopamine release will result in weak or unsuccessful reinforcement at the cellular level, a deficit that will be displayed as rapid extinction in patients' response to rewarding stimuli (Tripp & Wickens, 2008). In this context, one can argue that administration of methylphenidate restored goal-directed behavior by increasing the magnitude of the anticipatory dopamine neuronal firing to predictive cues (pressing the lever) (Tripp & Wickens, 2012; Tripp & Wickens, 2009). Further studies examining the neural circuits activated in SHR rats during instrumental performance and the site of action of methylphenidate will help to better understand the neural mechanisms underlying altered goal-directed action in ADHD.

6.3.2. D2R agonist and D1R antagonist restore goal-directed behavior in SHR rats

In SHR rats, stimulation of D2R (using Quinpirole) and inhibition of D1R (using SCH23390) restored goal-directed behavior. Conversely, stimulation of D1R (using SKF38393) and inhibition of D2R (using Raclopride) did not improve goal-directed action control in SHR rats. These results are in line with our *first hypothesis* of imbalance in D1R and D2R activation in SHR. We argue that an over-activation of D1R (direct pathway) at the expense of D2R (indirect pathway) might account for SHR tendency towards habitual behavior during outcome devaluation test (**Figure 6.2**). Inhibition of D1R and/or activation of D2R brought SHR rat responses under the control of goal-directed behavior. On the other hand, although WKY rats displayed goal-directed action control under normal

saline, this behavior was impaired under all D1R and D2R agonists and antagonists. These results support our *second* hypothesis that a balanced activation of D1R and D2R is essential to translate the optimal levels of dopamine to display goal-directed behavior. Hence, altering the level of activation in direct and/or indirect pathways impaired action control in WKY rats, while restoring the balance in direct/indirect pathways remediated the deficit in goal-directed behavior in SHR rats.

Consistent with these arguments, studies have suggested that D2R has higher affinity to dopamine as compared to D1R (Marcellino, Kehr, Agnati, & Fuxe, 2012). Methylphenidate is known to increase dopamine availability in the striatum (Volkow et al., 2002; Wilens, 2008). Accordingly, we expect that methylphenidate exerts its effects by increasing D2R activation (inhibiting the indirect pathway), rather than D1R activation (activating the direct pathway), given D2R's higher affinity to dopamine (Marcellino et al., 2012). Therefore, our earlier findings where methylphenidate remediated goal-directed behavior in SHR could be explained by increased activation of D2R in response to methylphenidate. This provides further evidence that hyper-activation/hypo-inhibition of the direct/indirect pathways, respectively, might account for impaired action control in SHR.

Although our findings support the direct/indirect pathway activation theory, they cannot be explained using ADHD dopamine hypofunction theory. Dopamine hypofunction is hypothesized to underlie the behavioral symptoms associated with ADHD (Russell, 2003). However, this theory fails to explain (1) the

remediation of goal-directed behavior in SHR rats after administration of dopamine (D1R) *antagonist* (SCH23390) or (2) the inefficacy of dopamine (D1R) *agonist* (SKF38393) in restoring goal-directed behavior in SHR rats.

In sum, we argue that impaired action control can either result from (1) a disease trait (ADHD, SHR); implicated by over-activation of D1R (over-activation of the direct pathway) and under-activation of D2R (hypo-inhibition of the indirect pathway), or (2) a dopaminergic state implicated by altered activation of the direct and/or indirect pathway as observed in health states (WKY rats).

6.3.3. Neural activity in the dorsomedial striatum is higher than that in the dorsolateral striatum in WKY on saline and SHR on methylphenidate.

We found that neural activity, measured by the immediate early gene product c-fos, was higher in the dorsomedial striatum (goal-directed system) as compared to dorsolateral striatum (habitual system) in WKY rats under normal saline injections and in SHR rats under methylphenidate injections. Conversely, c-fos neural activation was higher in the dorsolateral striatum (habitual system) as compared to dorsomedial striatum (goal-directed system) in SHR rats under normal saline and in WKY rats under methylphenidate injections. These results are consistent with our previous findings as follows: (1) Behaviorally, we showed that goal-directed behavior is impaired in both SHR rats and methylphenidate treated WKY rats. Instead, they relied on habitual behavior to carry out their instrumental response. At the neural level, both rat strains showed dominance in neural activation of the brain region that is implicated in habitual behavior; the

dorsolateral striatum. (2) We also showed that WKY rats as well as methylphenidate treated SHR rats displayed goal-directed action control. Accordingly, at the neural level, both rat strains exhibited dominance in neural activation of the brain region that is implicated in goal-directed behavior, the dorsomedial striatum. Although our sample size was small and our results did not reach statistical significance, we show strong trends that further support our proposed hypothesis of selective impairment in action control in SHR rats not only at the behavioral level, but also at the neural level.

To further understand SHR dorsal striatal activation during instrumental training and in response to methylphenidate, future studies are vital to investigate: (1) the role of interneuron activation/inhibition in modulating striatal medium spiny neurons; medium spiny neurons and (2) specific activation patterns of dopamine D1R (direct) and D2R (indirect) pathways.

Although the dorsal striatum is evidently involved in action control processes, many studies have shown that other brain regions might also be implicated in this behavior. Lesions in the prefrontal cortex significantly decreased sensitivity to outcome devaluation in healthy animals (Hitchcott et al., 2007). Further, altering neural excitation of the thalamostriatal pathway produced a deficit in goal-directed behavior (Aoki, Liu, Zucca, Zucca, & Wickens, 2015; Bradfield, Bertran-Gonzalez, Chieng, & Balleine, 2013; Okada et al., 2014). Another study revealed that altering the connection between the basolateral amygdala and the nucleus accumbens impairs sensitivity to outcome value during instrumental responding (Shiflett & Balleine, 2010). Future studies

are essential to characterize neural activation in these brain regions in SHR rats during instrumental performance. Further, employing functional neuroimaging studies, activation in these brain regions as well as in the dorsal striatum should be investigated in patients with ADHD using outcome devaluation paradigms.

6.4. CONCLUSIONS AND LIMITATIONS

We conducted multidisciplinary experiments using behavioral, neuropharmacological and immunohistochemistry techniques in SHR rats as well as a cognitive experiment in patients with ADHD and healthy controls. Our results showed for the first time that goal-directed behavior is impaired in patients with ADHD and a rat model of ADHD. This deficit can be remediated in SHR rats with methylphenidate treatment and selective dopamine D2R agonists and D1R antagonists. Further, our studies revealed that neural activation in the dorsal striatum corresponds to these findings. These results suggest that the loss of motivation exhibited by ADHD patients may reflect impaired goal-directed action control and that dopaminergic medications may activate this system to re-establish goal-directed behavior.

To overcome the limitations inherent in studying patients with ADHD exclusively, we primarily used SHR rats to conduct our experiments. Although SHR is the most commonly used and most widely accepted rat model of ADHD (Sagvolden, Metzger, et al., 1992; Sagvolden et al., 1993), utilizing an animal model to study neurobehavioral aspects of diseases has many limitations. It is impossible for an animal model to completely portray all characteristics of a

disorder. Further, given that the pathophysiological mechanisms of ADHD are still unclear, it is especially difficult to find an animal model that will fully match its neurobehavioral correlates.

Another limitation of our rat studies was refraining from testing female rats. Previous studies have shown gender differences in operant responding in SHR (Berger & Sagvolden, 1998); however, we did not include a female group in our studies for the following reasons: (1) to control for sex as a covariate in interpreting our results, (2) most, if not all, of previous research on SHR was conducted in male rats, (3) the original breeding process of SHR rats showed that symptoms of ADHD were better displayed in male as compared to female rats and (4) ADHD affects males more frequently than females at a ratio of up to 4:1. Future studies including female SHR groups ought to investigate sex differences in goal-directed action control.

Finally, medication modulation in SHR and WKY rats was examined under acute administration (one dose). Many studies have reported significant variations in the therapeutic effects of acute versus chronic treatment with dopaminergic medications. Future studies with different treatment time points are required to address this issue.

6.5. IMPLICATIONS

By studying goal-directed action control in ADHD, we addressed a critical gap in the literature that has not been investigated before. This is the first study to characterize goal-directed response in ADHD and carefully dissociate the

behavioral patterns of action control from potential side effects exhibited after treatment in this disorder. Unraveling action control mechanisms in ADHD can broaden our understanding of the neural circuits underlying cognitive symptoms of ADHD. Here, we show for the first time that behavioral deficits in ADHD might arise from a specific dysfunction in the corticostriatal pathways implicated as hyper-functioning of the D1R signaling (direct pathway) at the expense of D2R signaling (indirect pathway). This can elucidate new mechanisms and potential treatments for ADHD.

This research can be clinically significant as it (1) characterized a decision-making deficit in ADHD as an integral part of the disorder and (2) revealed a novel use of selective dopaminergic medications to remediate action control deficit in SHR. Particularly, our results show that using dopamine D2R agonist selectively restores goal-directed behavior in SHR. Studying these aspects in humans might advance health care for patients with ADHD and inform the use of new lines of drug treatment and cognitive therapy. Further studies using pharmacological and neural imaging techniques are imperative to delineate behavioral and neural mechanisms as well as novel treatment options for ADHD.

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TABLES

CHAPTER 1

Operant Training	Outcome Devaluation	Extinction	Contingency Degradation	Extinction
A => O	Using specific satiety or pairing with illness; devalue: O =>	↓A => ϕ	Outcome is delivered independent of action: A => (O_1)*	↓A => ϕ

Table 1.1 A schematic paradigm that describes outcome devaluation and contingency degradation paradigms. A = Action O = Outcome
ϕ : No outcome (extinction) ↓ : Response rate decreases
* : Outcome is delivered independent of action (lever press)

CHAPTER 2

	Experiment #1	Experiment #2	Experiment #3
Subjects	SHR & WKY	Long Evans	SHR & WKY
Age	Adult, P75-P105	Adult, P75-P105	Adolescent, P30-P50
Sex	Male	Male	Male
Drug doses	MPH, 2.0mg/kg Saline	———	MPH, 0.5, 1.0, 4.0mg/kg Saline
Timeline*	• Habituation (Day 1-2) • Instrumental Conditioning (Day 3-12) • Outcome Devaluation & Choice Test (Day 13-15) • Contingency Degradation & Choice Test (Day 15-20) • Locomotor Activity Test (Day 21-24)	• Habituation (Day 1-2) • Instrumental Conditioning (Day 3-12) • Outcome Devaluation & Choice Test (Day 13-15)	• Habituation (Day 1-2) • Instrumental Conditioning (Day 3-12) • Outcome Devaluation & Choice Test (Day 13-15) • Locomotor Activity Test (Day 16-20)

Table 2.1 A roadmap for chapter 2 experiments.
* Experiment procedures are described in detail in the Methods and Results section.

Instrumental Conditioning	Outcome Devaluation	Choice Test	Contingency Degradation	Choice Test
$A_1 \Rightarrow O_1$ $A_2 \Rightarrow O_2$	Using specific satiety; devalue: $O_1 \Rightarrow$ OR $O_2 \Rightarrow$	$\uparrow A_1 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_2 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_1 \Rightarrow \phi$ $\uparrow A_2 \Rightarrow \phi$	$A_1 \Rightarrow O_1 + (O_2)^*$ $A_2 \Rightarrow O_2$ OR $A_1 \Rightarrow O_1$ $A_2 \Rightarrow O_2 + (O_1)^*$	$\uparrow A_1 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_2 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_1 \Rightarrow \phi$ $\uparrow A_2 \Rightarrow \phi$

Table 2.2 A schematic paradigm that describes behavioral procedures design.
A = Action O = Outcome ϕ : No outcome (extinction) $\uparrow$: Response rate
* : With no lever press, outcome was delivered non-contingently once/sec with 5% probability

Instrumental Conditioning	Outcome Devaluation	Choice Test		Contingency Degradation	Choice Test	
	Devalue:	**Lever A_1**	**Lever A_2**	Degrade:	**Lever A_1**	**Lever A_2**
$A_1 \Rightarrow O_1$ $A_2 \Rightarrow O_2$	$O_1 \Rightarrow$ OR $O_2 \Rightarrow$	Devalued Valued	Valued Devalued	A_1-$O_1 \Rightarrow$ OR A_2-$O_2 \Rightarrow$	Degraded Non-degraded	Non-degraded Degraded

Table 2.3 A schematic paradigm for lever assignment during outcome devaluation and contingency degradation tests. A: Action O: Outcome

Injection ➔ Strain ⬇	No injection At 30 min	Normal saline At 60 min	MPH At 60 min
SHR	13.167g ± 0.98	2.667g ± 0.56	1.75g ± 0.75
WKY	9.458g ± 0.21	2.833g ± 0.25	0.75g ± 0.64

Table 2.4 Number of food pellets consumed during satiety-induced devaluation. The mean amount of pellets consumed (in grams) (± SEM) in SHR (N=12) and WKY (N=17) rats. MPH: Methylphenidate, SHR: Spontaneous Hypertensive Rats, WKY: Wistar-Kyoto Rats

CHAPTER 3

	Experiment #1	Experiment #2
Subjects	SHR & WKY	SHR & WKY
Age	Adult, P49-P80	Adult, P49-P80
Sex	Male	Male
Drugs & Doses	• D1R antagonist: SCH23390, 0.0025mg/kg • D2R agonist: Quinpirole, 0.01, 0.001mg/kg • Saline	• D1R agonist: SKF38393, 1.0, 3.0mg/kg • D2R antagonist: Raclopride, 0.05, 1.0mg/kg • Saline
Timeline*	• Locomotor Activity Test (Day 1-4) • Habituation (Day5-6) • Instrumental Conditioning (Day 7-16) • Outcome Devaluation & Choice Test (Day 17-23)	• Locomotor Activity Test (Day 1-4) • Habituation (Day5-6) • Instrumental Conditioning (Day 7-16) • Outcome Devaluation & Choice Test (Day 17-23)

Table 3.1 A roadmap for chapter 3 experiments.
* Experiment procedures are described in detail in the Methods and Results section. D1R: Dopamine D1 receptor, D2R: Dopamine D2 receptor

	Saline Control state		SCH23390 D1R antagonist		Quinpirole D2R agonist		SKF38393 D1R agonist		Raclopride D2R antagonist	
WKY	**D1R**	**D2R**	D1R ↓ **D1R**	**D2R**	**D1R**	**D2R** ↑ D2R	**D1R** ↑ D1R	**D2R**	**D1R**	D2R ↓ **D2R**
SHR	**D1R**	**D2R**	D1R ↓ **D1R**	**D2R**	**D1R**	**D2R** ↑ D2R	**D1R** ↑ D1R	**D2R**	**D1R**	D2R ↓ **D2R**

Table 3.2. Theoretical framework for D1R/D2R balance in SHR and WKY rats following saline, SCH23390, Quinpirole, SKF38393 and Raclopride injections. If D1R and D2R are in balance (green) we expect that rats will show goal-directed behavior. If D1R and D2R are not in balance (red), we expect that rats will show habitual response.

Instrumental Conditioning	Outcome Devaluation	Choice Test
$A_1 \Rightarrow O_1$ $A_2 \Rightarrow O_2$	Using specific satiety; devalue: $O_1 \Rightarrow$ OR $O_2 \Rightarrow$	$\uparrow A_1 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_2 \Rightarrow \phi$ $\uparrow\uparrow\uparrow A_1 \Rightarrow \phi$ $\uparrow A_2 \Rightarrow \phi$

Table 3.3 A schematic paradigm for all the behavioral procedures in chapter 3.
A = Action O = Outcome ϕ : No outcome (extinction) $\uparrow$: Response rate

	SHR-Saline	SHR-Quin	SHR-SCH	WKY-Saline	WKY-Quin	WKY-SCH
Original N	24	12	12	24	12	12
Pellet Preference	6	4	3	4	2	3
Lever Preference	2	0	0	0	0	0
Response rate <1 per min	0	0	0	1	0	2
No response during IC and outcome devaluation	0	0	0	1	0	1
Included N	16	8	9	18	10	6

Table 3.4 Number of rats included across strain and medication status in experiment #1.

	Consumption at 40 min	Injections at min 40	Consumption after injection
SHR	10.1g	Quinpirole	2.0g
	10.4g	Saline	1.6g
	10.7g	SCH23390	2.3g
WKY	9.2g	Quinpirole	2.7g
	9.1g	Saline	2.2g
	8.6g	SCH23390	1.4g

Table 3.5 Amount of food pellets consumed during satiety-induced devaluation in SHR and WKY rats at 40 minutes and after injections with Quinpirole, normal saline or SCH23390.

	SHR-Saline	SHR-Rac	SHR-SKF	WKY-Saline	WKY-Rac	WKY-SKF
Original N	12	12	12	12	12	12
Pellet Preference	2	2	2	1	1	1
Lever Preference	0	0	0	0	2	2
Response rate <1 per min	0	0	1	2	4	1
No response during IC and outcome devaluation	1	1	1	0	0	0
Included N	9	9	8	9	5	8

Table 3.6 Number of rats included across strain and medication status in experiment #2

	Consumption at 40 min	Injections at min 40	Consumption after injection
SHR	10.1g	Quinpirole	2.0g
	10.4g	Saline	1.6g
	10.7g	SCH23390	2.3g
WKY	9.2g	Quinpirole	2.7g
	9.1g	Saline	2.2g
	8.6g	SCH23390	1.4g

Table 3.7 Amount of food pellets consumed during satiety-induced devaluation in SHR and WKY rats at 40 minutes and after injections with Quinpirole, normal saline or SCH23390.

CHAPTER 4

	Habituation	Instrumental training	Perfusion	Cryoprotection in 30% sucrose	Mounting & storing at -20°C	Sectioning & staining	Imaging
Day	1-2	3-10	10	10-15	15	16-17	18

Table 4.1 A timeline for behavioral and immunostaining procedures in chapter 4.

Rat group	Action	Outcome	Injection assignment & number of rats
Experimental Rats	Lever press ➔	Food pellet	WKY-saline (N=3)
			WKY-MPH (N=2)
			SHR-saline (N=3)
			SHR-MPH (N=3)
Yoked Controls	Lever press ➔	No outcome	SHR-Saline (N=1)
	&		
	No action ➔	Food pellet synced with Experimental rat's action	WKY-Saline (N=1)
Quiet Controls	No action ➔	No outcome	WKY-Saline (N=1)
			WKY-MPH (N=1)

Table 4.2 Rat groups according to different training paradigms and saline/MPH injection assignment.

CHAPTER 5

Acquisition	Devaluation	Choice test	
A (S1, S2, S3) ➔ O1 B (S4, S5, S6) ➔ O2	A (S1, S2, S3) ➔ O1 B (S4, S5, S6) ➔ O3	A or B ➔	If A>B ➔ GDB If A=B or A<B ➔ HB

Table 5.1 A simplified diagram of the human analogue of the outcome devaluation paradigm. A: group A of stimuli; three bunnies associated with outcome#1 in acquisition. B: group B of stimuli; three bunnies associated with outcome#2 in acquisition. S: stimulus. O1: carrot lunch box. O2: lettuce lunch box. O3: empty lunch box. Symbols of >, =, <: rate of selecting A stimuli versus B stimuli during the choice test. GDB: goal-directed behavior. HB: habitual behavior.

	Stimulus	**Action**	**Outcome**
Animal outcome devaluation	Lever inserted in the operant chamber	Lever press	Food pellet
Human outcome devaluation	Bunny appears on the screen	Touching bunny's face	Picture of full lunch box

Table 5.2 Stimulus-action-outcome comparison between animal and human outcome devaluation paradigms.

ADHD subtype	Parent Cutoff	Group	M±SD	P-value
ADHD-In	≥1.78	ADHD	2.37±0.42	0.000*
		HC	0.45±0.27	
ADHD-H/Im	≥1.44	ADHD	2.03±0.67	0.000*
		HC	0.53±0.53	
ADHD-C	≥1.67	ADHD	2.36±0.36	0.000*
		HC	0.49±0.27	

Table 5.3 Parent tentative 5% cutoff, mean and standard deviation for subscale score of ADHD subtypes in the SNAP-IV-C rating scale in patients with ADHD (N=19) and HC subjects (N=21) (* = significant at $p<0.05$).

Disorder	Group	M±SD
Oppositional Defiant Disorder*	ADHD	2.14±0.55
	HC	0.65±0.44
Conduct Disorder	ADHD	0.51±0.74
	HC	0.03±0.08
Intermittent Explosive Disorder	ADHD	0.29±0.90
	HC	0.00±0.00
Tourette's Disorder	ADHD	0.67±1.06
	HC	0.00±0.00
Stereotypic Movement Disorder	ADHD	0.67±1.01
	HC	0.17±0.39
Obsessive Compulsive Disorder	ADHD	0.55±0.92
	HC	0.13±0.43
General Anxiety Disorder	ADHD	1.48±0.61
	HC	0.33±0.30
Narcolepsy	ADHD	0.48±0.93
	HC	0.00±0.00
Histrionic Personality Disorder	ADHD	1.95±1.16
	HC	0.58±0.67
Narcissistic Personality Disorder	ADHD	1.24±1.26
	HC	0.33±0.89
Borderline Personality Disorder	ADHD	0.86±1.11
	HC	0.17±0.39
Manic Episode	ADHD	0.70±0.75
	HC	0.12±0.20
Major Depressive Episode	ADHD	0.36±0.58
	HC	0.03±0.11
Dysthymic Disorder	ADHD	0.60±0.80
	HC	0.00±0.00
Post-traumatic Stress Disorder	ADHD	1.24±0.92
	HC	0.21±0.33
Adjustment Disorder	ADHD	0.90±0.98
	HC	0.33±0.72

Table 5.4 Mean and standard deviation of subscale score for possible comorbid disorders in the SNAP-IV-C rating scale in patients with ADHD (N=18) and HC subjects (N=12) (* = significant at $p<0.05$).

Academic	Group	M±SD
Orienting	ADHD	1.90±0.83
	HC	0.04±0.14
Maintaining	ADHD	2.33±0.84*
	HC	0.17±0.25
Directing	ADHD	1.86±0.82
	HC	0.13±0.23

Table 5.5 Mean and standard deviation of subscale scores for classroom academic performance in the SNAP-IV-C rating scale in patients with ADHD (N=18) and HC subjects (N=12) (* = significant at $p<0.05$).

Deportment	Group	M±SD
Attention to Other	ADHD	0.93±0.91
	HC	0.17±0.44
Attention to Rules	ADHD	1.74±0.10
	HC	0.13±0.31

Table 5.6 Mean and standard deviation of subscale score for classroom deportment in the SNAP-IV-C rating scale in patients with ADHD (N=18) and HC subjects (N=12) (* = significant at $p<0.05$).

ILLUSTRATIONS AND FIGURES

CHAPTER 1

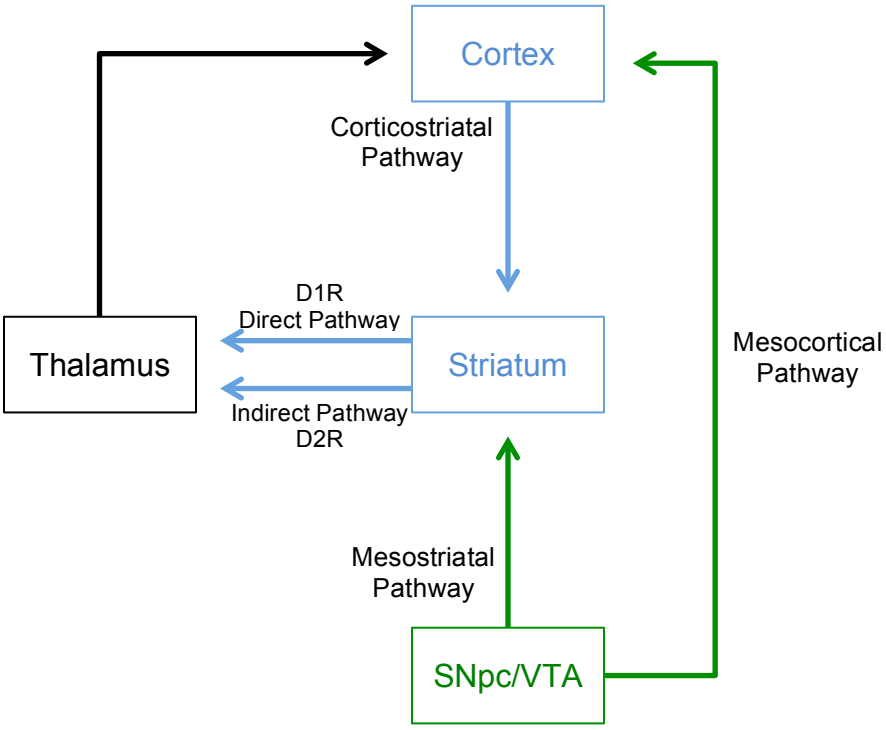


Figure 1.1 Illustration of dopamine and corticostriatal pathways.

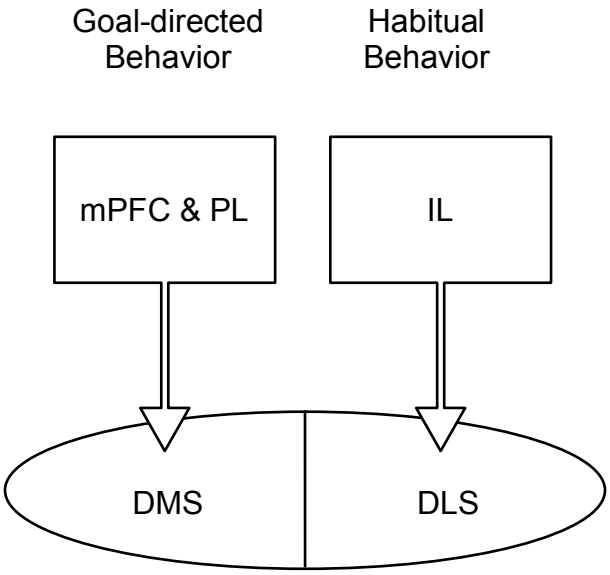


Figure 1.2 Corticostriatal pathways that underlie goal-directed and habitual behaviors. mPFC: medial prefrontal cortex; PL: Prelimbic prefrontal cortex; IL: Infralimbic cortex; DMS: Dorsomedial striatum; DMS: Dorsolateral striatum

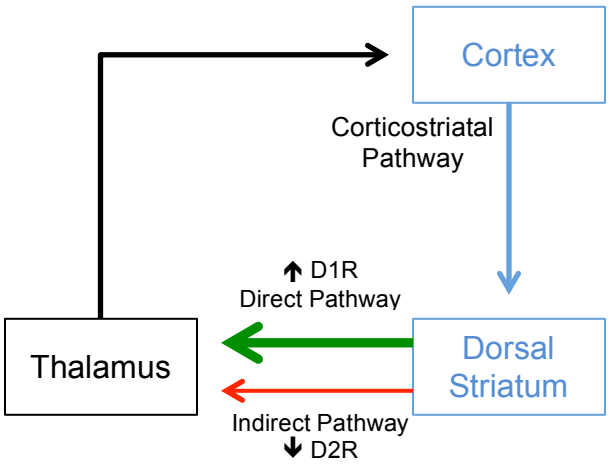


Figure 1.3 Illustration of misbalanced activation of D1R and D2R that might underlie a deficit in goal-directed behavior in ADHD. Green arrow: hyper-activation of the direct pathway). Red arrow: hypo-inhibition of the indirect pathway.

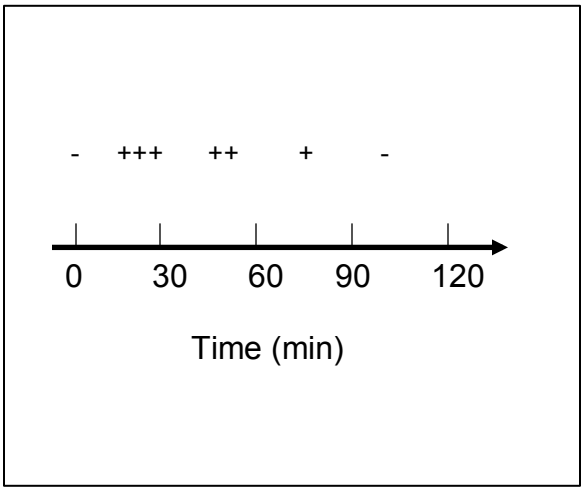


Figure 1.4 Simplified illustration of the time course of c-fos expression.

CHAPTER 2

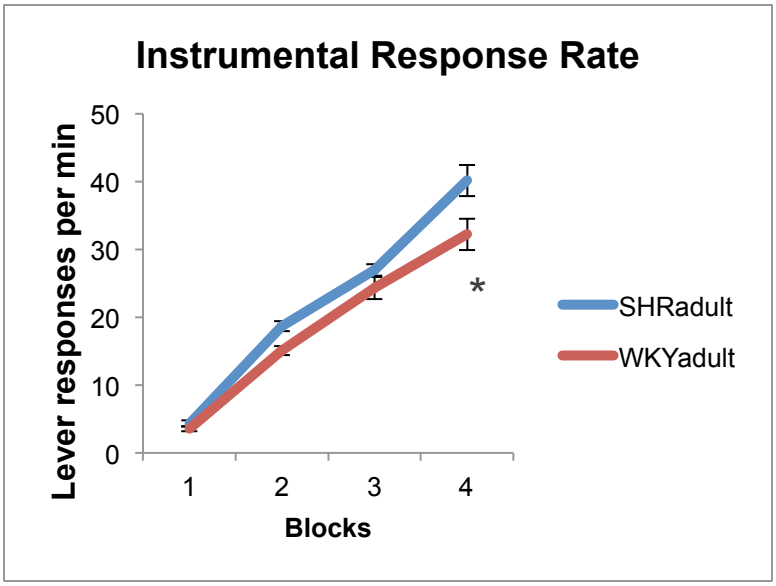


Figure 2.1 The mean number of presses per min on the four blocks of instrumental training in SHR (N=12) and WKY (N=17) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

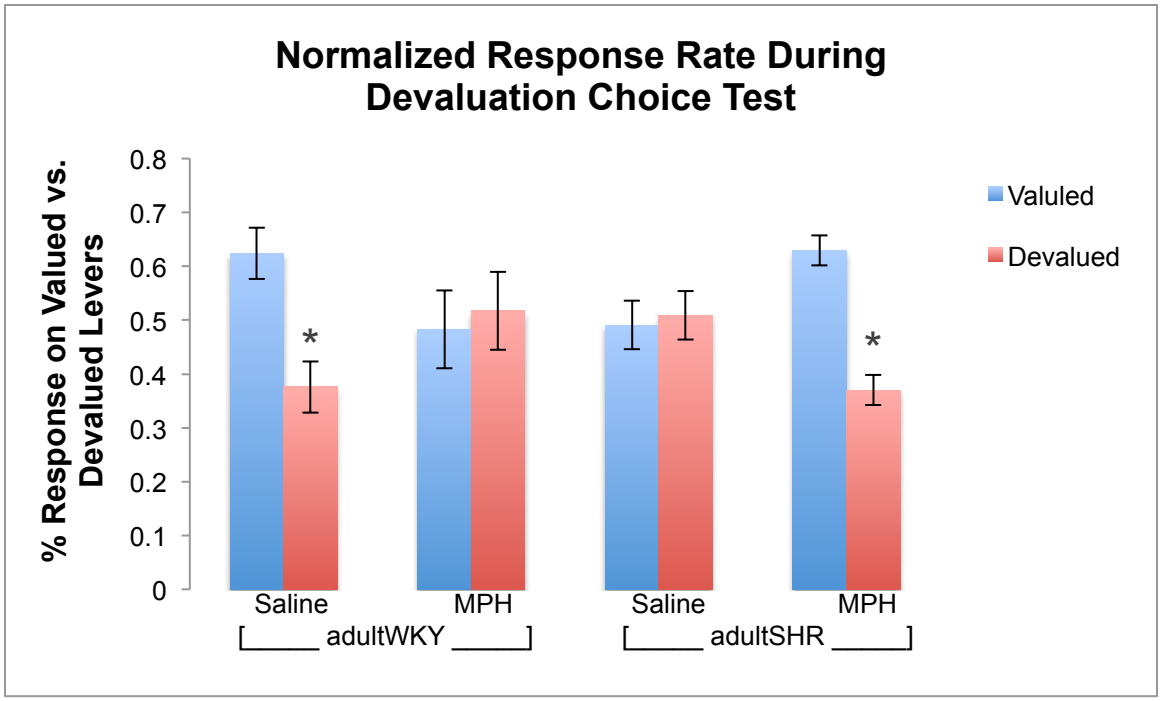


Figure 2.2 Normalized performance of SHR and WKY rats during the 10-min devaluation test. The percentage of responses on the valued and devalued levers for WKY (N=17) and SHR (N=12) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

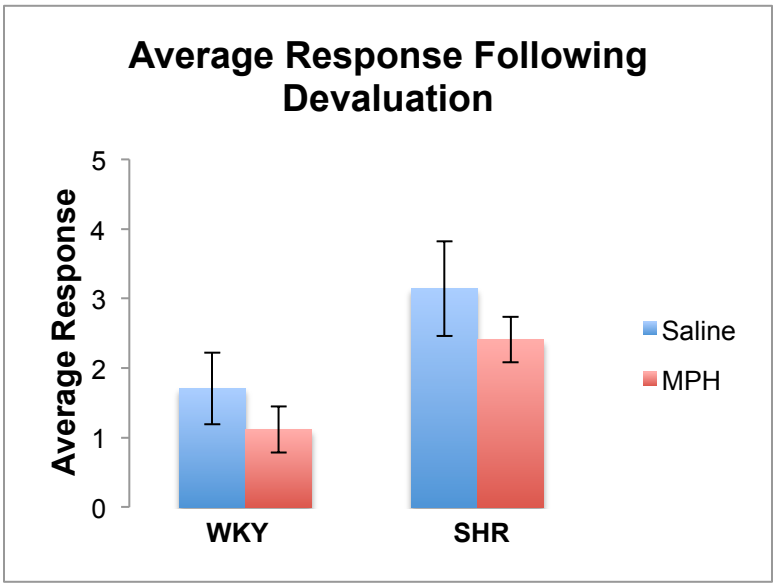


Figure 2.3 Overall response rate during the 10-min devaluation test, after receiving MPH or saline injections. The average of the response rates on both levers for SHR (N=12) and WKY (N=17) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

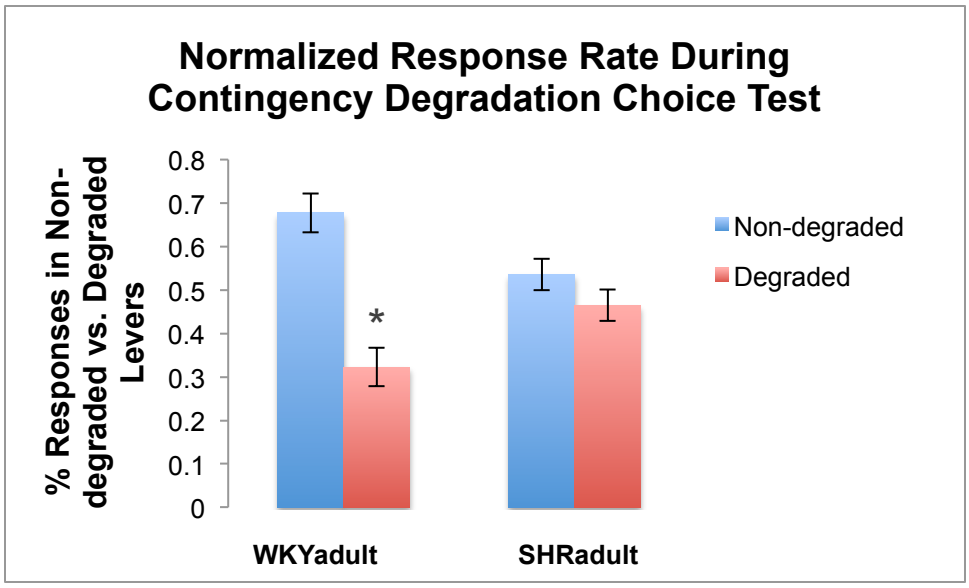


Figure 2.4 Normalized response rates during the extinction test after contingency degradation training. The percentage of responses on the degraded and non-degraded levers for WKY (N=17) and SHR (N=11) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

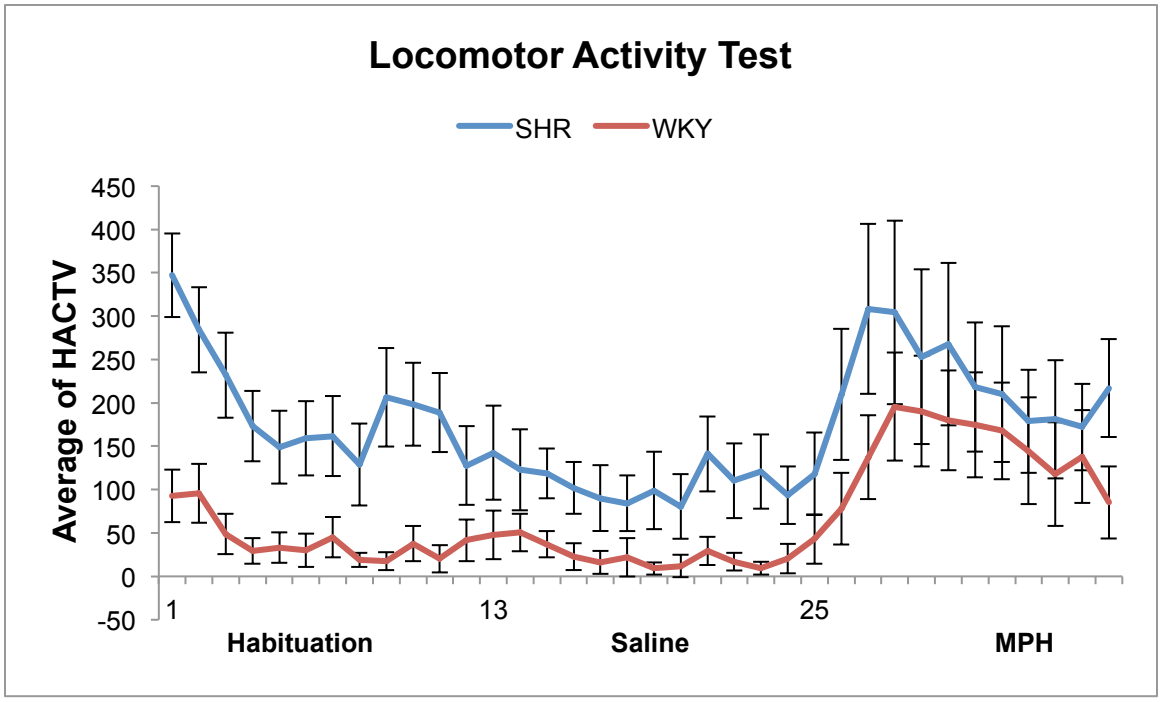


Figure 2.5 Locomotor activity in 5-min blocks over (1) habituation phase, block 1–12, (2) saline injection phase, block 12–24, and (3) MPH injection phase, block 24–36 in SHR (N=12) and WKY (N =12) (error bars = ±SEM) (∗significant at $p < 0.05$) (HACTV: average horizontal activity).

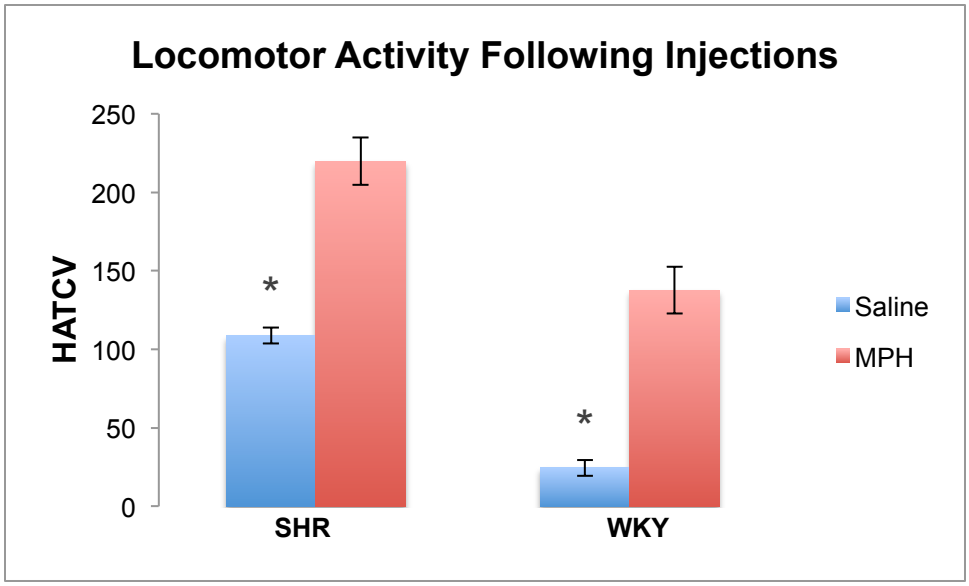


Figure 2.6 Locomotor activity expressed as average horizontal activity (HACTV) for one hour after saline and MPH injections in SHR (N=12) and WKY (N =12) (error bars = ±SEM) (∗significant at $p < 0.05$).

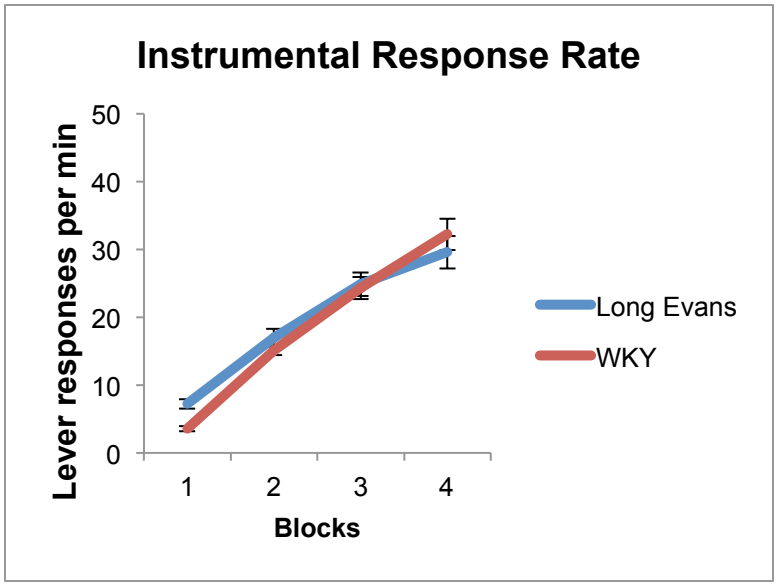


Figure 2.7 The mean number of presses per min on the four blocks of instrumental training in WKY (N=17) and Long Evans (N=32) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

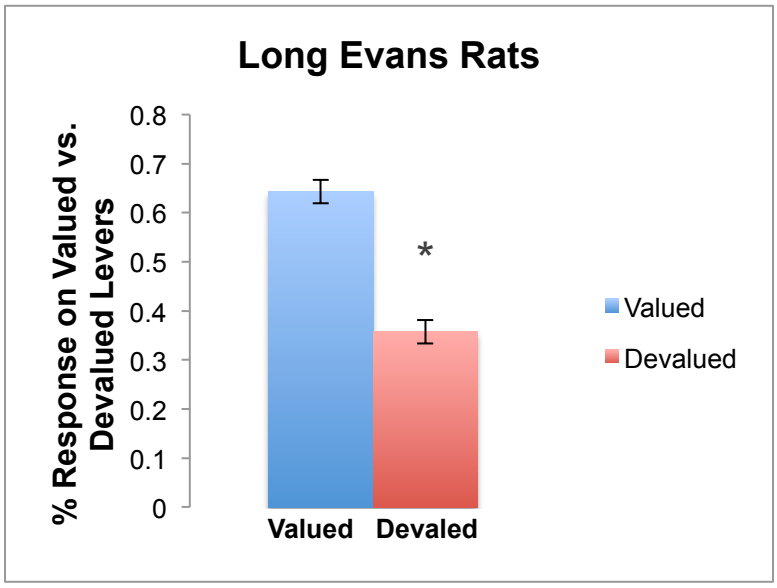


Figure 2.8 Normalized performance of Long Evans rats (N=32) during the 10-min devaluation test. The percentage of responses on the valued and devalued levers (error bars = ±SEM) (* = significant at $p < 0.05$).

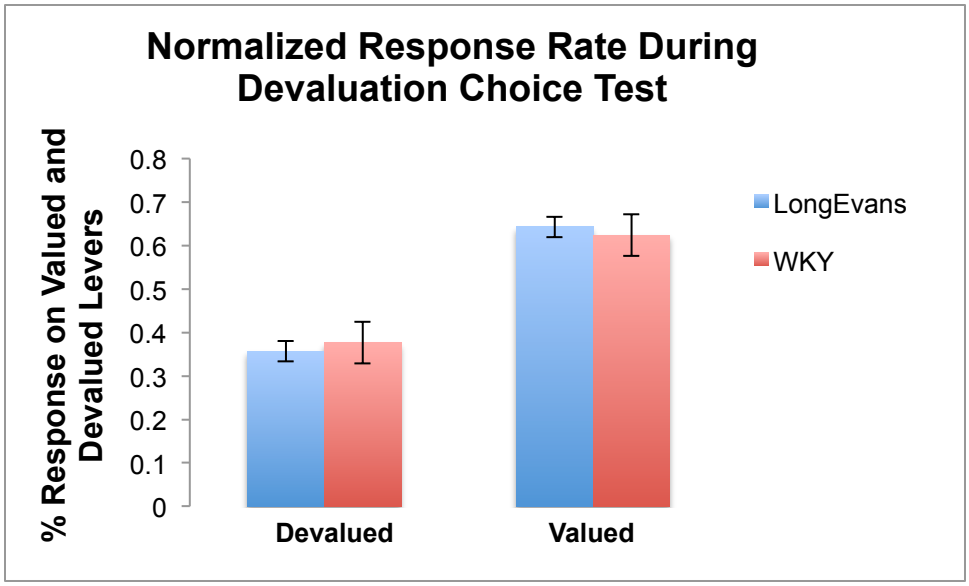


Figure 2.9 Normalized performance of Long Evans and WKY rats during the 10-min devaluation test. The percentage of responses on the valued and devalued levers for WKY (N=17) and Long Evans (N=32) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

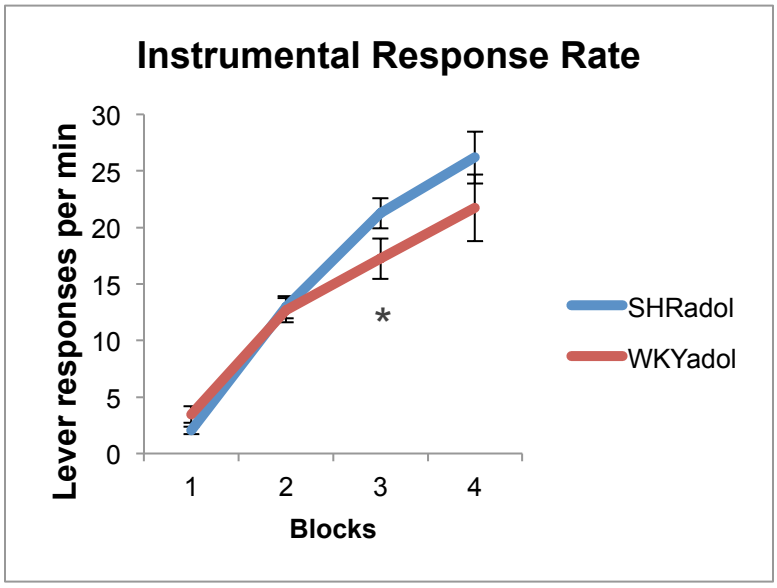


Figure 2.10 The mean number of presses per min on the four blocks of instrumental training in adolescent SHR (N=18) and WKY (N=18) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

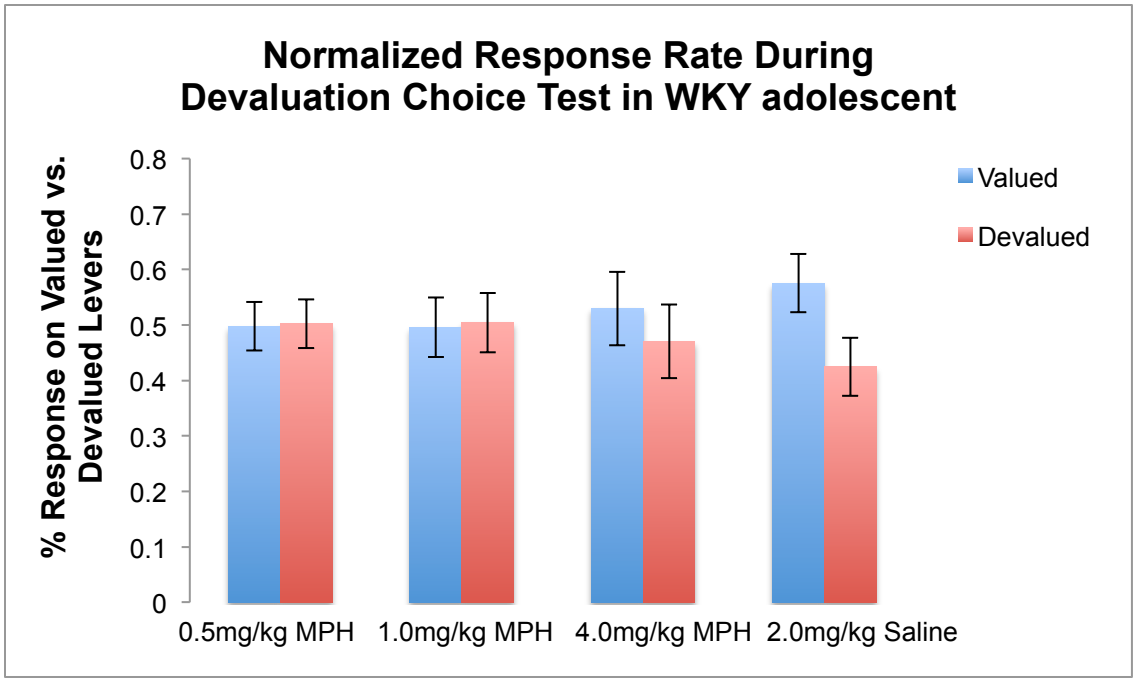


Figure 2.11 Normalized performance of adolescent WKY rats during the 10-min devaluation test after receiving a dose of 0.5, 1.0, or 4.0 mg/kg methylphenidate, or 2.0 mg/kg normal saline. The percentage of responses on the valued and devalued levers for WKY (N=17) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

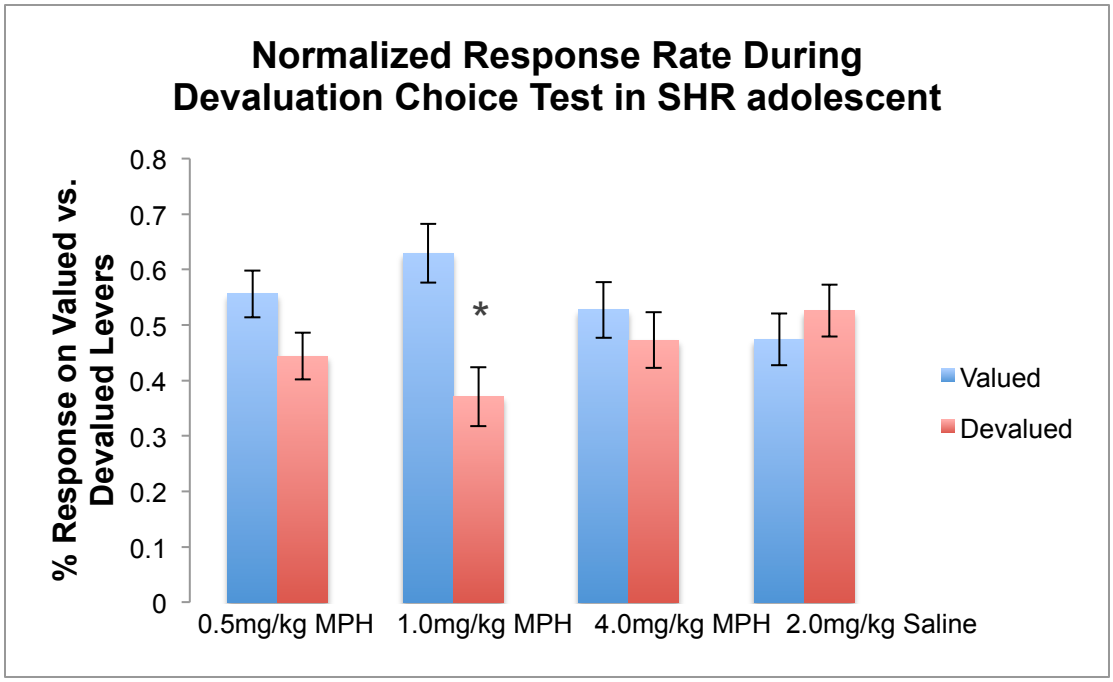


Figure 2.12 Normalized performance of adolescent SHR rats during the 10-min devaluation test after receiving a dose of 0.5, 1.0, or 4.0 mg/kg methylphenidate, or 2.0 mg/kg normal saline. The percentage of responses on the valued and devalued levers for SHR (N=17) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

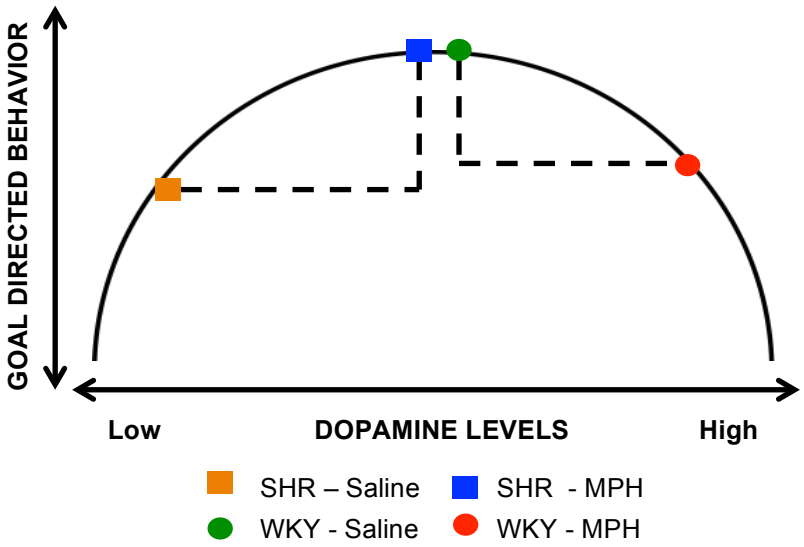


Figure 2.13 Inverted U-shape function of goal-directed behavior in SHR and WKY rats under the effect of MPH.

CHAPTER 3
Experiment 3.1

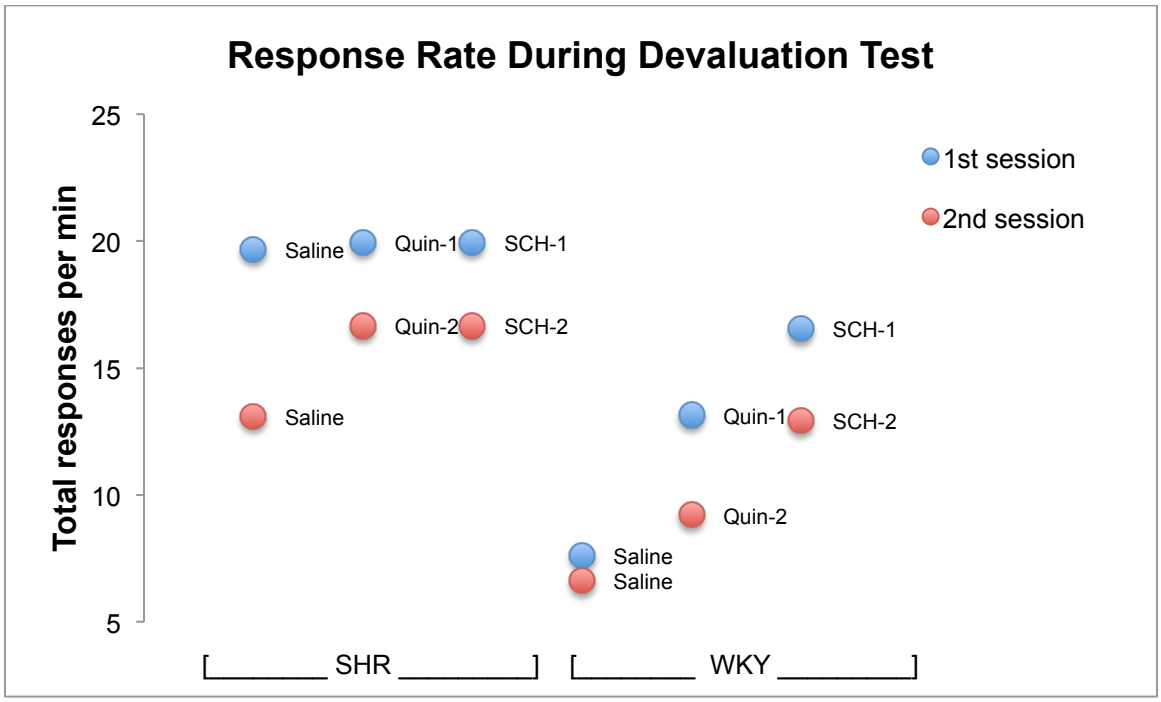


Figure 3.1 Average total responses per minute on valued + devalued levers during the first and second devaluation tests under injections with normal saline (Saline), Quinpirole (Quin) and SCH23390 (SCH) in SHR and WKY rats.

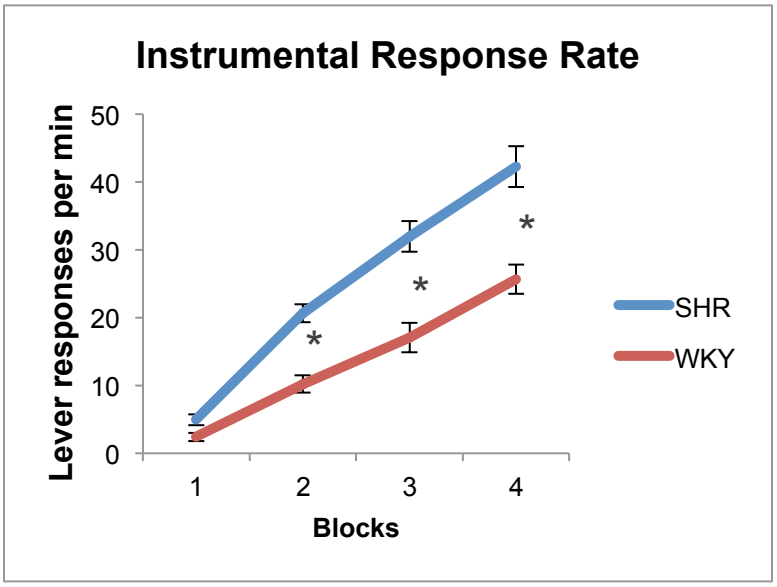


Figure 3.2 Performance of SHR and WKY rats during instrumental training blocks. Each block represents the average of two training sessions showed as the mean number of presses per min on each block of training in **SHR** (N=24) and **WKY** (N=24) rats (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

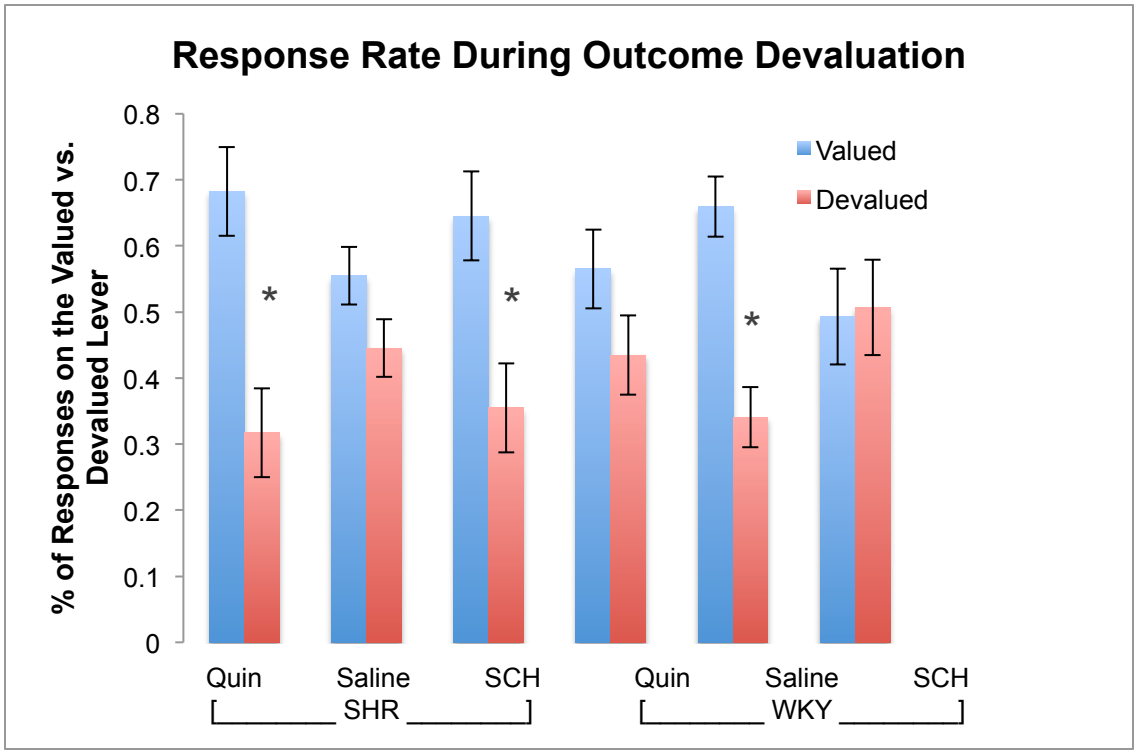


Figure 3.3 Outcome devaluation test. Normalized performance of SHR and WKY rats during the 5-min devaluation test under injections with normal saline (Saline), Quinpirole (Quin) or SCH23390 (SCH). The percentage of responses on the valued and devalued levers in **SHR** (Quin N=8, saline N=16, SCH N=9) and **WKY** (Quin N=10, Saline N=18, SCH N=6) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

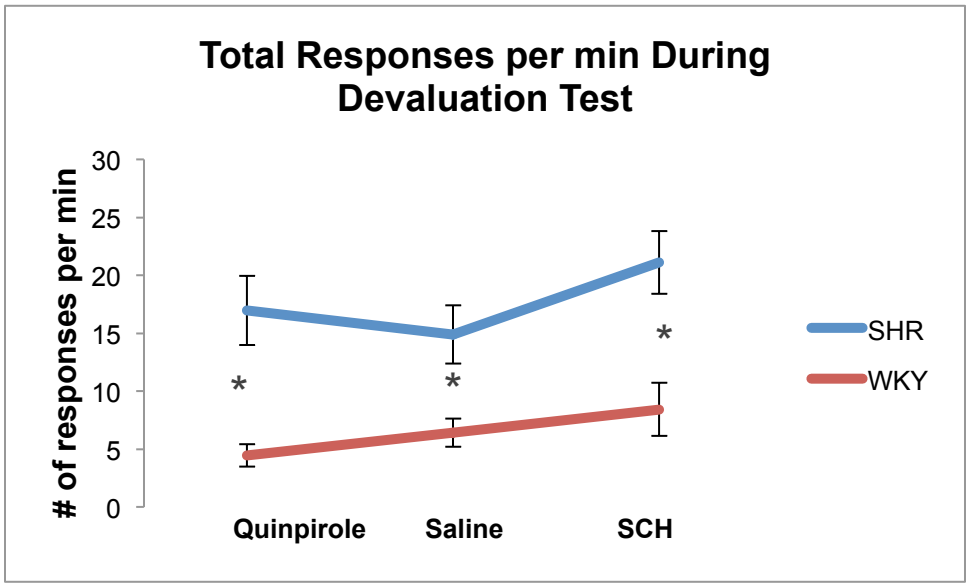


Figure 3.4 Total responses per min during devaluation test under injections with normal saline, Quinpirole, or SCH23390 in **SHR** (Quin N=8, saline N=16, SCH N=9) and **WKY** (Quin N=10, Saline N=18, SCH N=6) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

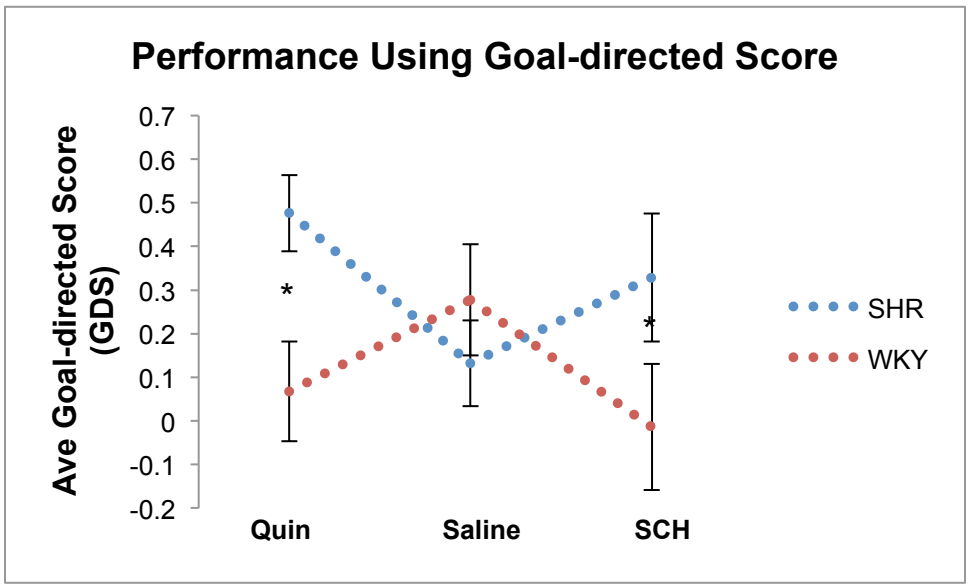


Figure 3.5 Goal-directed Score (GDS) calculated using the formula: [% of valued responses - % of devalued responses)/(% of valued responses + % of devalued responses)]. GDS under injections with normal saline (Saline), Quinpirole (Quin), or SCH23390 (SCH) in **SHR** (N=10) and **WKY** (N=10) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

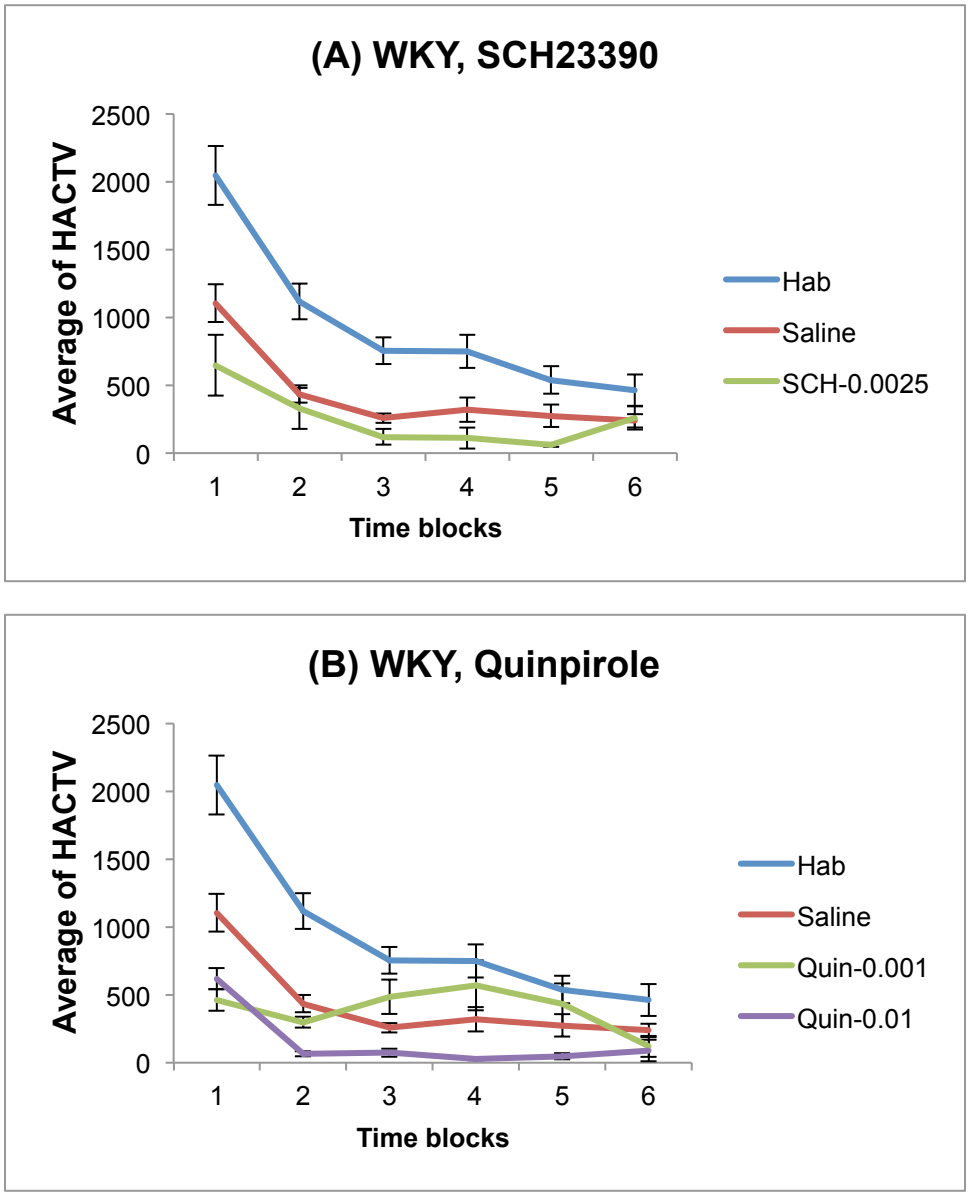
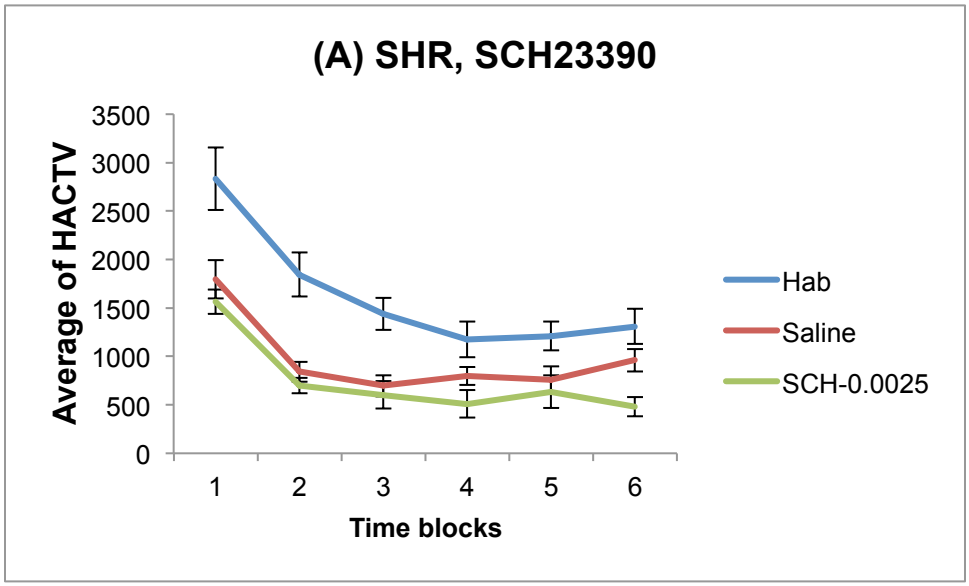


Figure 3.6 **A.** Locomotor activity in 5-min blocks over (1) habituation phase (N=12), (2) saline injection phase (N=12) and (3) SCH23390, 0.0025mg/kg injection phase (N=6) in WKY rats. **B.** Locomotor activity in 5-min blocks over (1) habituation phase (N=12), (2) saline injection phase (N=12), (3) Quinpirole, 0.001mg/kg injection phase (N=6) and (4) Quinpirole, 0.01mg/kg injection phase (N=6) in WKY rats. (error bars = ±SEM) (∗significant at $p < 0.05$) (HACTV: average horizontal activity).



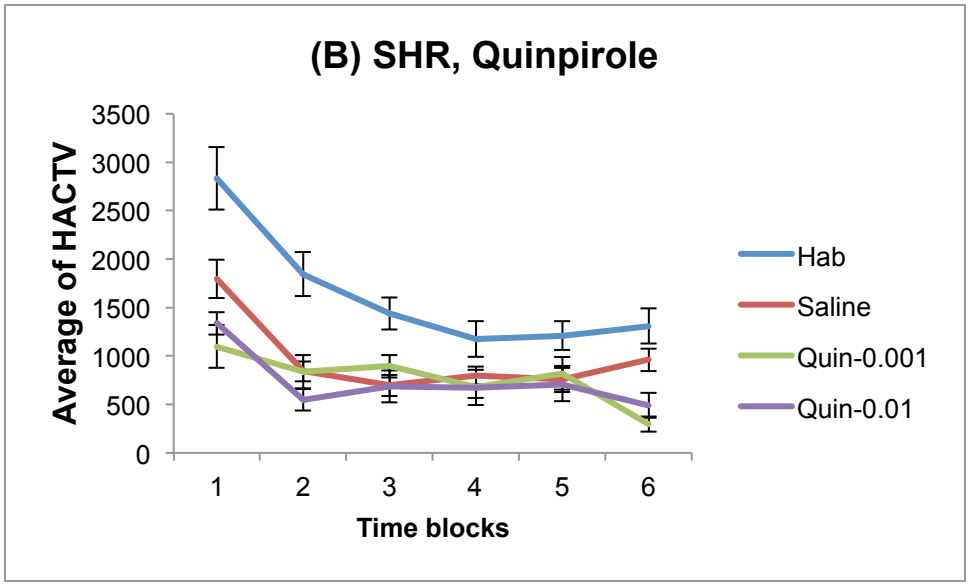


Figure 3.7 **A.** Locomotor activity in 5-min blocks over (1) habituation phase (N=12), (2) saline injection phase (N=12) and (3) SCH23390, 0.0025mg/kg injection phase (N=6) in SHR rats. **B.** Locomotor activity in 5-min blocks over (1) habituation phase (N=12), (2) saline injection phase (N=12), (3) Quinpirole, 0.001mg/kg injection phase (N=6) and (4) Quinpirole, 0.01mg/kg injection phase (N=6) in SHR rats. (error bars = ±SEM) (∗significant at $p < 0.05$) (HACTV: average horizontal activity).

Experiment 3.2

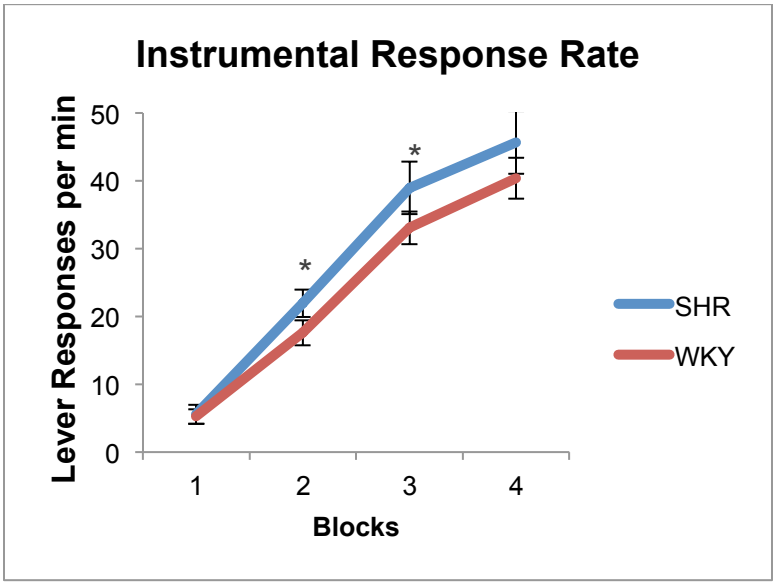


Figure 3.8 Performance of SHR and WKY rats during instrumental training blocks. Each block represents the average of two training sessions showed as the mean number of presses per min on each block of training in **SHR** (N=11) and **WKY** (N=12) rats (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

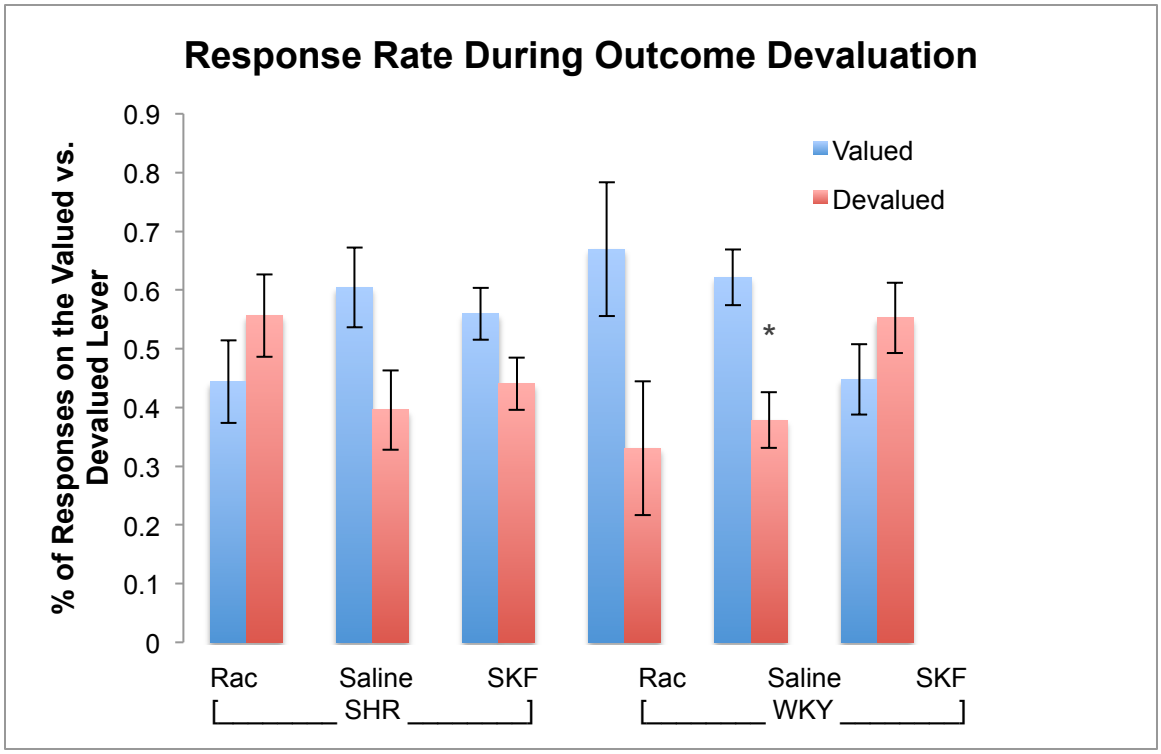


Figure 3.9 Outcome devaluation test. Normalized performance of SHR and WKY rats during the 5-min devaluation test under injections with normal saline (Saline), Raclopride (Rac) or SKF38393 (SKF). The percentage of responses on the valued and devalued levers in **SHR** (Rac N=9, saline N=9, SKF N=8) and **WKY** (Rac N=5, Saline N=9, SKH N=8) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

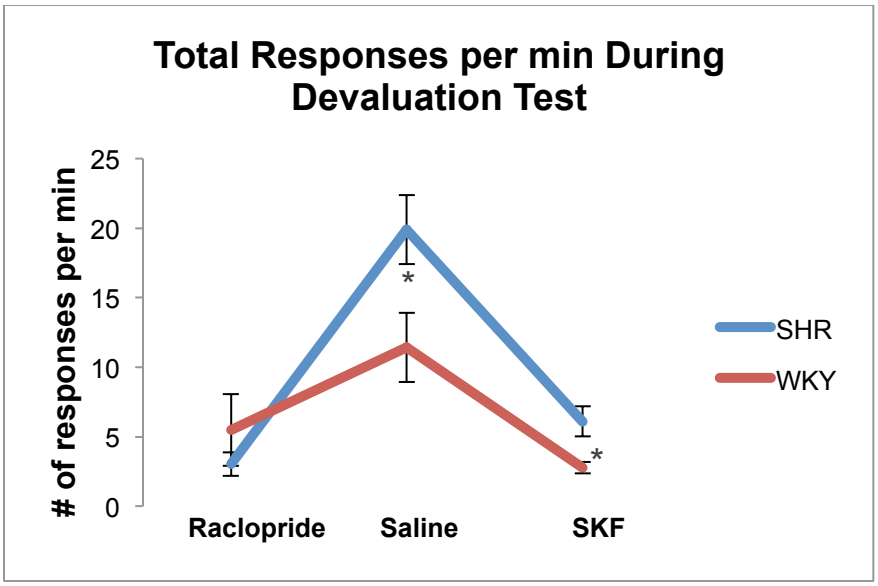


Figure 3.10 Total responses per min during devaluation test under injections with normal saline, Raclopride, or SKF38393 in **SHR** (Raclopride N=9, saline N=9, SKF N=8) and **WKY** (Raclopride N=5, Saline N=9, SKF N=5) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

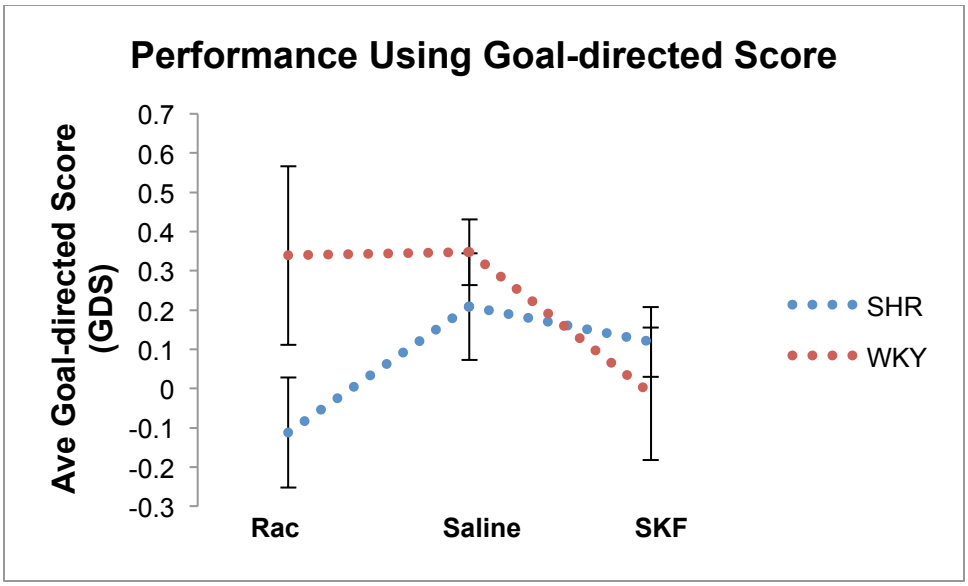


Figure 3.11 Goal-directed Score (GDS) calculated using the formula: [% of valued responses - % of devalued responses)/(% of valued responses + % of devalued responses)]. GDS under injections with normal saline, Raclopride or SKF38393 in **SHR** (N=9) and **WKY** (N=5) rats (error bars = ±SEM**)** (* = significant at $p<0.05$).

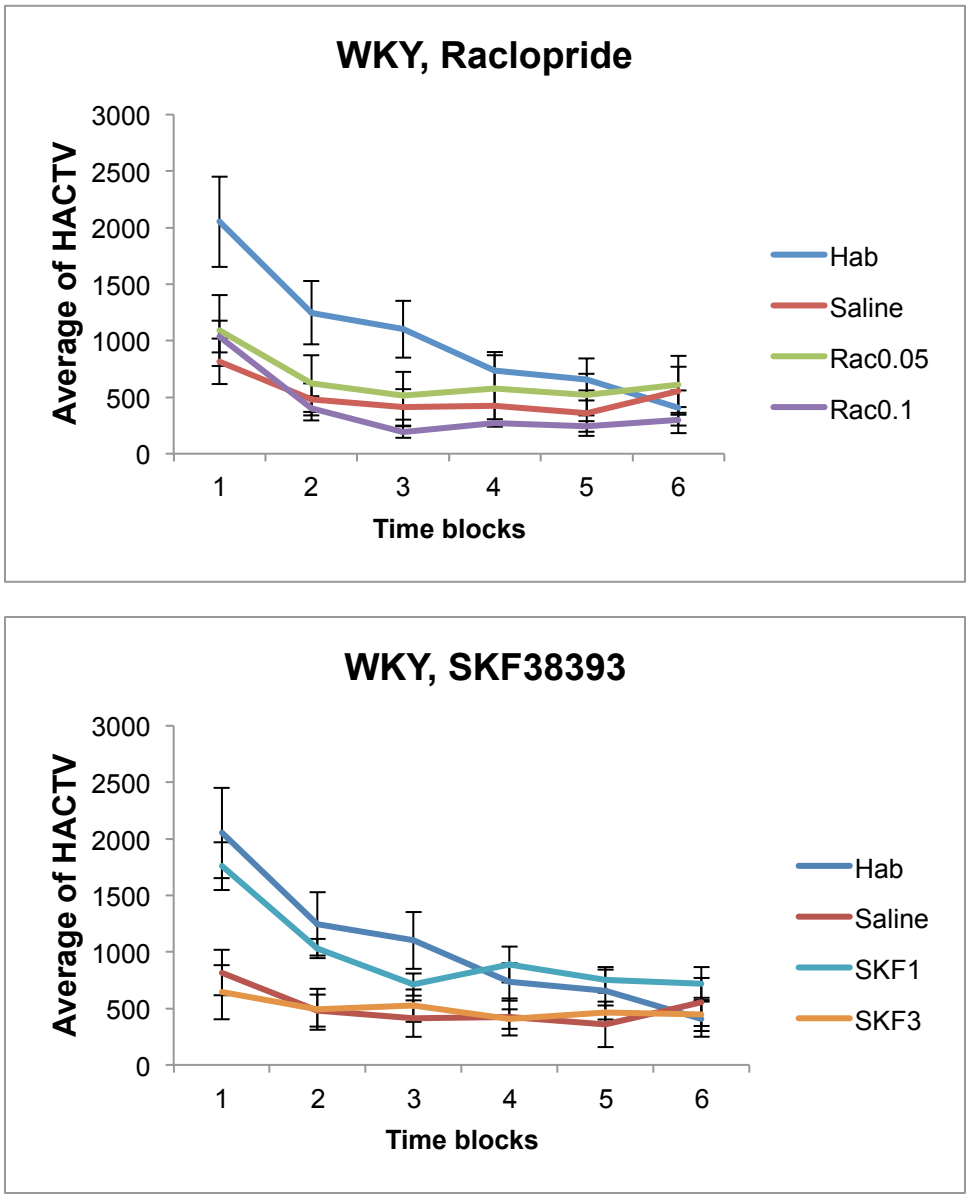
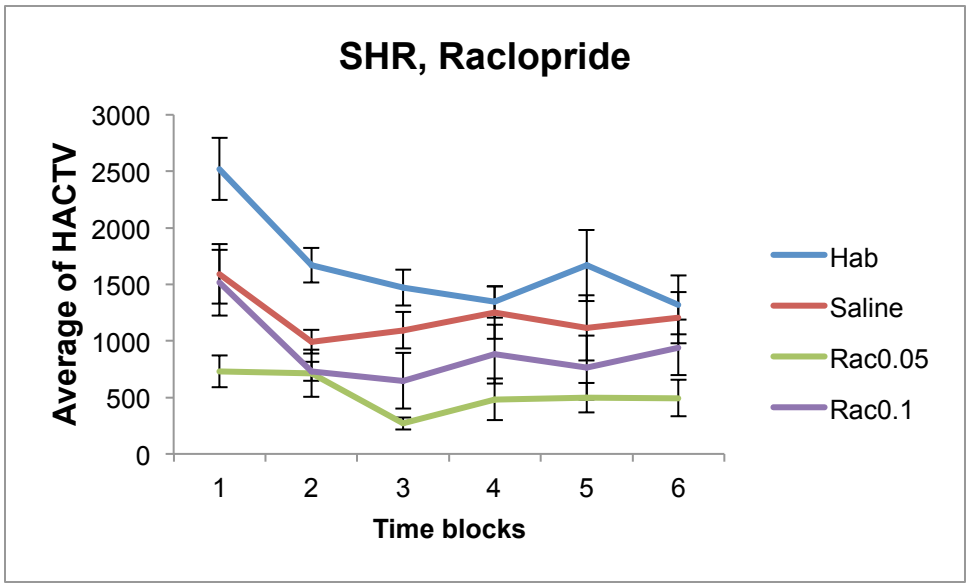


Figure 3.12 **A.** Locomotor activity in 5-min blocks over (1) habituation phase, (2) saline injection phase, (3) Raclopride, 0.05mg/kg injection phase and (4) Raclopride 0.1mg/kg injection phase in WKY (N=6) rats. **B.** Locomotor activity in 5-min blocks over (1) habituation phase, (2) saline injection phase, (3) SKF38393, 1.0mg/kg injection phase and (4) SKF38393 3.0mg/kg injection phase in WKY (N=6) rats. (error bars = ±SEM) (∗significant at $p < 0.05$) (HACTV: average horizontal activity).



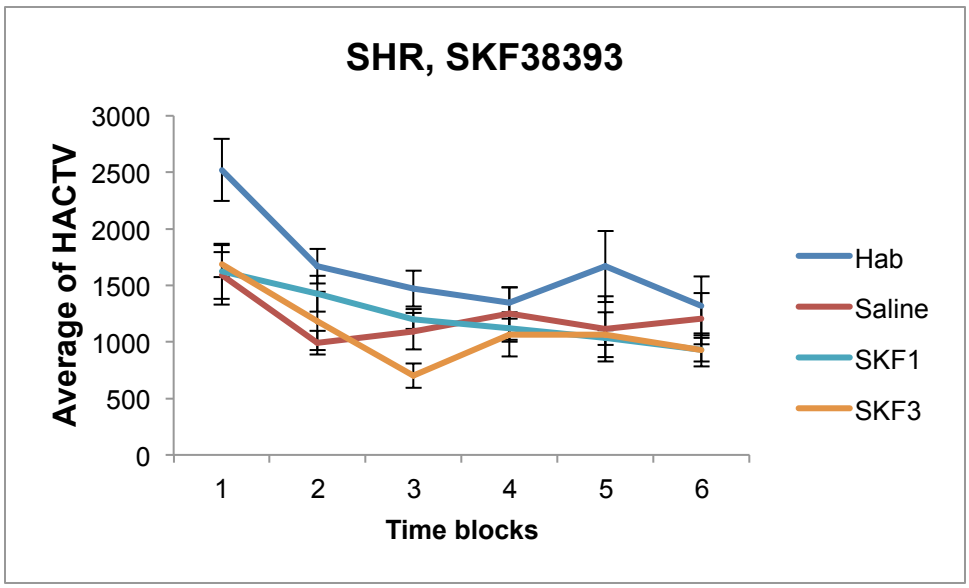


Figure 3.13 **A.** Locomotor activity in 5-min blocks over (1) habituation phase, (2) saline injection phase, (3) Raclopride, 0.05mg/kg injection phase and (4) Raclopride 0.1mg/kg injection phase in SHR (N=6) rats. **B.** Locomotor activity in 5-min blocks over (1) habituation phase, (2) saline injection phase, (3) SKF38393, 1.0mg/kg injection phase and (4) SKF38393 3.0mg/kg injection phase in SHR (N=6) rats. (error bars = ±SEM) (∗significant at $p < 0.05$) (HACTV: average horizontal activity).

CHAPTER 4

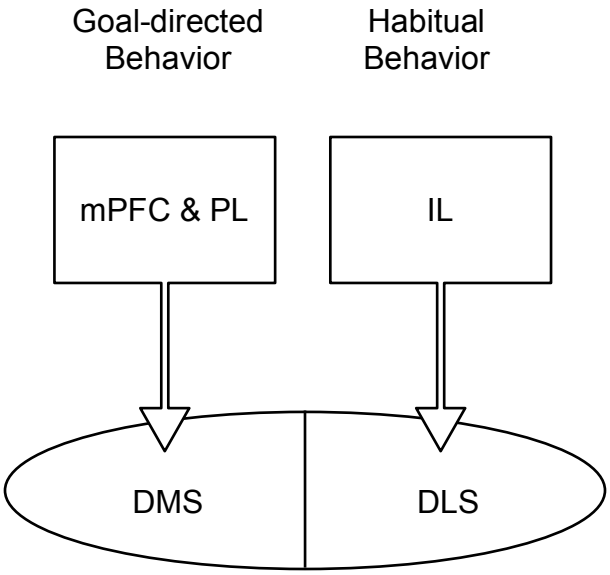


Figure 4.1 Corticostriatal pathways that underlie goal-directed and habitual behaviors. mPFC: medial prefrontal cortex; PL: Prelimbic prefrontal cortex; IL: Infralimbic cortex; DMS: Dorsomedial striatum; DMS: Dorsolateral striatum.

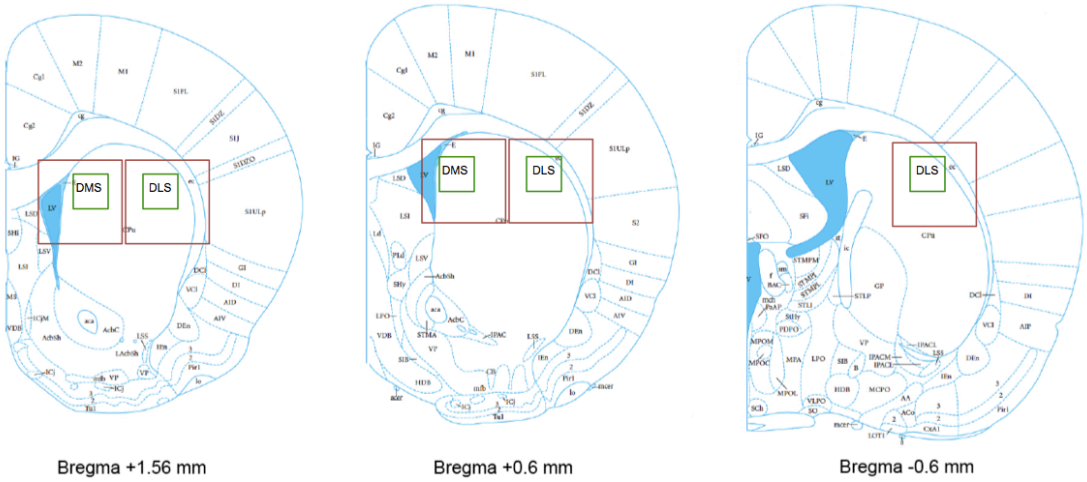


Figure 4.2 Schematic representation of the striatal regions where cell imaging and counts were performed (adapted from Paxinos and Watson, 2007). Bregma +1.56 mm: anterior striatum; Bregma +0.6 mm: middle striatum; Bregma -0.6 mm: posterior striatum. Red and green squares represent imaging and counting regions of interest, respectively.

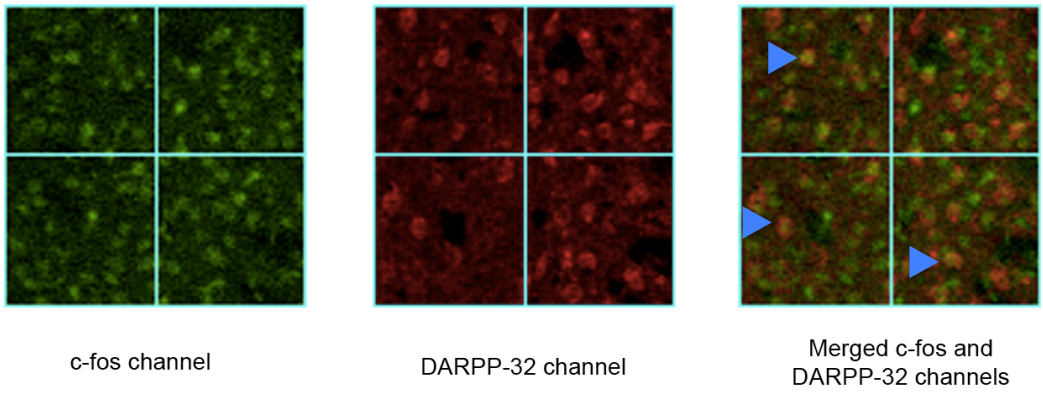


Figure 4.3 Confocal images of striatal expressing neurons immunostained for c-fos (green) and DARPP-32 (red). Images to the right illustrate the colocalization of c-fos and DARPP-32 in SHR rat brain striatum. Blue arrow indicates colocalization.

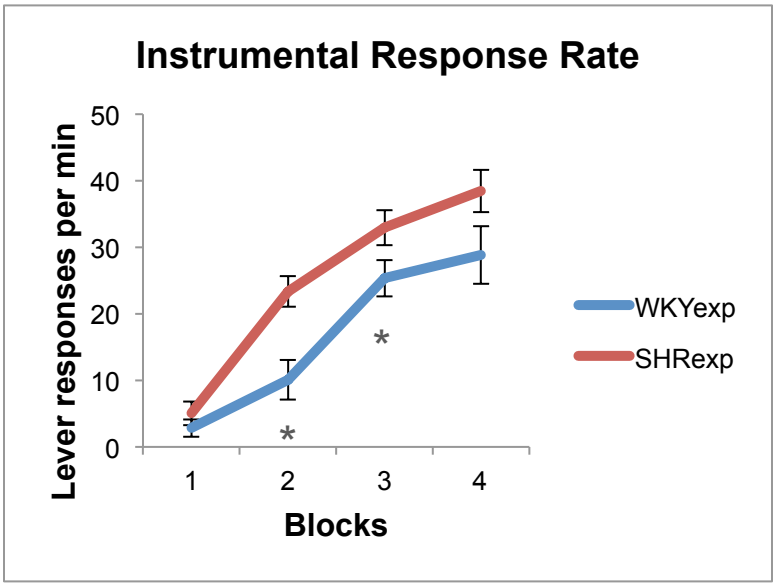


Figure 4.4 The mean number of presses per min on the four blocks of instrumental training in SHR (N=6) and WKY (N=5) rats (error bars = ±SEM) (* = significant at $p < 0.05$).

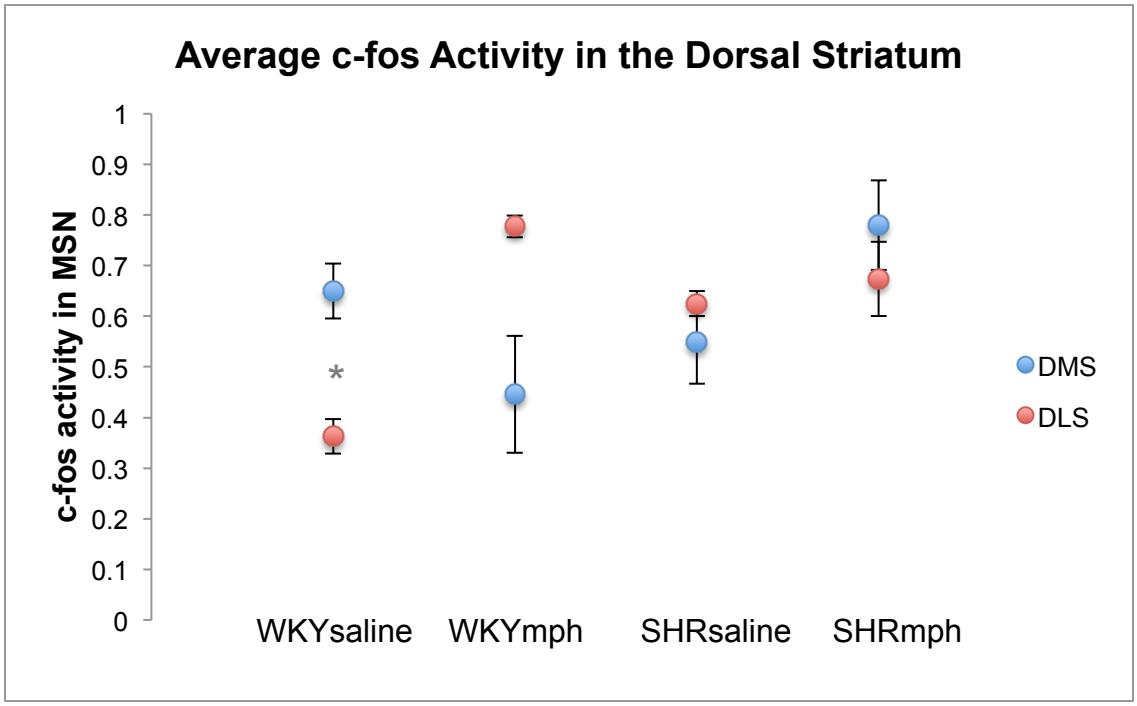


Figure 4.5 Average c-fos activity in medium spiny neurons (MSN) in the anterior, middle and posterior dorsomedial and dorsolateral striatum in experimental rats: **WKY** on saline (N=3), **WKY** on MPH (N=2), **SHR** on saline (N=3) and **SHR** on MPH (N=2) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

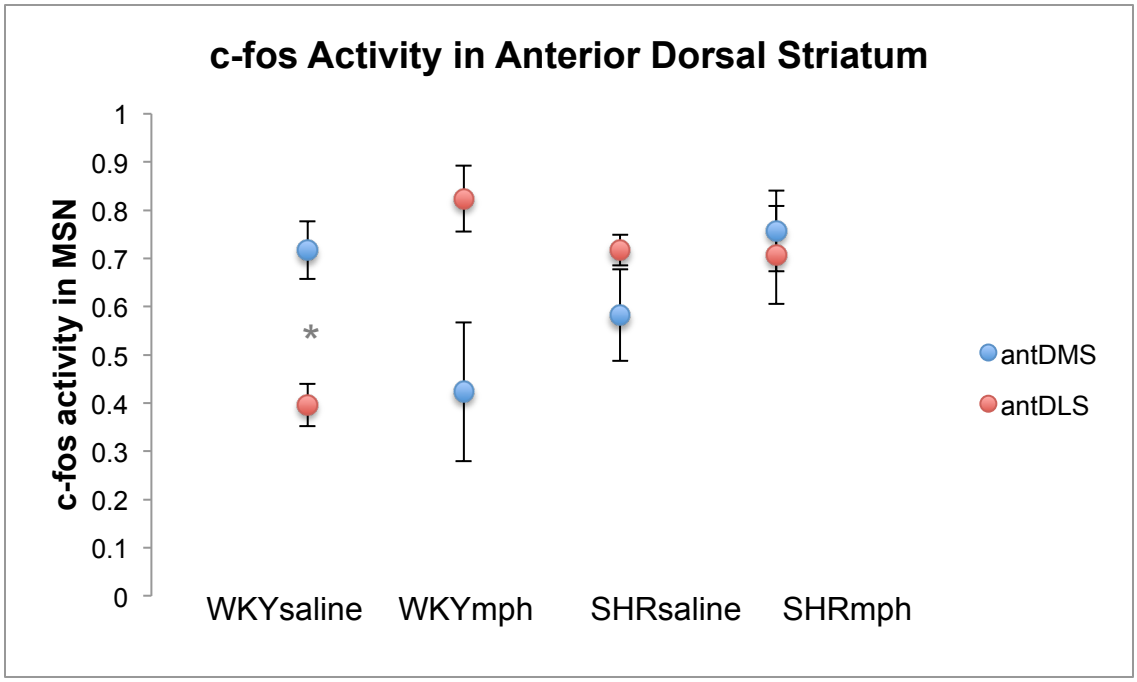


Figure 4.6 Average c-fos activity in medium spiny neurons (MSN) in anterior dorsomedial and dorsolateral striatum in experimental rats: **WKY** on saline (N=3), **WKY** on MPH (N=2), **SHR** on saline (N=3) and **SHR** on saline (N=3) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

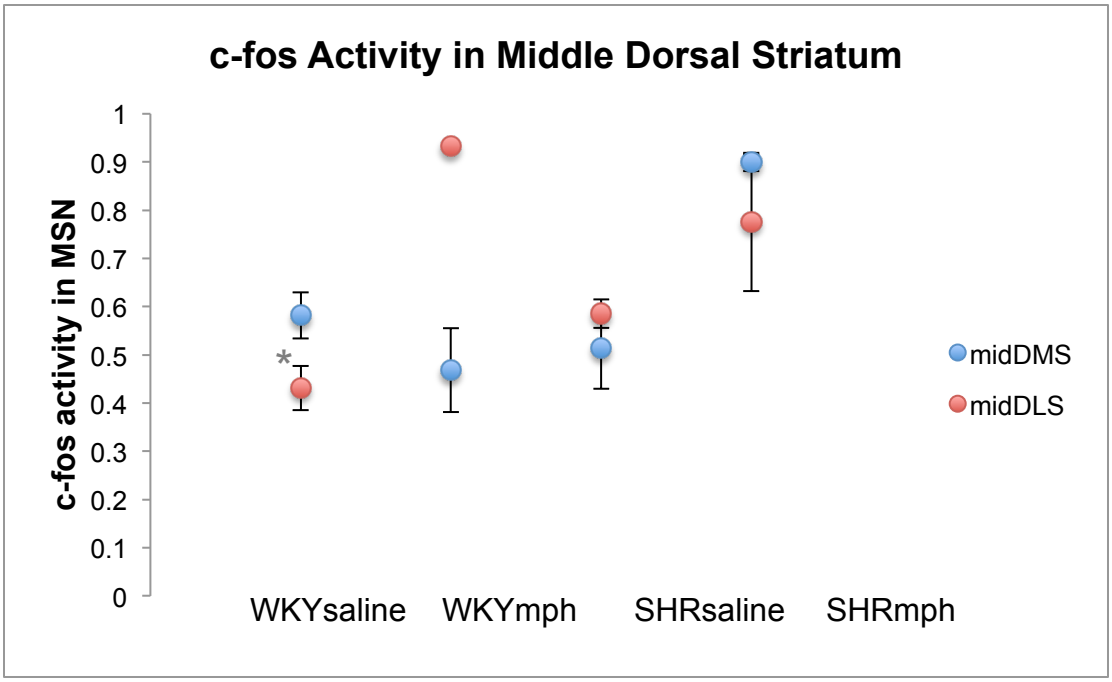


Figure 4.7 Average c-fos activity in medium spiny neurons (MSN) in middle dorsomedial and dorsolateral striatum in experimental rats: **WKY** on saline (N=3), **WKY** on MPH (N=2), **SHR** on saline (N=3) and **SHR** on MPH (N=2) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

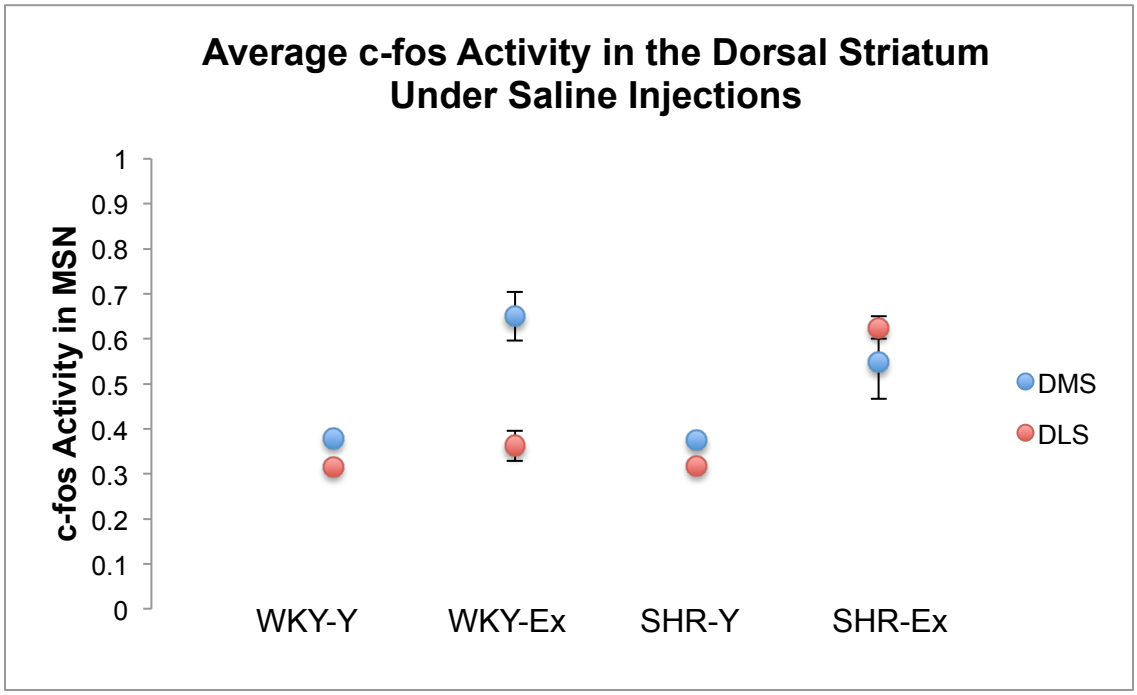


Figure 4.8 Average c-fos activity in medium spiny neurons (MSN) in the anterior, middle and posterior dorsomedial and dorsolateral striatum in experimental rats: **WKY** on saline (N=3), **SHR** on saline (N=3) and yoked controls: **WKY** on saline (N=1), **SHR** on saline (N=1). WKY-Y: WKY-Yoked, WKY-Ex: WKY-Experimental, SHR-Y: SHR-Yoked, SHR-Ex: SHR-Experimental.

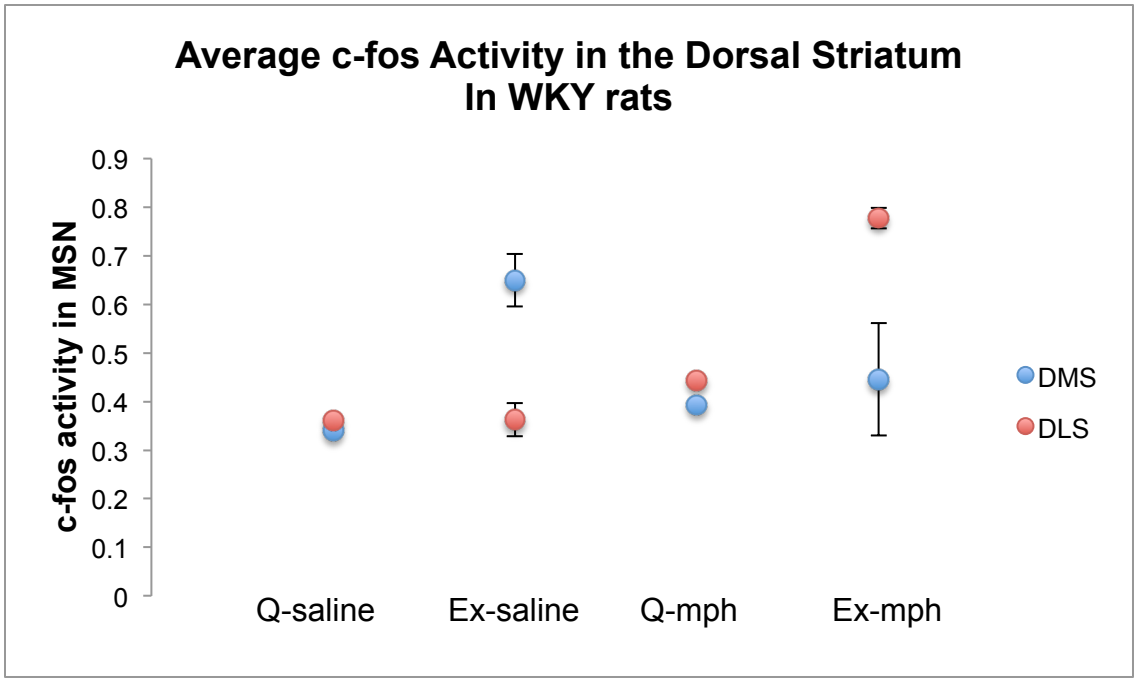


Figure 4.9 Average c-fos activity in medium spiny neurons (MSN) in the anterior, middle and posterior dorsomedial and dorsolateral striatum in experimental rats: **WKY** on saline (N=3), **WKY** on MPH (N=2) and quiet controls: **WKY** on saline (N=1), **WKY** on saline (N=1). Q-saline: WKY-Quiet-saline, Ex-saline: WKY-Experimental-saline, Q-mph: WKY-Quiet-mph, Ex-mph: WKY-Experimental-mph.

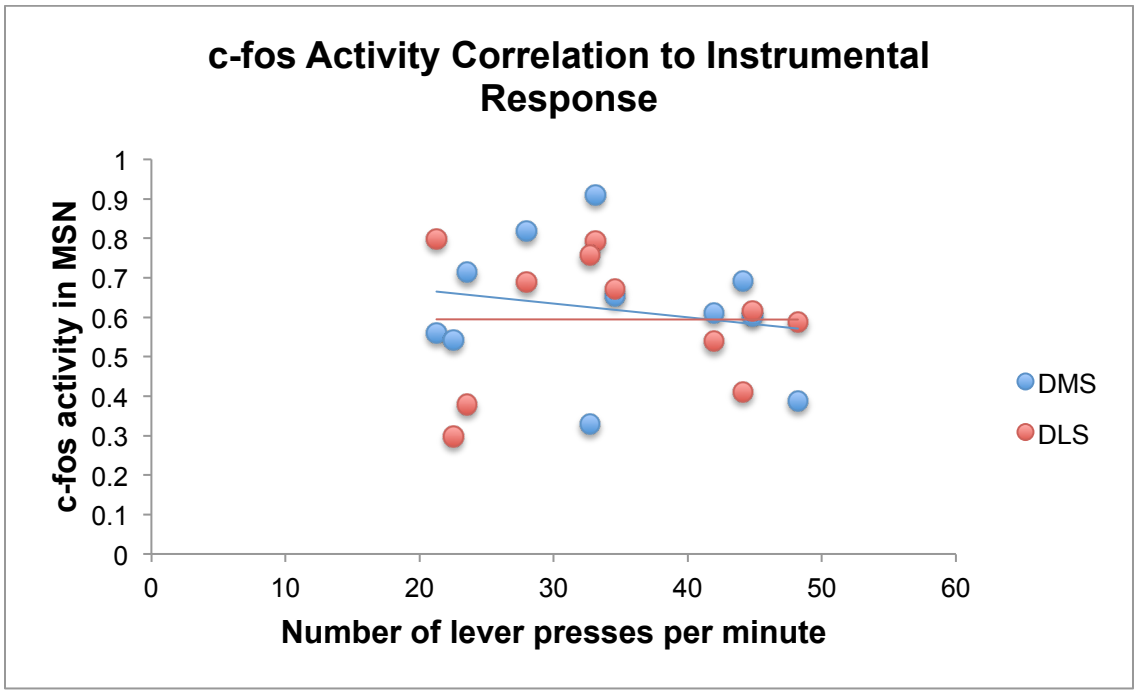
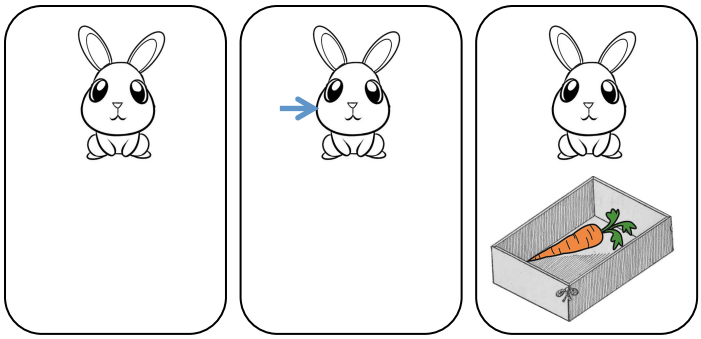


Figure 4.10 Average c-fos activity in medium spiny neurons (MSN) in the anterior, middle and posterior dorsomedial (DMS) and dorsolateral (DLS) striatum in experimental rats in correlation with lever response rate during the last instrumental training session. **N=11**.

CHAPTER 5

5.1.a
Acquisition
Devaluation



5.1.b
Choice test

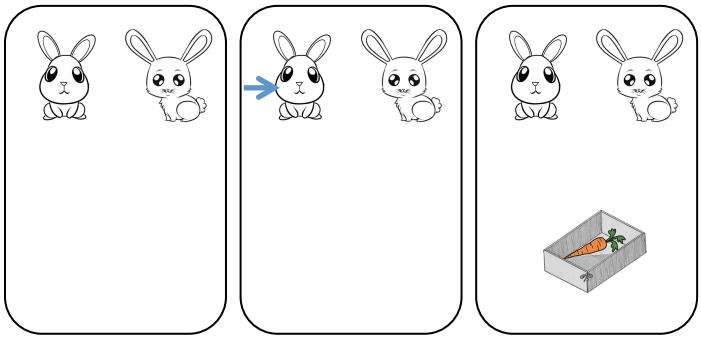


Figure 5.1 Illustration of the computer screen during acquisition and devaluation phases (5.1.a) and choice test (5.1.b). Blue arrow represents subjects' action, touching the bunny's face.

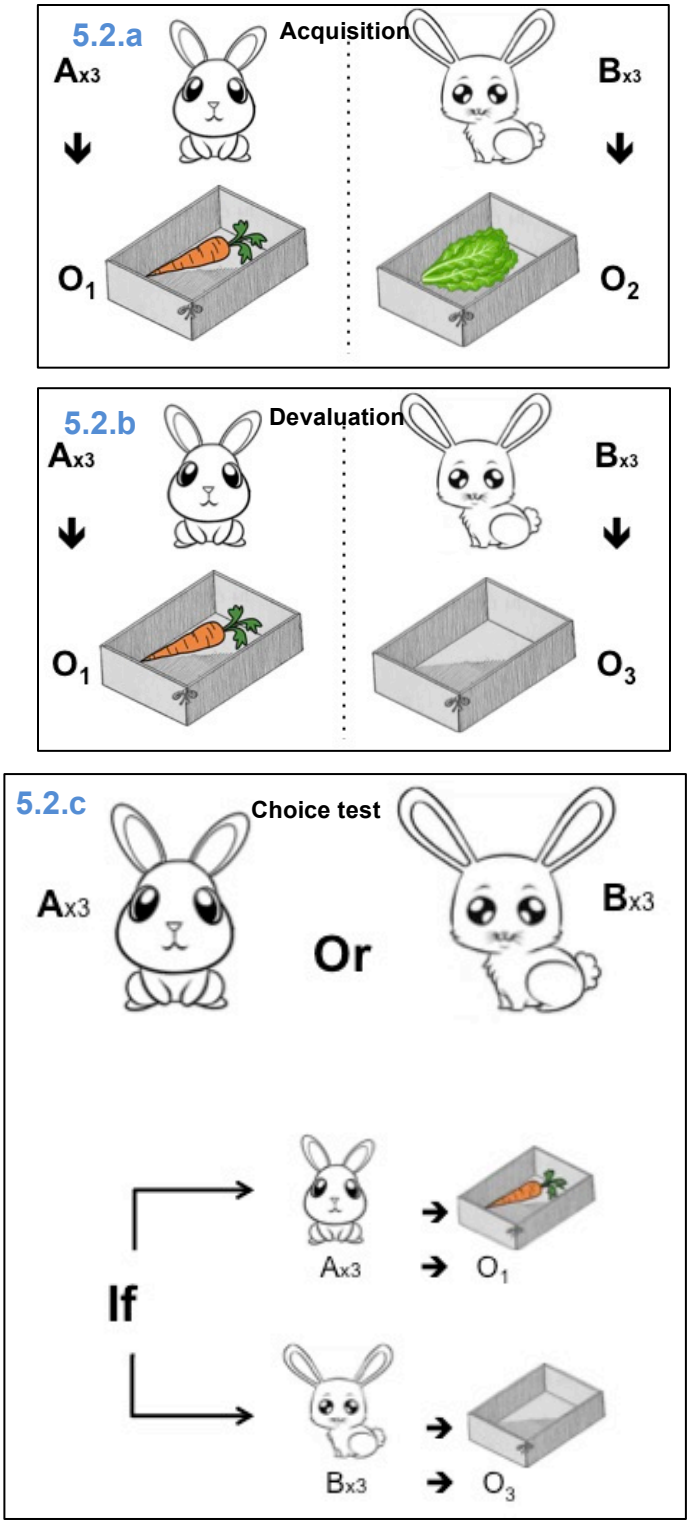


Figure 5.2 Illustration of the computer based task testing action control in patients with ADHD. A: group A of stimuli. B: group B of stimuli. S: stimulus. O1: carrot lunch box. O2: lettuce lunch box. O3: empty lunch box
5.2.a: Acquisition phase. 52.b: Devaluation phase. 5.2.c: Choice test.

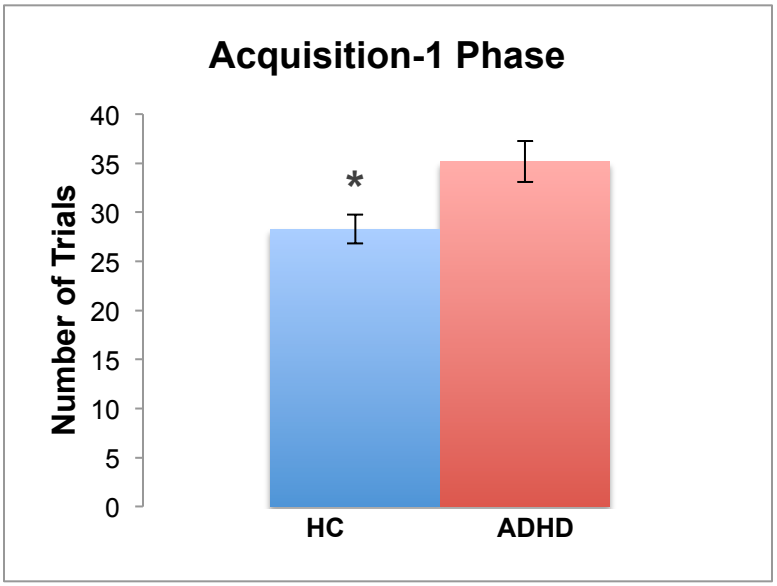


Figure 5.3 Mean number of trials that were needed to learn action-outcome associations in the first phase of the computer-based cognitive task; the Aqcuisition-1 phase, in patients with ADHD (N=19) and HC subjects (N=21) (error bars = ±SEM**)** (* = significant at $p<0.05$).

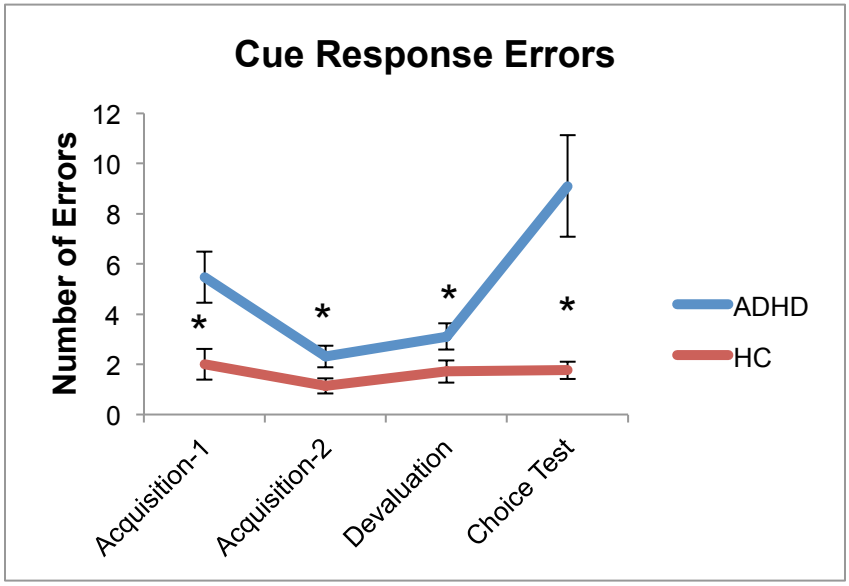


Figure 5.4 Number of errors during the four phases of the computer-based cognitive task showed as the mean number of errors per phase in patients with ADHD (N=19) and HC subjects (N=21) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

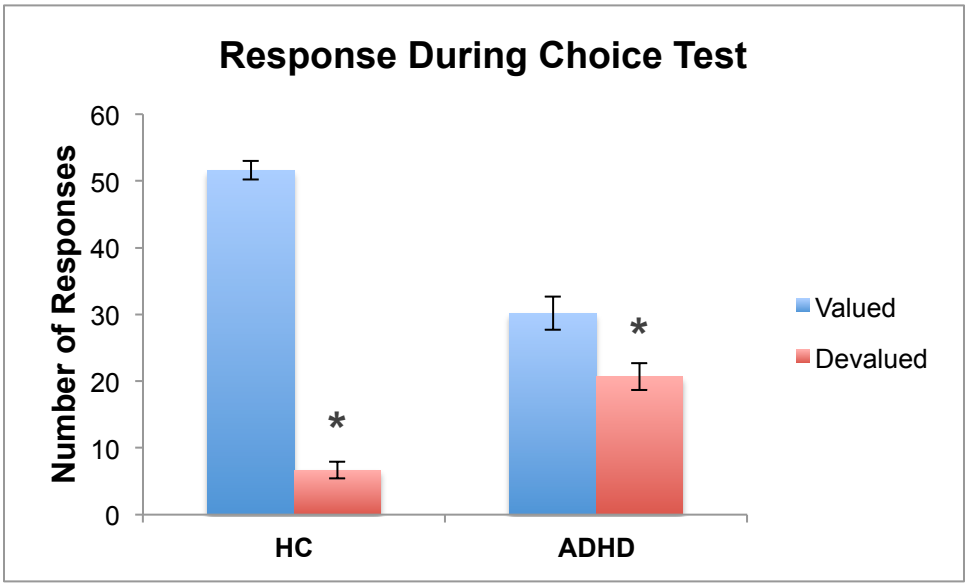


Figure 5.5 Choice test. Number of responses during the extinction test phase of the computer-based cognitive task choosing the valued (bunnies that are associated with a carrot lunch box) and the devalued (bunnies that are associated with an empty lunch box) outcomes in patients with ADHD (N=19) and HC subjects (N=21) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

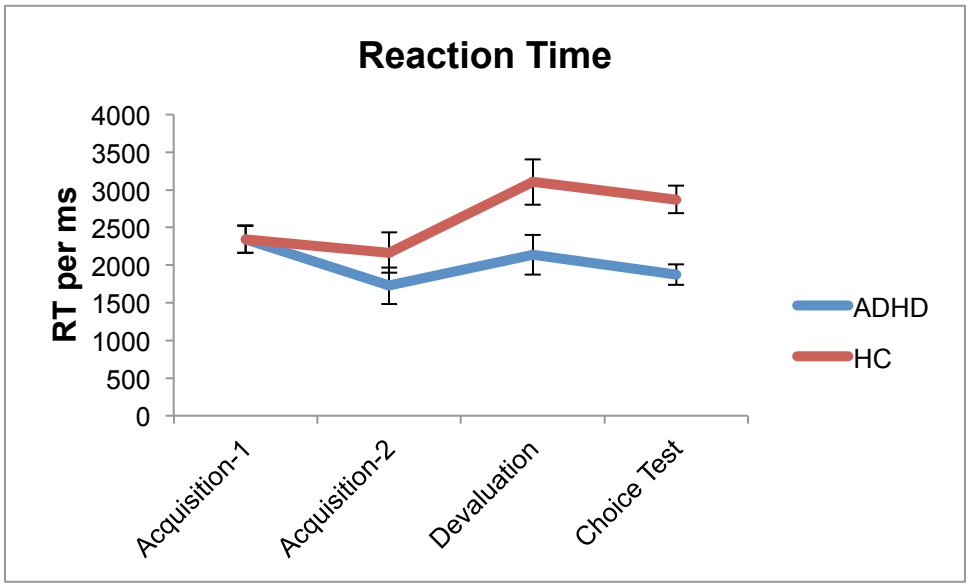


Figure 5.6 Reaction time during the four phases of the computer-based cognitive task showed as the mean number of RT per phase in patients with ADHD (N=19) and HC subjects (N=21) (error bars = ±SEM**)** (* = significant at $p<0.05$)**.**

CHAPTER 6

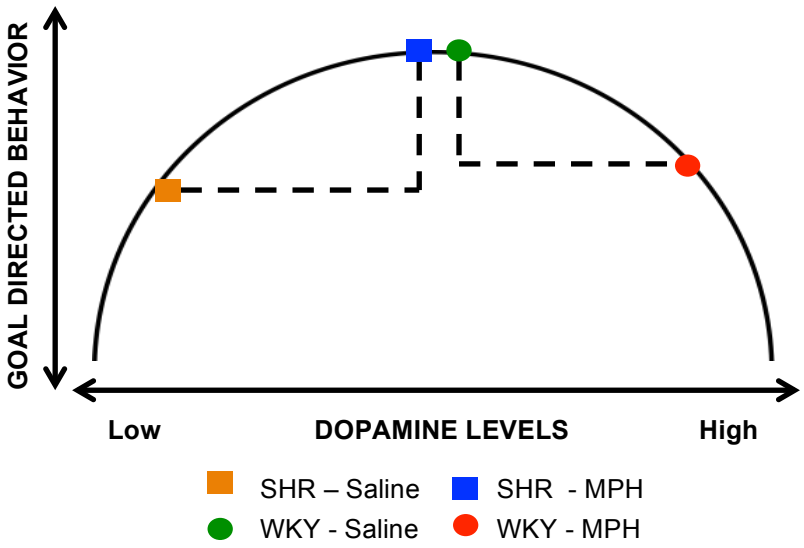


Figure 6.1 Inverted U-shape function of goal-directed behavior in SHR and WKY rats under the effect of MPH.

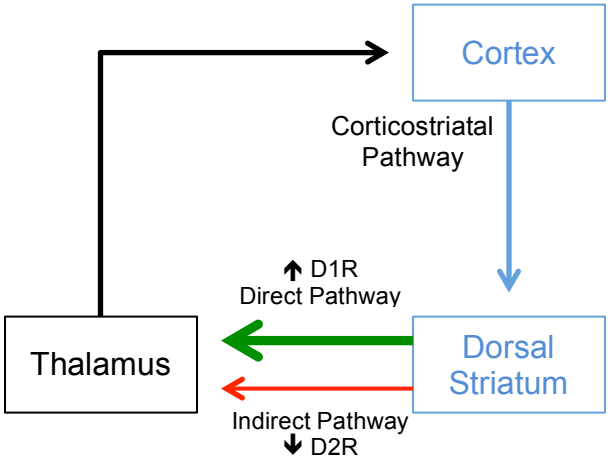


Figure 6.2 Illustration of misbalanced activation of D1R and D2R that might underlie a deficit in goal-directed behavior in ADHD. Green arrow: hyper-activation of the direct pathway). Red arrow: hypo-inhibition of the indirect pathway.