MOTIVATION IS DEFICIENT IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR), A RODENT MODEL OF ADHD: EVIDENCE FROM AN OPERANT BREAKPOINT PARADIGM

by

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ABSTRACT

MOTIVATION IS DEFICIENT IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR), A RODENT MODEL OF ADHD: EVIDENCE FROM AN OPERANT CHAMBER PARADIGM

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Attention Deficit Hyperactivity Disorder (ADHD) is one of the most commonly diagnosed childhood neurobehavioral disorders. ADHD is characterized by three core behavioral deficits (hyperactivity, inattention, and impulsivity) that significantly hinder the daily functioning of those diagnosed. Furthermore, children with ADHD have problems with motivation and often require larger, more frequent rewards in order to complete a task. In this study, we used the Spontaneously Hypertensive Rat (SHR), a rodent model of ADHD that exhibits all the core deficits of the disorder. The goal of the current study was to further validate the SHR as a model of ADHD by training rats in an operant conditioning breakpoint paradigm which is commonly used to assess motivation. Twelve male SHR and 12 male Wistar Kyoto (WKY) control rats were trained on a Progressive Ratio schedule that increased in difficulty until the rats reached their breakpoint, which was defined as the point at which the animals stopped working. The breakpoint served as a measure of motivation and the higher the breakpoint, the more motivated the animal was. Results show that the SHR animals had a significantly lower breakpoint compared to the control animals, indicating that the SHR animals gave up working on the task much sooner. While the etiology of the disorder is largely unknown, we do know that various areas of the brain, including the cerebellum, have abnormalities and warrants further investigation. In the current study, the dentate nucleus, one the three deep nuclei of the

cerebellum, was examined as it has previously been shown to have a role in motivational behavior. Findings indicate that the dentate nucleus volume was smaller in the SHR animals compared to the same structure in WKY rats. It is proposed that the smaller dentate nucleus in SHR rats may contribute to the motivational deficits expressed in these animals.

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LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
CPC	Corticopontocerebellar
CTC	Cerebellothalamocortical
DTI	Diffusor tensor imaging
EPM	Elevated plus maze
FA	Fractional anisotropy
FR	Fixed ratio
fMRI	Functional magnetic resonance imaging
HSV1	Herpes simplex virus type 1
IACUC	Institutional Animal Care and Use Committee
PET	Positron emission tomography
PFC	Prefrontal cortex
PR	Progressive ratio
ROI	Region of interest
SHR	Spontaneously hypertensive rat
WKY	Wistar Kyoto

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most commonly diagnosed childhood neurobehavioral disorders. The worldwide prevalence of children with ADHD is estimated at 5.3% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007) and in the United States, diagnostic rates range from 5-8% (Pliszka et al., 2007; Willcutt, 2012). This disorder is not restricted to childhood years as it has been reported that a majority (60-85%) of children diagnosed with ADHD will continue to show symptoms throughout the teenage years (Barkley, Fischer, Smallish, & Fletcher, 2002). Furthermore, more than 50% of children with ADHD will continue to meet criteria for this disorder throughout adulthood (Fischer, Barkley, Smallish, & Fletcher, 2002; Weiss, Hechtman, Milroy, & Perlman, 1985; Wilens, Faraone, & Biederman, 2004) and it is estimated to affect roughly 4.4% of the adult population (Faraone & Biederman, 2005). ADHD typically presents before the age of seven years (American Psychiatric Association, 2013), affects more boys than girls (Arnold, 1996; Hartung & Widiger, 1998; Ramtekkar, Reiersen, Todorov, & Todd, 2010) and is a highly pervasive disorder characterized by three core deficits- inattention, hyperactivity, and impulsivity. All aspects of this disorder significantly hinder performance of daily functioning in school, work, relationships, and personal well-being (American Academy of Pediatrics, 2000; American Psychiatric Association, 2013; National Institute of Mental Health, 2016a). Once diagnosed, combinations of behavioral and pharmacological (stimulant) interventions have been found to significantly alleviate symptoms and promote more normal behavioral function (Kratochvil et al., 2009, MTA Cooperative Group, 1999).

Although the etiology of ADHD is still unclear, family, twin, and adoption studies indicate that genetics strongly influence susceptibility with an estimated heritability of 70-80%

(Faraone et al., 2005; Franke et al., 2012; Sagvolden, Johansen, Aase, & Russell, 2005). Furthermore, ADHD diagnoses tend to aggregate in families within and across generations (Cantwell, 1972; Epstein et al., 2000; Morrison & Stewart, 1971). While it is most likely an interplay of several genes, some of the primary candidate genes extensively studied include those that code for catecholamines, such as *DAT1*, *DRD4*, *DRD5,* and serotonin-related genes like *5HTT* and *SNAP25* (Gizer, Ficks, & Waldman, 2009; Khan & Faraone, 2006). Genetics aside, additional evidence exists for the association between ADHD and brain structure, function, and neurotransmitter anomalies in various regions of the brain, including the prefrontal cortex (PFC) and cerebellum. In order to further the study of this disruptive and pervasive neurobehavioral disorder, a valid and incorporative animal model of ADHD is needed. While many exist, the Spontaneously Hypertensive Rat (SHR) is currently the best and most commonly used animal model in ADHD research (Russell, Sagvolden, & Johansen, 2005). In addition to the SHR exhibiting behaviors similar to the core symptoms of ADHD (inattention, impulsivity, and hyperactivity), it also portrays many neurobiological hallmarks comparable to humans with ADHD. While the SHR model appears to express the three core symptoms of ADHD, it is the purpose of the current study to add to the validation of the SHR model by determining whether SHR rats display additional symptoms, such as motivational deficiencies, which are commonly described in the human ADHD literature.

Literature Review

Brain Alterations in ADHD

A number of neuroimaging studies have examined the anatomical brain differences between those with and without ADHD. It has consistently been reported that those with ADHD have a significant global reduction in brain volume, around a 3-4% decrease, compared to

controls (Castellanos et al., 2002; Durston et al., 2004; Filipek et al., 1997; Hoogman et al., 2017). Other studies have reported specific reductions in white matter volume, corpus callosum area, and cortical thickness in ADHD (Castellanos et al., 2002; Krain & Castellanos, 2006). Diffusion Tensor Imaging (DTI) studies have confirmed that white matter abnormalities exist in various areas such as the corpus callosum (D'Agati, Casarelli, Pitzianti, & Pasini, 2010), fronto-temporal cortex (Silk, Vance, Rinehart, Bradshaw, & Cunnington, 2009), and premotor and striatal regions (Ashtari et al., 2005) in the brains of those with ADHD. This may reflect a decrease in myelination which could cause a reduction in the speed of neural communication between affected areas. Looking at brain-behavior relationships, it has been observed that there are clear and distinct morphological changes in brain areas, particularly in the PFC, that are known to be responsible for attention, executive functions, and inhibitory control, all of which are deficient in ADHD (Curatolo, D'Agati, & Moavero, 2010).

The PFC guides executive functions which include, but are not limited to, attention, working memory, planning, emotion, and impulse control (Arnsten & Li, 2005) and these functions critically depend on a sufficient catecholamine supply. Brozoski, Brown, and Goldman (1979) reported that depletion of dopamine and norepinephrine from the PFC in rhesus monkeys resulted in a significant reduction of correct responses on a delayed spatial alternation task. The reduction was so severe that performance levels approached those observed in the group of animals who received complete surgical removal of the PFC. In ADHD patients, the stimulant medications that are prescribed to treat ADHD symptoms have been found to have a profound effect on catecholamine neurotransmitter availability within the brain, either through the enhancement of the release or the inhibition of the reuptake of necessary catecholamines (Brennan & Arnston, 2008; Wilens, 2008). Therefore, ADHD symptomology may be due to the

disruption of catecholamine signaling systems, and this may be genetic, especially in the genes coding for dopamine receptors and transporters (Faraone et al., 2005; Faraone & Mick, 2010).

There is surmounting evidence that ADHD is associated with impairment in catecholamine neurotransmission, particularly in the PFC (Arnsten, 2006; 2009; Brennan & Arnsten, 2008). It has been reported that in the brains of those with ADHD, certain dopaminergic neural pathways mature at a slower rate and that the functioning of these neurons and their projection areas is compromised (Castellanos, 1997; Castellanos & Tannock, 2002). Further studies reveal that dopamine receptors and transporter proteins are significantly lower in the fronto-subcortical circuits (prefrontal cortex, anterior cingulate cortex, caudate, and putamen) in children with ADHD and this may underlie the symptoms of inattention and impulsivity (Curatolo, D'Agati, & Moavero, 2010).

With the advancement of imaging techniques such as Functional Magnetic Resonance Imaging (fMRI), researchers have found that those with ADHD tend to have abnormal resting-state baselines (Lou, Henriksen, & Bruhn, 1990; Yu-Feng et al., 2007) and hyper and hypo-activation in various brain regions (Cortese et al., 2012). In ADHD, under-activation occurs almost exclusively in the fronto-parietal region, which again is involved in executive functions, whereas over-activation is found in the default mode, visual, and dorsal attentional networks (Cortese et al., 2012). Interestingly, while examining working memory in adults with ADHD, Wolf and colleagues (2009) discovered that there was a reduced activation between the prefrontal cortex and the cerebellum compared to controls. In addition, it has been reported that during a variety of attentional tasks performed in conjunction with fMRI, those with ADHD have reduced activation and functional connectivity between fronto-striato-parieto-cerebellar areas. Furthermore, methylphenidate, the most commonly prescribed ADHD medication, normalized

the activation state of this network compared to healthy controls and improved performance on these attentional tasks in those with ADHD (Rubia et al., 2009). This link between prefrontal areas and the cerebellum has been of particular interest in ADHD research.

Cerebellum Connections

There is detailed evidence that the cerebellum is connected, through closed-loop circuits, to various non-motor forebrain regions, including those associated with higher order cognitive functions, in both rat and primate (Middleton & Strick, 1994; Schmahmann, 1996; 1997). Although many pathways exist, two of the most investigated circuits include the corticopontocerebellar (CPC) and cerebellothalamocortical (CTC) pathways, both of which include feedforward and feedback limbs, creating closed-looped pathways between the cerebral cortex and the cerebellum. The first pathway, the CPC, includes projections from the cerebral cortex (sensorimotor, paralimbic, and associative regions) that reach the pontine nuclei in the pons, and then a ponto-cerebellar pathway conveys information to the cerebellar cortex. The cerebellum cortical projections then make contact with the deep cerebellar nuclei (dentate, interpositus, and fastigial) (Andreasen, Paradiso, & O'Leary, 1998). This pathway is closed-looped and information can flow both ways. This connectivity provides evidence that the cerebellum can exert influence over prefrontal areas (Palesi et al., 2017; Schmahmann & Pandyat, 1997; Zemanick, Strick, & Dix, 1991).

Furthermore, the dentate or lateral nuclei of the cerebellum, which are the largest of the deep nuclei, are heavily connected to multiple regions throughout the cerebral cortex (Chan-Palay, 1997). The dentate nucleus contains various types of cells, including large neurons, small neurons, and neuroglia. Output from the cells within the cerebellum, mainly originating from the dentate nucleus, passes through the superior cerebellar peduncle, decussates in the rostral

midbrain, and projects via the ventrolateral thalamus to various brain areas, including prefrontal regions, thus creating the cerebellothalamocortical (CTC) pathway (Andreasen, Paradiso, & O'Leary, 1998).

There is accumulating evidence, in both animal and human literature, that the dentate nucleus is topographically organized, is comprised of motor and nonmotor regions, and that it contains several output channels that are directed at different areas of the prefrontal cortex. For example, injections of retrograde trans-neuronal transport of herpes simplex virus type 1 (HSV1) into motor areas such as M1, ventral premotor area, and dorsal premotor area were found to be traced back to the more dorsal regions of the dentate nucleus (Middleton & Strick, 2001). HSV1 injections in the frontal eye fields labeled neurons in the more caudal and lateral regions of the dentate (Hoover & Strick, 1999; Lynch, Hoover, & Strick, 1994; Zemanick, Strick, & Dix, 1991). These results indicated that cerebellar projections to the prefrontal, oculomotor, and skeleto-motor areas of the cortex all appear to be derived from distinct regions of the dentate nucleus.

In contrast, the nonmotor domain of the dentate nucleus is in the ventral portion and it contains output channels that are associated with aspects of cognition and visuospatial functions. Furthermore, within the ventral region of the dentate nucleus, the neurons that project to the prefrontal and posterior parietal areas are clustered together into distinct and non-overlapping regions (Dum & Strick, 2003). It is important to note that neurons in this ventral region of the dentate nucleus seldomly respond to activity related to limb movements (Schieber & Thach, 1985; Wetts, Kalaska, & Smith, 1985). More than 20% of the volume of the ventral dentate nucleus is occupied by output channels that project to areas 9, 46, and 7 of the prefrontal cortex (Clower, West, Lynch, & Strick, 2001; Middleton & Strick, 1994; 1997; 2001). These areas have

been shown to be involved in cognitive functions, such as attentional mechanisms and working memory, and in visuospatial functions, such as the creation of extra-personal space maps and recalibration of eye-hand coordination. These seminal findings that the cerebellum connects to areas involved in cognition defied the long-standing theory that the cerebellum was only involved in motor processes (Middleton & Strick, 1994).

In humans, Kim, Ugurbil, and Strick (1994) examined fMRI activation of the dentate nucleus during the Insanity Task and the Visually Guided Task. They found that subjects displayed a large ventral activation of the dentate nuclei while attempting to solve the Insanity Task, a task that required skilled movement of colored pegs conforming to a set of rules (involves cognition and motor movement). This increase was three to four times larger than the activation of the dentate nuclei during the Visually Guided Task, which just required subjects to move colored pegs from one side of a board to the other (involves just motor movement). It was found that these two tasks activated distinctly different portions of the dentate nuclei suggesting that regions of the dentate nuclei involved in cognitive processing are distinct from those involved in movement of limbs and eyes. Jueptner and colleagues (1997a; 1997b) reported that during a task that required learning new sequences of finger movement, there was significant activation in ventrolateral portions of the dentate nuclei, as well portions of areas 9 and 46.

Given that the cerebellum is heavily connected to prefrontal regions through the dentate nucleus, one would expect that global damage to the dentate nucleus would also have damaging effects on those prefrontal areas it projects to. Recently, Bauer, Peterson, & Swain (2013) disrupted output connections from the cerebellum by lesioning the dentate nucleus. One month after the lesion, animals were sacrificed, and the microstructure of the prefrontal cortex was examined. In response to the lesion, the proportion of long to short dendrites was decreased. This

finding indicated that damage to the cerebellum, particularly the dentate nucleus, and thus cerebellar output, resulted in synaptic alterations in prefrontal areas. In addition, given the fact that the ventral portion of the dentate nucleus is involved in cognition, one might predict that damage to this area, or regions that innervate it, may result in cognitive deficits. In humans, damage to the lateral portion of cerebellar cortex resulted in impaired performance on rule-based practice learning tasks (Fiez, Petersen, Cheny, & Raichle, 1992) and on the Tower of Hanoi, a task requiring cognitive planning (Grafman et al., 1992). As such, projections from the dentate nucleus have been implicated in cognitive processes and have an influence on prefrontal functioning separate from motor tasks. Damage to the dentate nucleus disrupts the activity of the CTC pathway, and cognitive deficits may be the result (Kelly & Strick, 2003) Because of these findings, some ADHD researchers have focused on the cerebellum and findings indicate that abnormalities in this structure may be one of the etiologic causes of the disorder.

Cerebellum Abnormalities in ADHD

It has been proposed that the deficits and symptoms of ADHD are attributable to a disruption or a poor connection in the pathways that connect prefrontal, and other parieto-occipital areas, to the cerebellum (Silk et al., 2009). Previously thought of as strictly a motor region, it is important to acknowledge that the structure and connectivity of the cerebellum has been found to be altered and correlates with abnormal non-motor processes in those with ADHD. It has been reported that there is an overall reduction in total cerebellar volume (Castellanos et al., 1996; Durston et al, 2004; Valera, Faraone, Murray, & Seidman, 2007). In fact, some report almost a four to six percent decrease in total cerebellar volume when compared to controls (Berquin et al., 1998; Castellanos et al., 2001). More specifically, multiple studies have found that the posterior-inferior cerebellar vermis (lobules VIII-X) have significantly less volume

(around 2%) in both males and females with ADHD (Berquin et al., 1998; Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002; Castellanos et al., 2001; Hill et al., 2003; Mostofsky, Reiss, Lockhart, & Denckla, 1998). Furthermore, this cerebellar total volume and specific vermal volume loss has been shown to significantly correlate with parent and teacher ratings of ADHD symptom severity and clinical outcomes (Castellanos et al., 2002; Mackie et al., 2007).

Using DTI, Ashtari and colleagues (2005) examined the white matter integrity in the cerebellum and reported that those with ADHD had decreased fractional anisotropy (FA) in the left cerebellar peduncle and left cerebellar hemisphere. A low FA value is an indication of decreased myelination, which may hinder the functionality of the CPC or CTC pathways, thus hampering the cross-talk between cerebellum and cerebral cortex. The authors suggest that the low FA in the left middle cerebellar peduncle might represent the point of entry of the fibers from the ponto-cerebellar pathway into the cerebellum. The left cerebellar hemisphere had lower FA at the level of the dentate nucleus which may explain a deficit in the output from this region which further implicates the CTC pathway in ADHD.

The stimulant medications that are typically prescribed to subdue the symptoms of ADHD are known to have a widespread effect on the brain, including the cerebellum. The effects of methylphenidate administration on the cerebellar vermis were examined using fMRI. Methylphenidate had a dose-dependent effect on the T_2 relaxation time and paramagnetic properties of the cerebellar vermis, which presumably is the result of changes in blood flow to this area (Anderson, Polcari, Lowen, Renshaw, & Teicher, 2002). Bledsoe, Semrud-Clikeman, & Pliszka (2009) reported that chronically-medicated children with ADHD had cerebellar vermis sizes comparable to the healthy controls, suggesting that chronic stimulant treatments helps to normalize the development of the cerebellar vermis in ADHD. Moreover, it has been discovered

that stimulant medications are associated with larger regional volumes in the left hemisphere of the cerebellum and that there is an inverse relationship between symptom severity and regional vermis volume (Ivanov, Murrough, Bansal, Hao, & Peterson, 2014). The cerebellar connectivity and its structural and functional response to medications has been a popular line of ADHD research in human subjects. However, in order to further study this disorder more in depth, an ADHD animal model is needed.

The Spontaneously Hypertensive Rat (SHR)

The Spontaneously Hypertensive Rat (SHR) is one of the most widely studied and accepted animal models of ADHD. SHRs were originally created as an animal model of hypertension. Wistar Kyoto (WKY) rats with high blood pressure were selectively mated and after many repeated inbreeding sessions, the development of a strain of rats that displayed spontaneous hypertension, before 15 weeks of age, was successful (Okamoto & Aoki, 1963). Because of their genetic similarities, WKY rats are commonly used as the control animals in SHR-based studies (Russell, 2005; Sagvolden, 2000; Sagvolden et al., 2009). Adult SHRs are typically used in cardiovascular research; however, by happenstance, it was found that juvenile SHRs, around three to four weeks of age, begin to demonstrate hallmark features of ADHD prior to developing hypertension (Christiansen, Roald, Tenstad, & Iversen, 2002). As the most investigated model of ADHD, the SHR has been shown to fulfil most aspects of face, construct, and predictive validity (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005).

Face validity pertains to whether something “looks valid”. In other words, does the model appear to measure what it claims to measure? Face validity is achieved if the animal model essentially mimics the human behavioral-clinical characteristics of ADHD that are typically observed in humans diagnosed with the disorder, namely hyperactivity, impulsivity, and

inattention (Berger & Sagvolden, 1998; Sagvolden, 2000; Sagvolden et al., 1992; Sagvolden, Pettersen, & Larsen, 1993). In addition, SHR behavior is variable and includes response-reengagement deficits similar to human children with ADHD (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005).

A valid ADHD animal model must also display construct validity, which requires that the model sufficiently represents of the construct of interest. With ADHD as the construct, the model should portray the pathophysiological, behavioral, and biochemical sequelae of ADHD. It is important to note that construct validity is difficult to fully obtain because many theories of the etiology of ADHD exist. Criteria that are considered for construct validity include neuropathology, neurotransmitter dysfunction, sex differences, genetics, and medication effects that are similar to those found in humans with ADHD (Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). Regarding neuropathology, the brains of SHRs show similar structural and functional abnormalities as humans, such as smaller overall brain volume, smaller cerebellar vermis, reduced cortical thickness, a reduction in frontal lobe activity, and an abnormal caudate nucleus (Li et al., 2007; Tajima et al., 1993). SHRs also display neurotransmitter anomalies as hypo-functionality of their dopamine and norepinephrine systems have been reported (Russell, 2003; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995; Sagvolden, Johansen, Aase, & Russell, 2005). When examining gender and ADHD, just like humans, both male and female SHRs display differential behavioral and learning disparities (Berger & Sagvolden, 1998; Bucci, Hopkins, Keene, Sharma, & Orr, 2008; Sagvolden & Berger, 1996). Genetically, SHRs show DNA sequence changes in dopaminergic genes compared to the WKY control strain (Mill, Sagvolden, & Asherson, 2005; Sagvolden, 2000). Finally, it has been shown that SHR motor activity and impulsiveness is reduced in response to ADHD medications, which increase

dopamine availability (Sagvolden, 2006; Sagvolden et al., 1992; Sagvolden & Xu, 2008). While the SHR exhibits features of ADHD, we cannot conclude the validity of SHR as a model of ADHD based solely on construct validity.

Finally, the predictive validity of a model is defined as the ability to provide information not previously known about the behavior, genetics, and neurobiology of ADHD, and to predict future events associated with ADHD. The SHR model of ADHD has provided us with further knowledge in areas of the behavioral, etiological, and pharmacological aspects. However, the limitation of the predictive validity of this model is the fact that SHRs develop hypertension, which becomes a potential confound in the interpretation of results (van den Bergh et al., 2006). For this reason, this model has a small-time frame of valid use, from three weeks to 10 weeks of age, before hypertension becomes a confound (De Jong, Linthorst, & Versteeg, 1995; Russell, Sagvolden, Johansen, 2005). While this animal displays the three core symptoms of ADHD, it has not yet been determined if SHRs also exhibit deficiencies of motivation, a behavioral hallmark that is commonly displayed in humans with ADHD. Furthermore, the cerebellum, now known to have non-motor roles, has also been suggested to be involved in motivational processes, but its exact role in motivation has yet to be analyzed in the SHR. Without validation of the presentation of this symptom and its neurological basis, the SHR lacks in face and construct validity, thus decreasing the overall strength of the SHR as a good model of ADHD.

Motivation

The complex concept of "motivation" has been discussed for centuries, dating back to Plato, and its definition is widely disputed (Cooper, 1984; Stellar & Stellar, 1985). Broadly stated, a motive is an impulse/need/desire that causes a person to act (Motive, n.d.). Motivation, on the other hand, is an internal process that guides behavior toward a specific goal. In humans,

motivation is demonstrated by actions and/or decisions (Elliot & Covington, 2001). In animals, motivation is defined as the amount of effort an animal is willing to extend to receive reinforcement (Hodos, 1961; Skjoldager, Pierre, & Mittleman, 1993).

The neurotransmitter, dopamine, plays a role in motivational processes (Ikemoto, Yang, & Tan, 2015). While there are multiple dopamine pathways, the main pathway is the mesolimbic pathway which connects the ventral tegmental area (VTA) in the midbrain to the nucleus accumbens (NAcc) in the ventral striatum. Under normal conditions, this pathway is sensitive to natural rewards, such as food, sex, and social interactions, thus its proper functioning is an important determinant of motivation and sensitivity to incentives (Wise, 2002). Dysfunction in this pathway affects dopamine transmission and affects the response requirements of a task which may bias an animal's behavior towards lower effort alternatives (Salamone et al., 2016) and reduces operant responding for food (Wise, 2004).

ADHD, typically characterized by symptoms of inattention, hyperactivity, and impulsivity, is now thought to also include deficiencies with motivation. Thirty years ago, a hypothesis surfaced that ADHD is a result of the dysfunction of the pathways involved in reward and motivation (Haenlein & Caul, 1987) and there is increasing evidence that this dysfunction does indeed play a role in ADHD (Johansen et al., 2009). Children with ADHD tend to need stronger and more appealing rewards in order to modify their behaviors compared to those without ADHD (Kollins, Lane, & Shapiro, 1997). Furthermore, those with this disorder show impaired responses to schedules of reinforcement, a preference for a smaller, sooner reward over a larger, delayed one (Antrop et al., 2006), and show a failure to delay gratification (Freibergs & Douglas, 1969; Parry & Douglas, 1983)

Multiple studies have provided evidence that disruption of dopamine reward pathways may underlie the motivational deficits seen in ADHD (Plichta et al., 2009; Scheres, Milham, Knutson & Castellanos, 2007; Sonuga-Barke, 2003; Tripp & Wickens, 2008). Volkow and colleagues (2011), using PET imaging, found that within the reward/motivation pathway of the brain, humans with ADHD, compared to those without, have less dopamine, as well as a less dopamine receptors, both of which could explain these deficits. Furthermore, a positive correlation between dopamine availability and scores on a motivation level assessment was found in those with ADHD but not in the controls.

In attempts to understand which brain regions are associated with motivation, researchers have used non-human animal models to ablate or inactivate specific areas of the brain to determine their function in this process. The non-motor roles of the cerebellum have been of interest and there is increasing evidence that the cerebellum is also related to and involved in the regulation of motivation. Bauer, Kerr, & Swain (2011) reported that lesions of the dentate nucleus in rats significantly reduced breaking points compared to sham controls, indicating that the animals had decreased willingness to physically work, i.e, lever pressing, for reinforcement. In addition, dentate-lesioned animals displayed significantly fewer exploratory behaviors in an open field paradigm. These results indicate that the cerebellum is important in tasks that involve motivation and that the disruption of cerebellar output can lead to deficits in other forms of cognition apart from motor learning. Another study found that after the temporary deactivation of the dentate nucleus using bupivacaine, animals were not as willing to climb ever-increasing barriers to receive a food reward compared to the baseline trials in which they received saline. Essentially, with suppression of the activity of the dentate nuclei, rats found the amount of effort required to climb a high barrier was not worth the reinforcement; thus demonstrating a decrease

in motivational behaviors and reaching a breakpoint much sooner than control conditions (Peterson et al., 2012). From these studies, it was concluded that cerebellar damage in rodents reduces motivational behavior. In humans, focal cerebellar lesions result in significantly impaired reward-based reversal learning (Thoma, Bellebaum, Koch, Schwarz, & Daum, 2008). Children with autism were found to exhibit significantly less explorative behavior, indicative of motivation, compared to non-autistic children and it was correlated to the hypoplasia in the cerebellar vermis (Pierce & Courchesne, 2001). Together, these experiments lend to the understanding that the cerebellum has more than just a motor-based role, but in fact, the abnormities of the cerebellum in ADHD could explain why deficient motivation is a symptom of this disorder.

Purpose

Currently the SHR is one of the most validated models of ADHD as it displays the core behavioral symptoms (hyperactivity, impulsivity, attentional deficits), conforms to a theoretical rationale of ADHD, and has several structural, functional, genetic, and biochemical changes that are also observed in humans. To our knowledge, no study has attempted to investigate if the SHR model displays motivational problems like those seen in humans with ADHD. Therefore, the primary behavioral goal of the present study was to determine if the SHR model of ADHD displays motivation deficiencies, compared to Wistar Kyoto (WKY) control animals, in a progressive ratio (PR) breakpoint-type experiment, which is classically used to assess motivation. A PR schedule, in which the animals' response requirement gradually increases throughout the session, measures how hard the animals are willing to work for a reward (Hodos, 1961). A secondary evaluation of behavior in the SHRs was conducted using an open field apparatus, which has been commonly used to assess purposive motivational behavior such as exploration (Caston, Chianale, Delhaye-Bouchaud, & Mariani, 1998; D'Agata, Drago, Serapide,

& Cicirata, 1993; Ferguson & Cada, 2003). To test anxiety-like behaviors, animals were subjected to a session on the elevated-plus maze (EPM), a raised apparatus consisting of four arms, two open and two enclosed. The EPM relies upon rodents' proclivity toward dark and enclosed spaces opposed to those that are elevated and open (Montgomery, 1958). Finally, because of the cerebellar connectivity to the rest of the brain, the non-motor roles this structure is known to have, such as motivation, and based off the results of previous dentate lesion and deactivation studies, the primary anatomical goal was to examine and characterize the cerebellar dentate nucleus in the SHR animals. The overall purpose of the study was to further ADHD research by adding novel behavioral data, that the SHRs display decreased levels of motivation, and anatomical data, that there are abnormalities in the SHR cerebellar dentate nuclei, to the face and construct validity of the SHR model of ADHD.

Hypotheses

Operant Chamber

The primary hypothesis in the operant chamber was that the SHRs, used as a model of ADHD, would demonstrate lower breaking points, due to decreased motivation. The assessment of the strength of animal's motivation was determined by the highest breakpoint ratio an animal would reach. Breaking points were defined as the value of the ratio (the number of lever presses required to receive a food pellet) toward which the rat was working but failed to achieve due to either reaching the end of a 1 hr training session or a 15 min period of inactivity (absence of lever pressing).

In addition, we aimed to provide evidence in support of the manifestations of the inattentive, hyperactive, and impulsive symptoms while the SHR animals were in the chamber. Regarding inattention**,** we used the amount of time the animals spent off task as a measure of

inattention to the lever-pressing task. Off-task behaviors were defined as any behavior other than lever pressing, retrieving a food pellet from the food cup, and chewing a pellet. It was hypothesized that the SHR animals would spend more time off-task compared to the WKY. Hyperactivity was assessed by measuring the number of instances that the animals engaged in off-task behavior and therefore, we hypothesized that the SHR animals would engage in more instances off off-task behaviors compared to the WKY. As a secondary measure of hyperactivity in the chamber, we examined the run rate (lever presses/min), which was used as a measure of bar-pressing frequency (Bauer, Kerr, & Swain, 2011). It was predicted that the SHR animals would display increased run rates compared to the WKY. Impulsivity was measured by examining the pre-ratio pause defined as the amount of time after receipt of a reward and prior to the start of a ratio for subsequent rewards (the first lever depression in a ratio). We hypothesized that the SHR animals would demonstrate shorter pre-ratio pauses compared to the WKY.

Open Field

As a secondary behavioral measure, all animals were exposed to the open field apparatus. Previous studies have reported that in a free-exploration open field, the SHR animals ambulated more (Sagvolden, Hendley, & Knardahl, 1992; Sagvolden, Pettersen, & Larsen, 1993). As a measure of hyperactivity, it was therefore hypothesized that the SHR animals would cross more grid squares overall compared to the WKY. We further hypothesized that the SHR animals would exhibit non-purposeful explorative behavior by spending significantly less time in the inner squares of the open field compared to the WKY. Finally, we hypothesized that there would be no difference in the amount of rears, grooms, defecations, or urinations.

Elevated Plus Maze

The elevated plus maze (EPM) was used as an assessment of anxiety-related behaviors. It was hypothesized that there would be no differences in the number of open arm entries, total time spent in open vs. closed arms, time spent in distal portion of open arms, or time spent in center of maze between the SHR and WKY groups. However, entries into closed arms were to be used as measure of locomotion. It was hypothesized that SHRs will exhibit more entries into the closed arms due the increased hyperactivity of these animals.

Cerebellum Histology

Because of the hypothesized behavioral differences between the SHR and WKY groups and the role of the dentate nucleus in motivation-related behavior, the primary histological hypothesis was that the volume of the dentate nucleus would be significantly smaller in the SHR group compared to the control WKYs. To control for any training-related changes in the dentate nuclear volume, naïve animals were included in the analyses. It was also hypothesized that the volume of the dentate nucleus in the training-naïve animals would be smaller than that of trained, but that the volume in the SHR would remain smaller than WKY regardless of training condition. It was hypothesized that the SHR animals would also exhibit significantly fewer neurons in the dentate nucleus compared to the WKY controls and that this finding would underlie any observed changes in cerebellar dentate nucleus volume.

Humans with ADHD and SHR animals have a significantly smaller cerebellar vermis compared to neuro-typical controls and this has been reported several times (Berquin et al., 1998; Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002; Castellanos et al., 2001; Hill et al., 2003; Li et al., 2007; Mostofsky, Reiss, Lockhart, & Denckla, 1998). Therefore, the cerebellar vermis was also examined in this study. It was hypothesized that the finding would remain consistent

with the literature and that the volume of the cerebellar vermis in the SHR animals would be significantly smaller than the WKY control animals.

Purkinje cells, which are large neurons with many branching extensions, are found in the cortex of the cerebellum between the granule and molecular layer. The axons of the Purkinje cells project to the deep cerebellar nuclei, including the dentate nucleus, and inhibit the activity of these structures. Purkinje cells are the sole output source of the cerebellar cortex (Purves et al., 2004). Dysfunction or lack of Purkinje cells has been shown to play a role in various disorders, including ADHD (Fatemi et al., 2002; Fukutani, Cairns, Rosser, & Lantos, 1996; Xia et al., 2013). Purkinje cells were investigated in this study and it was hypothesized that the SHR animals would exhibit fewer Purkinje cells in the cerebellar vermis lending to the overall smaller volume of the cerebellar vermis.

Methods

Subjects

Young (postnatal day 21), male, Spontaneously Hypertensive Rats (SHRs) (N=27) and Wistar Kyotos (WKY) (N=27) were obtained from Charles River Laboratories (Wilmington, MA). All animals were coded upon arrival to ensure blind treatment of animals. Six of the animals served as free-feeding controls and were not used in analysis, therefore bringing the sample size to 24 for both the SHR and WKY. The final breakdown of animals was as follows: Experimental SHR: N=12, Experimental WKY: N=12, Control SHR: N=12, Control WKY: N=12. Upon arrival, animals were placed in pairs (1 WKY and 1 SHR) into individual standard shoebox cages and given one week to acclimate. During this period, all rats were allowed free-access to food and water in order to establish a free-feeding baseline weight. Animals were weighed and handled daily during this acclimation week and throughout the experimental

procedure. Animals were tested in three separate cohorts. All animal care and experimental procedures were overseen and approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee (IACUC) (Protocol 17-18 #13).

Materials

Operant Chamber. Two single-lever chambers (Gerbrands, Model C; 29 cm by 24 cm by 19 cm) served as the experimental apparatuses. The chamber is shown in Figure 1. The lever was centered on the front wall above the grid floor and required a minimum force of 0.2 N to complete a response. Reinforced responses triggered a 1000 Hz, 1-sec tone accompanied by delivery of a grain food pellet to a recessed food cup positioned to the left of the lever and near the floor. The training rooms were dark, but illumination was provided via a single house-light located directly about the food cup. A computer connected to the operant chamber ran the program and collected data. The program used to control the chambers and collect data was written and tested specifically for this experiment by University of Wisconsin-Milwaukee Psychology personnel. Collected data by the program included break points, pre-ratio pauses, and run-rates. All operant chamber sessions were monitored by an experimenter and all off-task behaviors and time off task were collected live.

Open Field. A second behavioral measure was conducted using an open field apparatus. The open field is a large, white-painted wooden box that measures 91.5 cm by 91.5 cm and has walls extending 57.5 cm high. The open field apparatus is shown in Figure 2. To facilitate data collection, the floor of the apparatus is marked off into 36 equal squares. A video camera was mounted over the apparatus to record each session. Collected data included total grid squares cross, total time spent in inner zone, rears, grooms, defecations, and urinations.

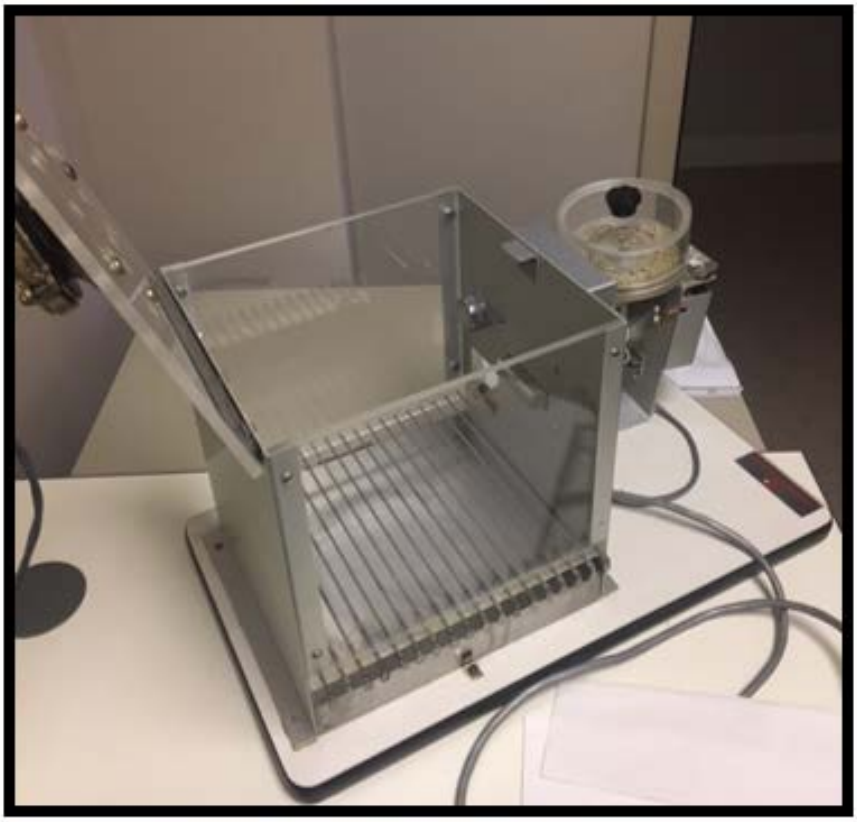

Figure 1. **Operant chamber.** The recessed food cup is positioned in the bottom left of the cage. The lever is centrally located, and the illumination light is directly above the food cup.

Elevated Plus Maze. Anxiety was assessed using the EPM; a cross maze with two closed and two open arms that stands 21.5 inches off the ground. The EPM is shown in Figure 3. Each animal received one 10 min videotaped trial that was scored at a later point. Parameters that were quantified included: number of entries into closed and open arms, total time spent in closed and open arms, time spent in distal portion of open arms, and time spent in the center of the four arms. An entry into any given arm was defined as having all four paws in the arm.

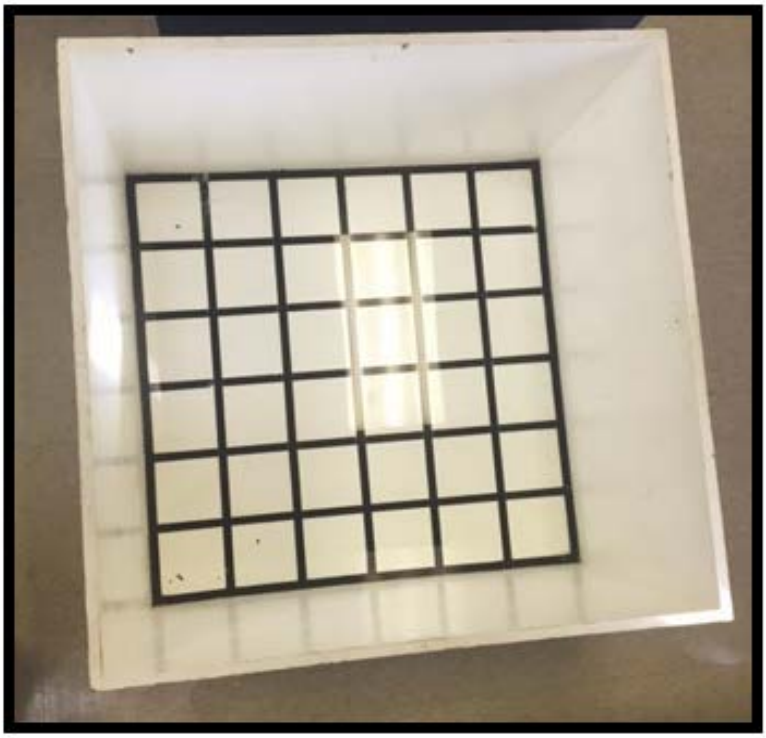

Figure 2. **Open field.** The open field has four white walls that surround a plex-glass floor marked into 36 squares.

Figure 3. **Elevated plus maze.** The elevated plus maze has two open arms, two closed arms, and is raised 21.5" off the ground.

Procedure

Acclimation. Upon arrival, all animals acclimated in their home cages for one week during which they received unlimited access to standard food pellets and water. Immediately following the week of home-cage acclimation, all rats, both experimental and control, began food restriction that remained throughout the experiment to ensure that rats were maximally hungry during training sessions. Water was provided *ad libitum* except while in the behavior apparatuses. Animals acclimated to the food restriction protocol for one week prior to the onset of training. For the last three days of the food restriction acclimation period, prior to beginning lever-press training, experimental animals were placed in the operant chambers for 10 min daily for acclimation. Animals were exposed to both of the behavioral chambers and which chamber they trained in alternated daily. After chamber acclimation, a protocol was followed in order to shape and train the animals to press a lever for grain food pellets (TestDiet- LabTab®, MLab Rodent 45mg). To prevent pellet neophobia in the operant chamber, food pellets were sprinkled into the home cages during the three days of chamber acclimation. All animals consumed the food pellets. The acclimation protocol can be seen in Figure 4. All control animals remained in the home cage and were not exposed to the operant chamber, open field, or elevated plus maze.

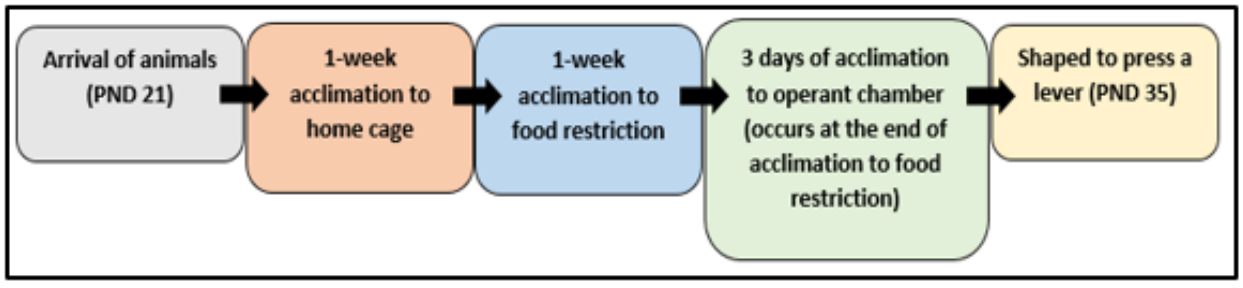

Figure 4. **Schematic of acclimation protocol**

Food restriction and daily feeding. Immediately following cessation of training each day, animals were allowed access to standard rat chow food pellets in their home cages. In order to determine how much food to feed the experimental and control animals, one animal in each condition, which were not included in data collection, were allowed free-access to food and water throughout the duration of the training procedure. These animals were deemed the "free-feeding control animals" and were used to ensure that the food-restricted animals were not losing greater than 20% of their free-feeding weights. Experimental, control, and free-feeding animals were all weighed daily. In addition, the amount of food that the free-feeding animals consumed was measured each morning. Experimental and control animals were fed 15% less than what the free-feeding animals consumed daily. If a food-restricted animal fell below 80% of the free-feeding animal weight, 3% more food was given. Conversely, if animals were over the 80% of free-feeding weight, these animals were given 3% less food.

Operant training. Operant training sessions occurred during the animals' light cycle. The only animals who completed the training were the experimental WKY (n=12) and experimental SHR animals (n=12). The first step in training was to shape the rats to press the lever. Experimenters monitored rats while they were in the operant chambers and responses that got closer to the desired lever-pressing response were reinforced. For example, this first included delivering food pellets approximately 1x per minute to train the rat where the food was delivered. Once the rat learned this, food pellets would start to be delivered when the rat was in the general vicinity of the lever, when it oriented to the lever, when it pressed its nose to the lever, etc., with multiple trials at each step. Most rats acquired the lever-press response within one to two shaping sessions each lasting one hour. If shaping was taking longer, the experimenters moved back several steps in the protocol to retrain and/or ensure the rat is properly motivated to consume

food pellets. If a rat did not master lever-pressing after four shaping sessions, it was removed from the study. A control animal was then substituted as an experimental animal and underwent the shaping protocol.

Following successful shaping, the rats completed training sessions of up to 50 trials on a fixed ratio (FR) schedule of 1, 2, 5, 10, and 20, one ratio each day for a total of 5 days. As an example, an animal on a FR5 schedule had to press the lever 5 times in order to receive a single food pellet reward. The rat either completed 50 trials of an FR schedule or reached the 1 hr max to conclude one training session. All rats were subjected to only one training session daily. The chamber in which the rats were trained in were counterbalanced daily. On the FR1-FR5 schedules, rats remained in the chamber until all rewards were earned (50 pellets) or until they timed out the 1 hr session. Rats either earned up to 50 pellets for the FR10 and FR20 schedules or the experimenter ended the training after 1 hr.

Upon completion of the 5 days of FR training, rats switched to training on a progressive ratio (PR) schedule. While the step size increased slowly in the FR schedule paradigm, the rats moved directly into a PR20 when they completed the FR training. On a PR20 schedule, the difficulty to achieve a food pellet increased with each trial. On trial 1, animals were required to press the lever 20 times in order to get reinforcement. On trial 2, it required 40 lever presses, trial 3, 60 lever presses, and so on. A PR20 session lasted 1 hour or was terminated if a rat sat inactive for 15 min. Rats remained on the PR20 schedule, one session a day, for the remainder of the experiment until a consistent breakpoint was established. The breakpoint was defined as the point at which a rat stopped lever pressing for food for a period of 15 min or reaching the 1 hr session time limit. If a rat sat inactive for 15 min, the session was terminated. If the rat remained active throughout the session, the breakpoint was the last completed ratio of the session. A

consistent breakpoint was defined as three consecutive days during which the breaking points differed by no more than a single ratio. For example, breaking points of 80, 100, and 80 on three consecutive days would meet criterion for the establishment of consistency. An average of these three numbers was the breakpoint ratio and it served as the measure of the rats' motivation (e.g. 80+100+80 = 260. Breakpoint: 260/3 = 86.67).

During the training sessions, the number of instances of off-task behavior and time off task were recorded. Experimenters watched each operant session live. A rat was defined as being off task if it was not actively pressing the lever, retrieving food pellet from food cup, or eating a food pellet. As soon as the rat finished pressing the lever and walked away or as soon he finished chewing, a stopwatch was started to collect a running total of time off task for the training session. Conversely, if the rat began pressing again or started to eat a food pellet, the stopwatch was stopped immediately. Additionally, all instances of off-task behaviors were tallied to allow for an in-depth analysis of what type of behaviors animals were engaging in while in the chamber. These behaviors included the following instances: biting, grooming, rearing, sitting inactive, smelling, and walking around.

Open Field. After a consistent breakpoint was established, animals were subjected to the open field as a secondary measure of motivation. In order to assess exploratory behaviors without anxiety of a novel environment, animals were acclimated to the open field apparatus for a five-minute period for two consecutive days. On the third day, animals were placed into the open field for a 10 min session. Each animals' session was video-recorded. Collected data included total grid squares crossed, total time spent in inner zone, rears, grooms, defecations, and urinations.

Elevated Plus Maze. After the open field session, animals immediately completed the EPM. Animals were placed in the center of the maze facing a closed arm and were left undisturbed to explore for 10 min. The videotaped session was scored later. Parameters that were quantified included: number of entries into closed and open arms, total time spent in closed and open arms, time spent in distal portion of open arms (defined as all four limbs in open arm), and time spent in the center of the four arms. An entry into any given arm was defined as having two forelimbs in the arm. The order of training events can be visualized in Figure 5.

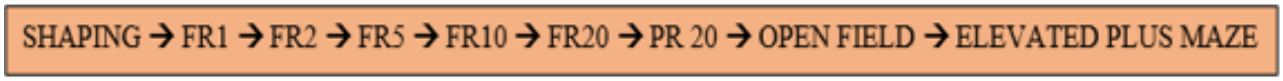

Figure 5. **Behavioral training protocol**

Tissue Analysis. At the conclusion of behavioral testing, all animals were euthanized by immersion in a carbon dioxide chamber and underwent pericardial perfusion using 200mL of phosphate buffer and 400mL of paraformaldehyde. Animals were decapitated and brains were extracted and stored in paraformaldehyde for 24 hours before being moved to a 30% sucrose solution for cryoprotection until further processing.

The entire perfused cerebellum was sectioned in sequential 40 μm coronal sections using a cryostat (3050s, Leica, Bannockburn, IL) and slices were mounted onto gelled slides. Tissue slices were dried overnight, stained with Cresyl Violet using a graded hydration process followed by a graded dehydration process, and then coverslipped. All microscope slides were labeled with the codes given to the animals upon arrival to avoid condition bias.

Dentate Nucleus Volume. To conduct volumetric analyses of the dentate nucleus, tissue slices were imaged using a light microscope (Olympus, America, Inc., Center Valley, PA) with an attached SPOT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI) at 10x

magnification. Images were taken of all slices that contained the dentate nucleus. Volumetric measures of the cerebellar dentate nucleus were conducted using ImageJ software. The area of the dentate nucleus visible on all sections was outlined by tracing the border of the structure. See Figure 6. This procedure yielded a measure of surface area in pixels, which was subsequently converted to microns. The sum of these areas in microns were then multiplied by the thickness of the tissue slice and number of tissue sections imaged, which resulted in the total volume of the dentate nucleus.

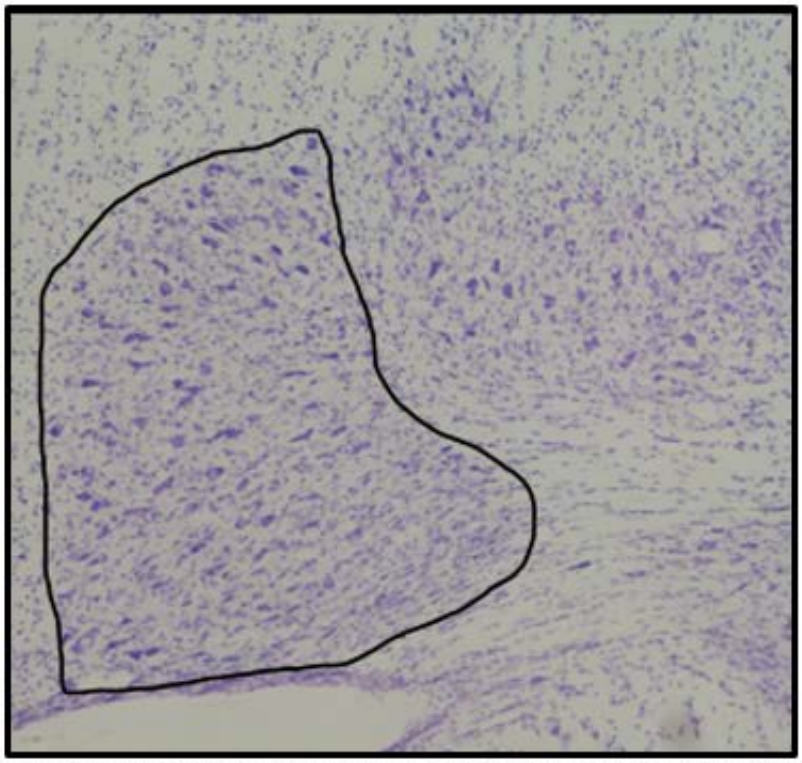

Figure 6. **Sample from a representative animal showing boundaries of the dentate nucleus.** The border of the dentate nucleus was determined based off the orientation of the cells and the presence of white matter.

Neuron Estimate in the Dentate Nucleus. In order to further study the expected volumetric differences of the dentate nucleus, the total area fraction of neurons in the dentate nucleus using unbiased stereology was calculated. The same tissue slices that were obtained for the volumetric analyses of the dentate nucleus were re-imaged using 40x magnification. Imaging began on the first tissue slice in which the dentate nucleus appeared. From that slice, every other slice was imaged until the complete structure (dentate nucleus) was captured. After tissue sections were imaged, images from each section were randomly selected for unbiased stereology to determine the total area fraction of neurons in the dentate nucleus. Images remained coded such that investigators remained blind to the animals' conditions during quantification. For each image, a black line was traced, using ImageJ software, around the dentate nucleus creating a Region of Interest (ROI). The ROI was determined by the orientation of cells, the presence of white matter, and guidance from the rat brain atlas (Paxinos & Watson, 1986). Once an ROI was placed, a 475-point grid was superimposed using ImageJ software. The number of crosses that fell into the ROI were counted. Then, if a cross of the point grid fell on a neuron cell body, the neuron was counted. For each animal, a minimum of 100 points that fell on neurons was counted so the number of images sampled per animal varied. Area fractions were calculated for the dentate nucleus by dividing the total number of points that fell on neurons by the total number of points on the grid that fell into the ROI. See Figure 7.

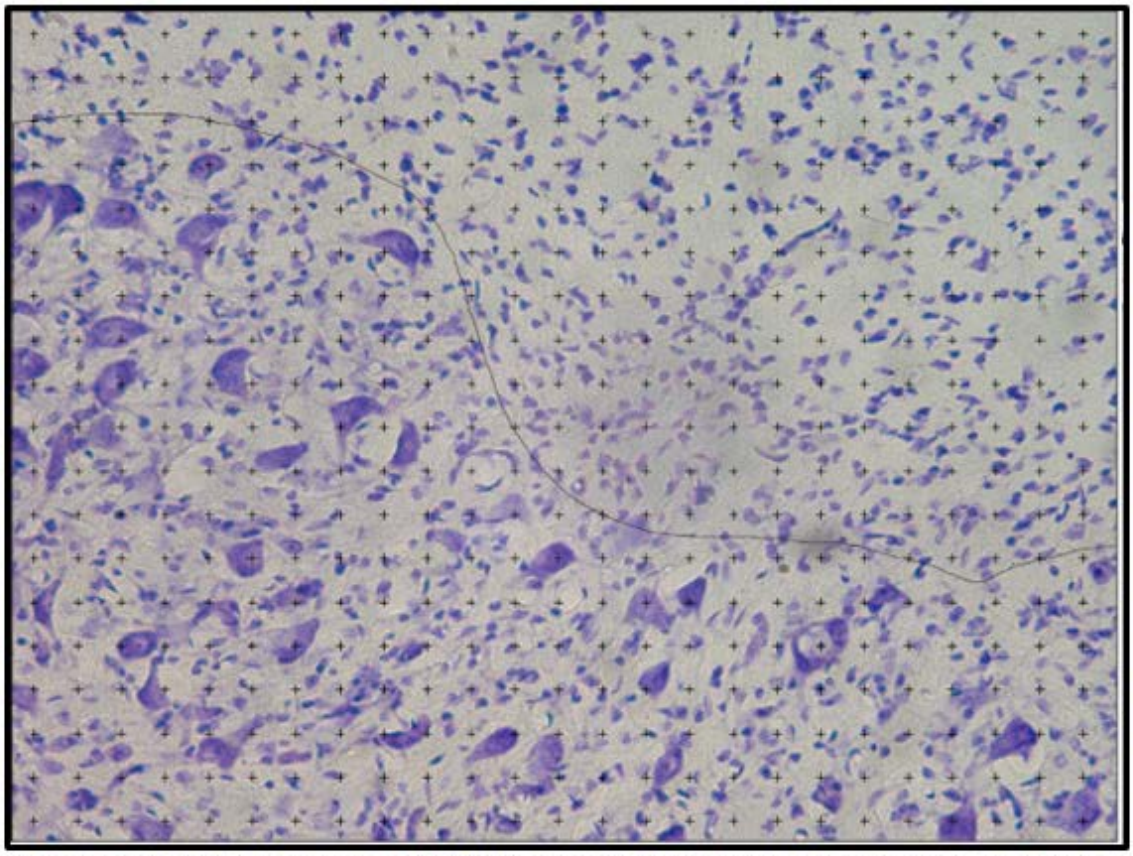

Figure 7. **Point grid over the dentate nucleus.** The ROI was drawn on using the orientation of the cells and the presence of white matter. A 475-point grid was overlaid using ImageJ software. A neuron was counted if the center of the crossed touched the large neurons.

Cerebellar Vermis Volume. Reduced cerebellar vermis volume is a well-established finding in the both the human and SHR literature (Anderson, Polcari, Lowen, Renshaw, & Teicher, 2002; Berquin et al., 1998; Bledsoe, Semrud-Clikeman, & Pliszka, 2009; Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002; Castellanos et al., 2001; Hill et al., 2003; Mostofsky, Reiss, Lockhart, & Denckla, 1998). In this study, we aimed to replicate these findings by investigating the cerebellar vermis volume in the experimental SHR and WKY and the control SHR and WKY groups. Starting from the first slice of cerebellum, images were taken

of the cerebellum vermis at 10x magnification. Volumetric measures of the cerebellar vermis were calculated using ImageJ software. The area of the vermis visible on the slice images was outlined by tracing the border of the structure. This tracing produced a surface area in pixels, which was subsequently converted to microns. The sum of these areas in microns were then multiplied by the thickness of the tissue slice and number of tissue sections imaged, which resulted in the total volume of the cerebellum vermis. See Figure 8.

Purkinje Cell Count in Cerebellar Vermis Lobule 8. If the volume of the cerebellum was smaller in the SHR animals, our hypothesis was that this volume difference would be driven by the number of Purkinje cells found in the vermis. Our initial aim was to count Purkinje cells throughout the entire vermis. However, a previous study reported that the SHR animals have fewer Purkinje cells in specifically and only Lobule 8 of the cerebellum vermis compared to control animals (Yun et al., 2014). Therefore, we aimed to replicate this study's finding. Lobule 8 was imaged in its entirety using a light microscope at 40x. All Purkinje cells within the cerebellum vermis lobule 8 were counted using ImageJ software to yield a total Purkinje cell count. See Figure 9.

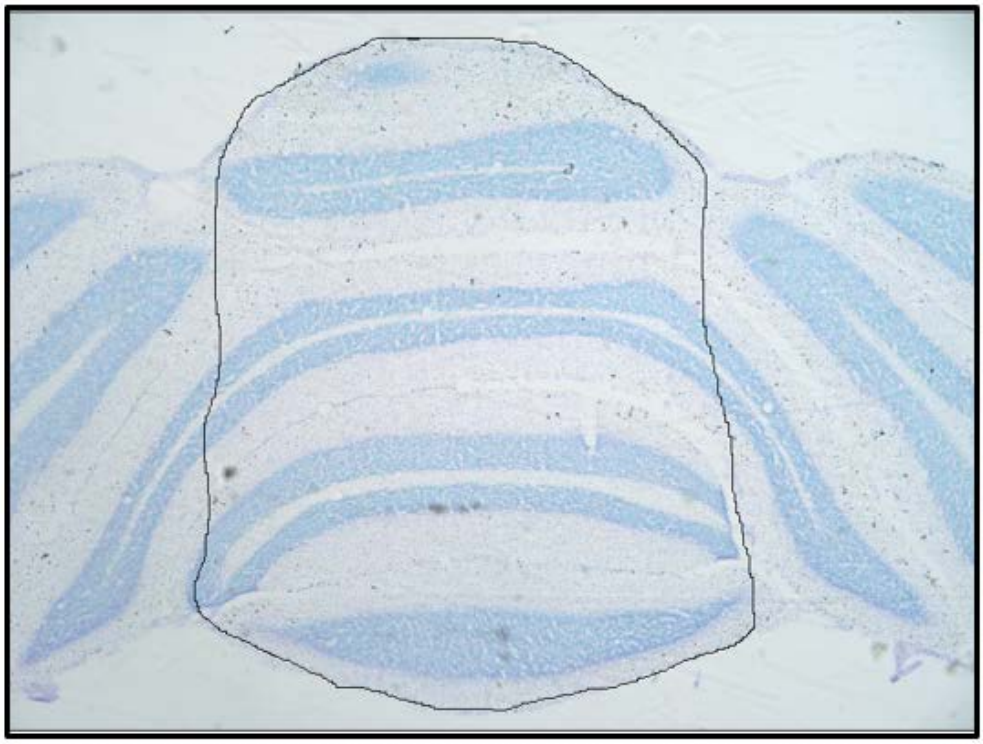

Figure 8. **Cerebellar vermis volume**- The vermis of each cerebellum slice was traced using ImageJ.

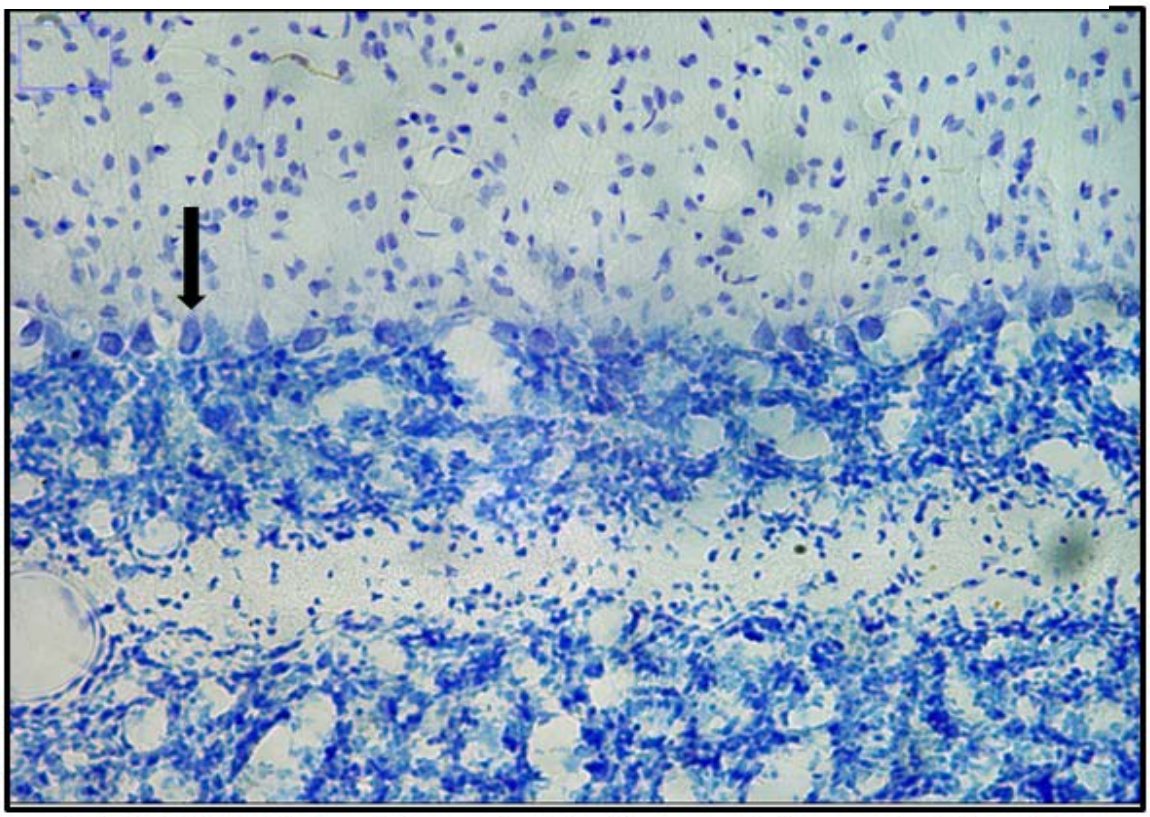

Figure 9. **Lobule 8 Purkinje cells imaged at 40x.** The arrow indicates a Purkinje cell.

Results

Operant Chamber

Twenty-four animals were exposed to the operant chamber and all were included in data analyses. Data presented here are from the operant chamber from the three days of consistency during the PR20 schedule. All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) Version 25.

We first analyzed the average body weights of animals during the three days of consistency on the PR20 schedules to ensure that the animals were not differentially affected by the food deprivation protocol. A difference in body weight may affect the amount of food an animal would eat, therefore impacting the interpretation of our breakpoint ratio data. An independent samples t-test of the average body weights of animals, during the three days of consistency on the PR20 schedules, showed no significant difference in weight between the SHR (M=157.528, SD=28.565) and WKY (M=150.667, SD=29.682) experimental animals ($t(22) = -.577$, $p = .570$).

In addition, we examined the number of days it took for animals to acquire shaping to rule out a difference in the ability to learn the task. The SHR (M=2.417, SD=.793) and WKY (M=2.083, SD=.900) did not differ in the number of days it took to shape the rats to accurately press a lever for a food pellet as indicated by an independent samples t-test, $t(22)= -.962$, p=.346. Furthermore, only three of the original experimental animals, 2 SHR and 1 WKY, failed to meet the requirements of shaping in 4 or less 1 hr sessions. These animals were replaced control animals by an experimenter in the lab naïve to the experiment and not involved in data collection or analysis. The new experimental animals then began shaping in the operant chamber and were included in the data analysis. We then analyzed if there was a difference between the groups in the number of days it took the rats to reach a breakpoint consistency once on a PR20 ratio. An

independent t-test revealed no significant difference between the SHR (*M*=5.667, *SD*=1.875) and WKY (*M*=4.583, *SD*= 1.881) groups on the number of days it took them to reach the criterion for breakpoint consistency (*t* (22) =-1.413, *p*=.172). With no difference in the weight of the animals, the days required to shape, and the days to reach consistency on the PR20, we were able to better interpret our initial hypotheses.

Our primary behavioral goal was to examine if the SHR animals displayed reduced motivation in the PR20 breakpoint paradigm, and the breakpoint ratio was used as a measure of motivation in the operant chamber. Breakpoint was defined as the value of the ratio toward which the rat was working but failed to achieve due reaching the 1 hr training session limit or due to a 15 min period of inactivity (absence of lever pressing). Rats were required to reach a consistent breakpoint on three consecutive days that did not differ by more than a single ratio. An independent samples t-test was conducted to compare the average breakpoint ratio for the SHR and WKY animals. In support of our original hypothesis, the SHR (*M*=113.167, *SEM*= 11.325) animals had a significantly lower average breakpoint ratio compared to the WKY animals ((*M*=205.000, *SEM*=66.188); *t*(22)=4.135, *p* = 0.0004). Figure 10 depicts the mean breakpoint ratio by group. This result provides evidence that motivation is indeed lower in the SHR animals compared to the WKY controls.

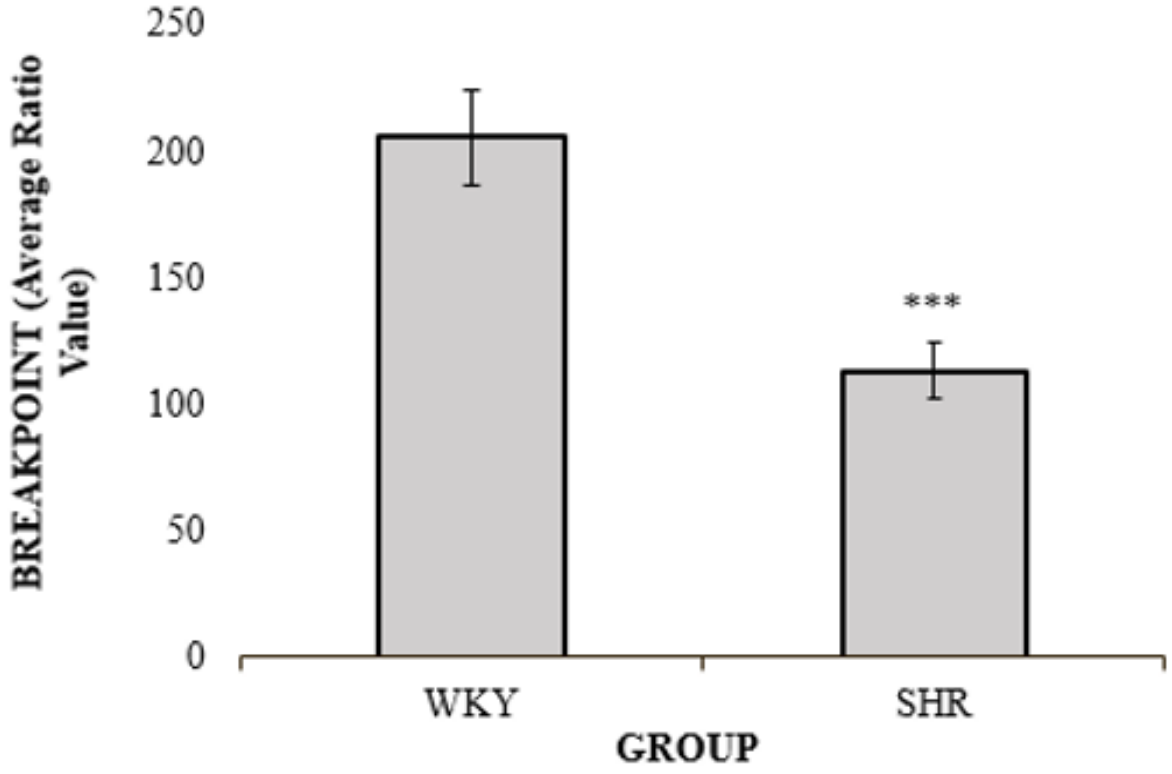

Figure 10. **Operant Chamber- Average breakpoint ratio**. SHR animals had a significantly lower breakpoint average compared to the WKY animals (p= .0004).

Our secondary goal was to provide evidence that the inattentive, hyperactive, and impulsive symptoms of ADHD could be replicated and displayed in the SHR model while in the operant chamber during a PR20 breakpoint paradigm. All behaviors in the operant chambers were recorded live and as an approximation of inattention, we calculated the amount of time the animals spent off task. As we initially predicted, an independent samples t-test showed a significant difference in the amount of time spent off task between the SHR ((M= 2052.583, SEM= 136.045) and the WKY (M=1387.808, SEM= 101.576) animals; $t(22) = -3.915$, p =.001). Figure 11 depicts the mean time spent off task by group.

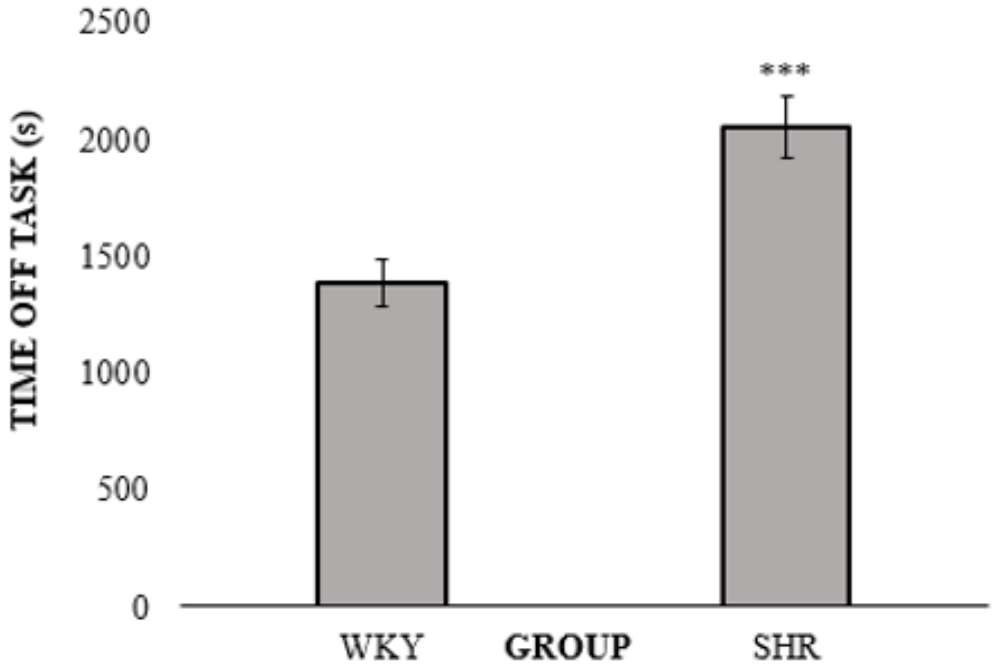

Figure 11. **Operant Chamber- Average amount of time spent off task (s).** The SHR animals spent significantly more time off task during the days of breakpoint consistency compared to the WKY animals (p=.001).

A secondary evaluation of inattention was measured using the average number of consecutive lever presses. Remaining at the lever for only a few bursts of presses was defined as our measure of inattention to the task. An independent samples t-test showed that the SHR animals (M=8.666, SEM= .972) pressed the lever significantly fewer times consecutively before taking a break (defined as 10 sec or more) compared to the WKY animals ((M= 22.184, SEM= 2.581); t(22)=4.902, $p < .001$). Figure 12 displays the mean of consecutive lever presses by group.

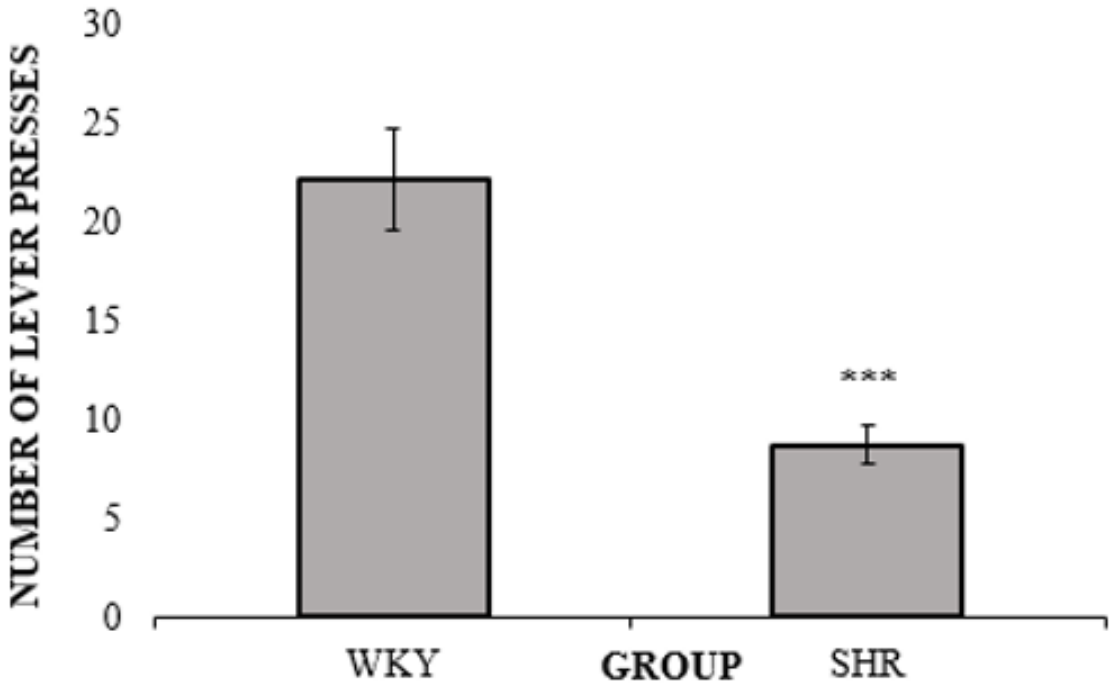

Figure 12. **Operant Chamber- Consecutive lever presses.** The SHR animals pressed the lever in shorter consecutive bursts before taking a break compared to the WKY animals ($p < .001$).

Hyperactivity was assessed by recording the number of instances the animals engaged in any type of off-task behavior. The data support our original hypothesis as the results of an independent samples t-test revealed that the SHR animals (*M*= 168.438, *SEM*= 18.147) engaged in significantly more instances of off-tasks behaviors compared to the WKY animals ((*M*= 106.139, *SEM*=8.847); $t(22) = -3.086$, $p = .005$). See Figure 13 for a visual representation of the mean instances of off-task behaviors by group.

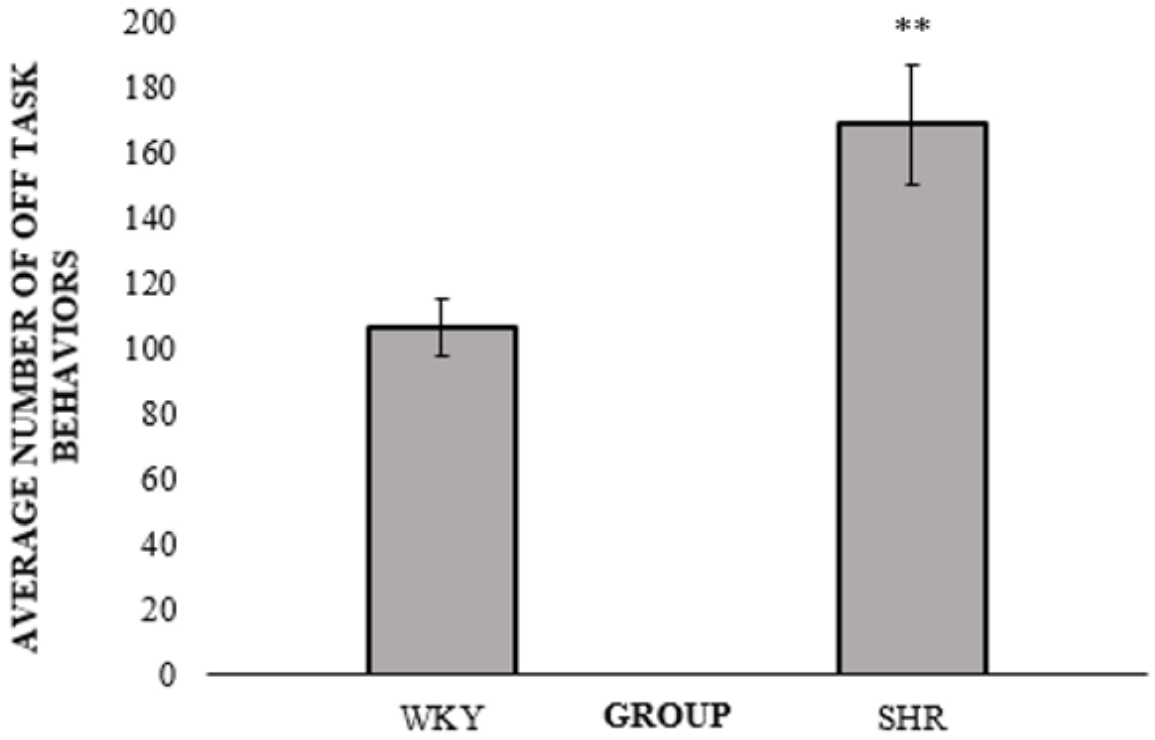

Figure 13. **Operant Chamber- Average number of instances of off-task behavior.** The SHR animals engage in significantly more instances of off-task behaviors compared to the WKY (p=.005).

The behavioral sessions in the operant chamber were viewed and scored live. The types of behaviors the animals engaged in while in the operant chamber were observed and broken down into the following categories: bite, groom, rear, sit inactive, smell, walk around. The results of a one-way multivariate analysis of variance (MANOVA) showed significant differences in the categories of behaviors between the SHR and WKY animals (F (6,17) = 3.054, p =.032; Wilks' λ=.481, partial ή=.519). A test of between-subjects effects revealed that the SHR animals (M=29.444, SD=23.208) engaged in significantly more instances of rearing compared to the WKY ((M=13.889, SD=4.887), F (1,22) =5.499, p=.028). Furthermore, the SHR animals (M=80.417, SD=34.437) engaged in significantly more sniffing compared to the WKY

((*M*=45.528, *SD*=17.462), *F* (1,22) =9.798, *p*=.005). Finally, the SHR animals (*M*=24.47, *SD*=8.630) walked around the cage significantly more than the WKY ((*M*=13.750, *SD*=10.206), *F* (1,22) =7.723, *p*=.011). Figure 14 depicts the categories of behaviors engaged in while in the chamber.

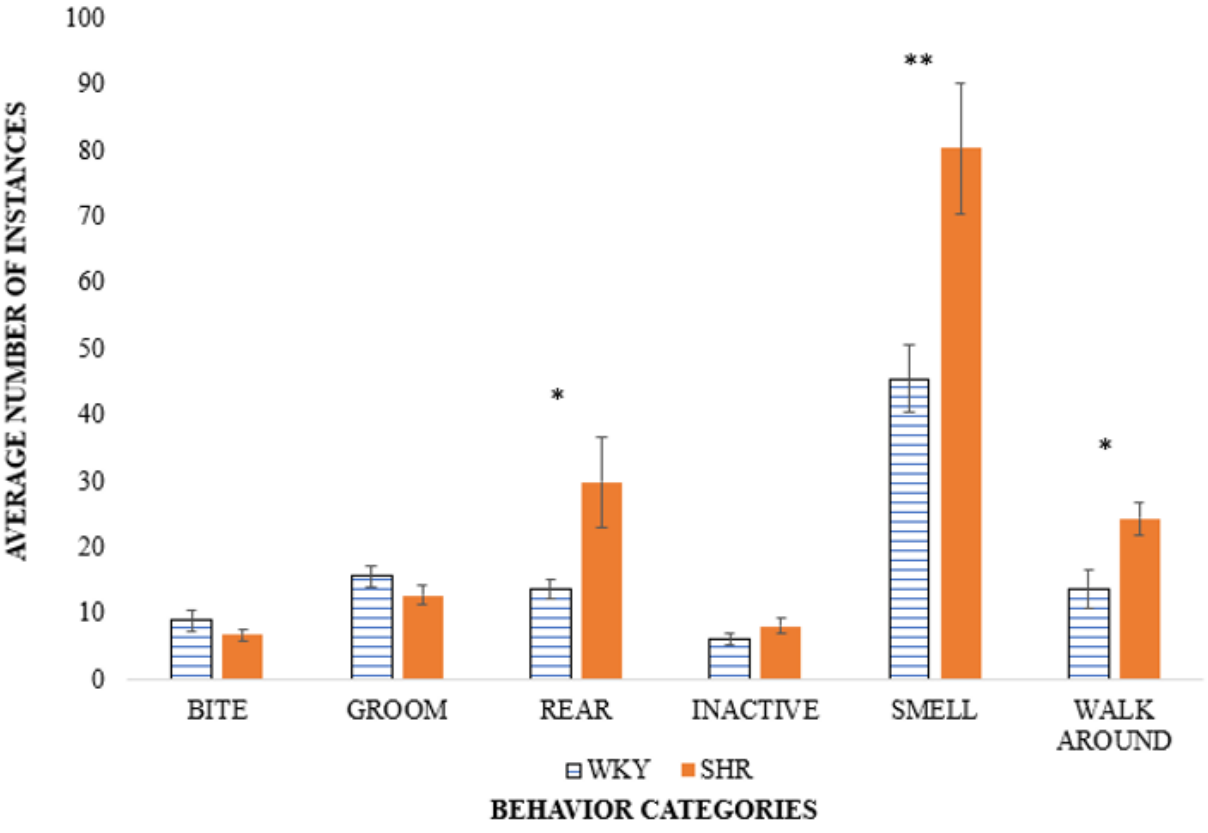

Figure 14. **Operant Chamber- Categories of behaviors while in operant chamber.** SHR animals engaged in significantly more rearing, smelling, and walking around while in the operant chamber compared to the WKY animals.

Hyperactive behavior while in the chamber was also assessed by calculating the run rate, which was defined as the number of lever presses per minute. To calculate the run rate accurately, the amount of time the animals spent off task and the pre-ratio pauses were subtracted out of the 60 min session. Therefore, we were left with the amount of time the animals spent pressing the lever. We then divided the total number of lever presses completed in that given training session by the number of minutes spent pressing the lever. A run rate was calculated for each day of constancy and then an average of the days was taken. This number was used a measure of hyperactivity, and it was initially predicated the SHR animals would exhibit hyperactivity evidenced by a higher run rate. The data do not support that hypothesis. In fact, an independent samples t-test revealed that the SHR animals (*M*=30.479, *SEM*=3.686) had a significantly lower run rate compared to the WKY animals ((*M*=49.378, *SEM*=5.101); *t*(22)=3.003, *p*=.007). The average run rates for each group are depicted in Figure 15.

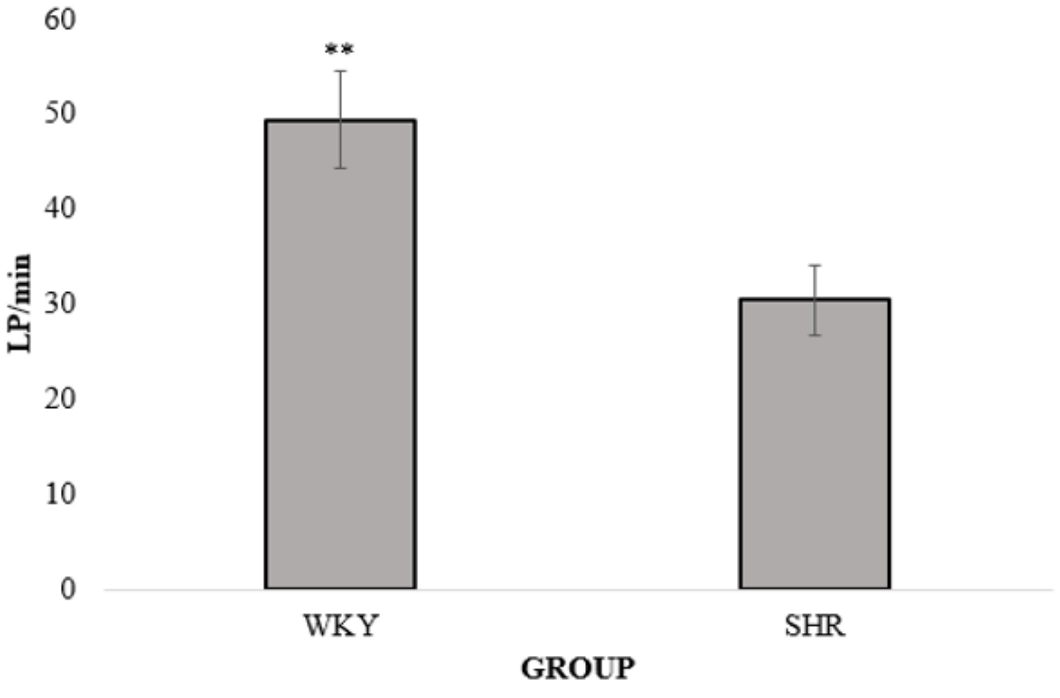

Figure 15. **Operant Chamber-Run rate.** The WKY animals had a significantly higher run rate compared to the SHR (*p*= .007).

Finally, we then used pre-ratio pauses as a measure of the impulsive symptom associated with ADHD, assuming that the impulsive nature of the SHR model would result in their inability to pause briefly prior to starting a new ratio. The data did not support our hypothesis that the SHR animals would exhibit lower average pre-ratio pauses. An independent samples t-test showed no difference in the length of the average pre-ratio pause between the SHR (*M*=48.480, *SEM*=5.909) and WKY animals ((*M*=41.696, *SEM*=5.245); *t*(22), -.859, *p* =.400).

Open Field

Twenty-four animals were subjected to the open field apparatus and all were included in data analysis. Data presented here from the open field is from one 10 min video-taped session. All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) Version 25. As a measure of hyperactivity, we analyzed the total number of squares crossed in the open field. The data support our original hypothesis and the SHR (*M*=258.417, *SEM*=27.884) animals significantly crossed approximately 3x more squares compared to the WKY ((*M*=93.000, *SEM*= 20.550); as revealed by an independent samples t-test, *t*(22)=4.776, *p*<.001). Figure 16 illustrates the average number of squares crossed by group.

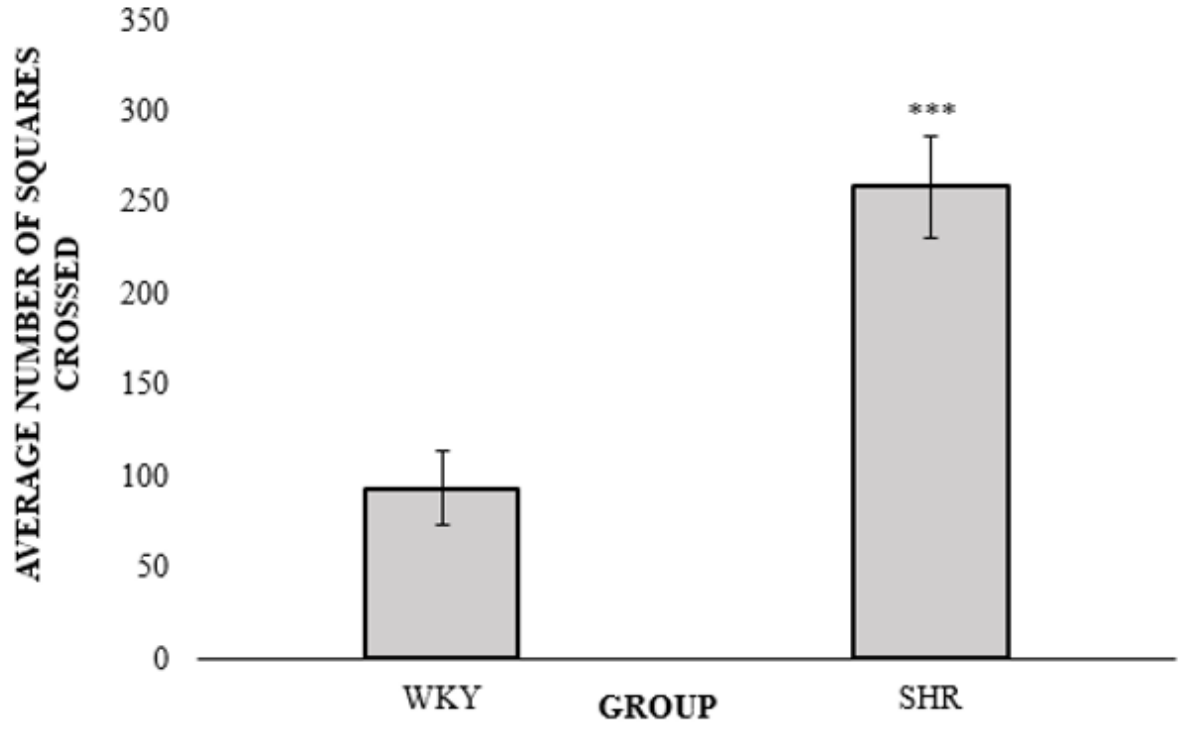

***Figure 16*. Open Field- Number of squares crossed.** The SHR animals crossed significantly more squares in the open field compared to the WKY ($p < .001$).

We also hypothesized that the SHR animals would exhibit non-purposeful explorative behavior by spending less time in the inner grids of the open field. The data did not support this hypothesis. However, the results of an independent samples t-test showed the opposite results as the SHR animals (*M*= 51.083, *SEM*= 10.345) spent significantly more time on the inside of the open field, defined as all of the squares that are not on the immediate border of the box, compared to the WKY animals ((*M*=10.167, *SEM*= 3.359), $t(22) = -3.762$, $p = .001$). The average amount of time spent in the inner squares by each group is displayed in Figure 17.

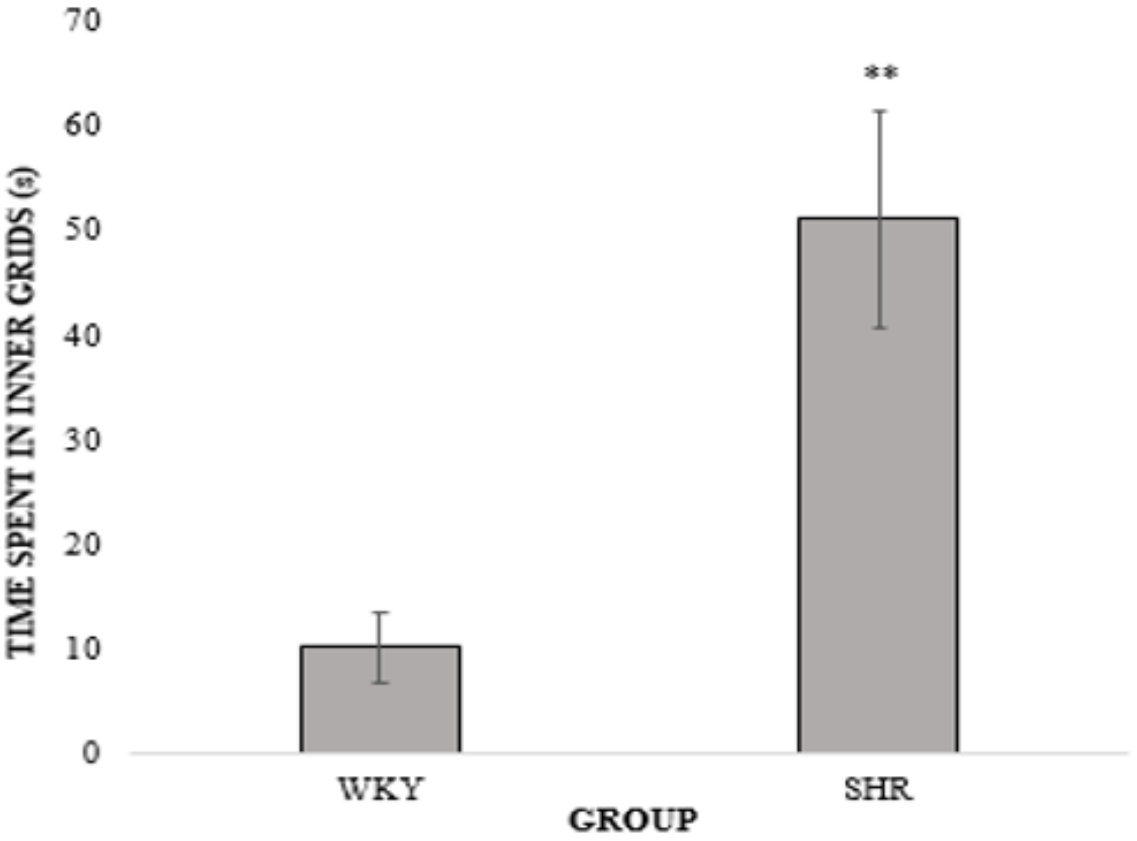

Figure 17. **Open Field- Time spent in inner squares.** The SHR animals spend significantly more time in the inner squares of the open field compared to the WKY (p =.001).

We had predicted no difference between the groups of animals on the number of rears, grooms, defecations, and urinations. These behaviors were grouped together and one-way MANOVA revealed statistically significant differences in these measures between the two animal groups, $F(4,19)= 6.935$, $p =.001$; Wilks' $\lambda=.406$, partial $\eta=.59$. A test of between-subjects effects revealed a significant difference in the number of rears between the SHR (M=48.250, SD=20.046) and WKY ((M= 14.083, SD=12.176), F (1,22)= 25.456, p <.001). Figure 18 displays the mean number of rears by group in the open field. In addition, the SHR (M=1, SD=1) animals defecated significantly more than WKY ((M= 0, SD=0.00), $F(1,22)= 6.000$, p =.023). There were no significant differences in grooms and urinations between the animals.

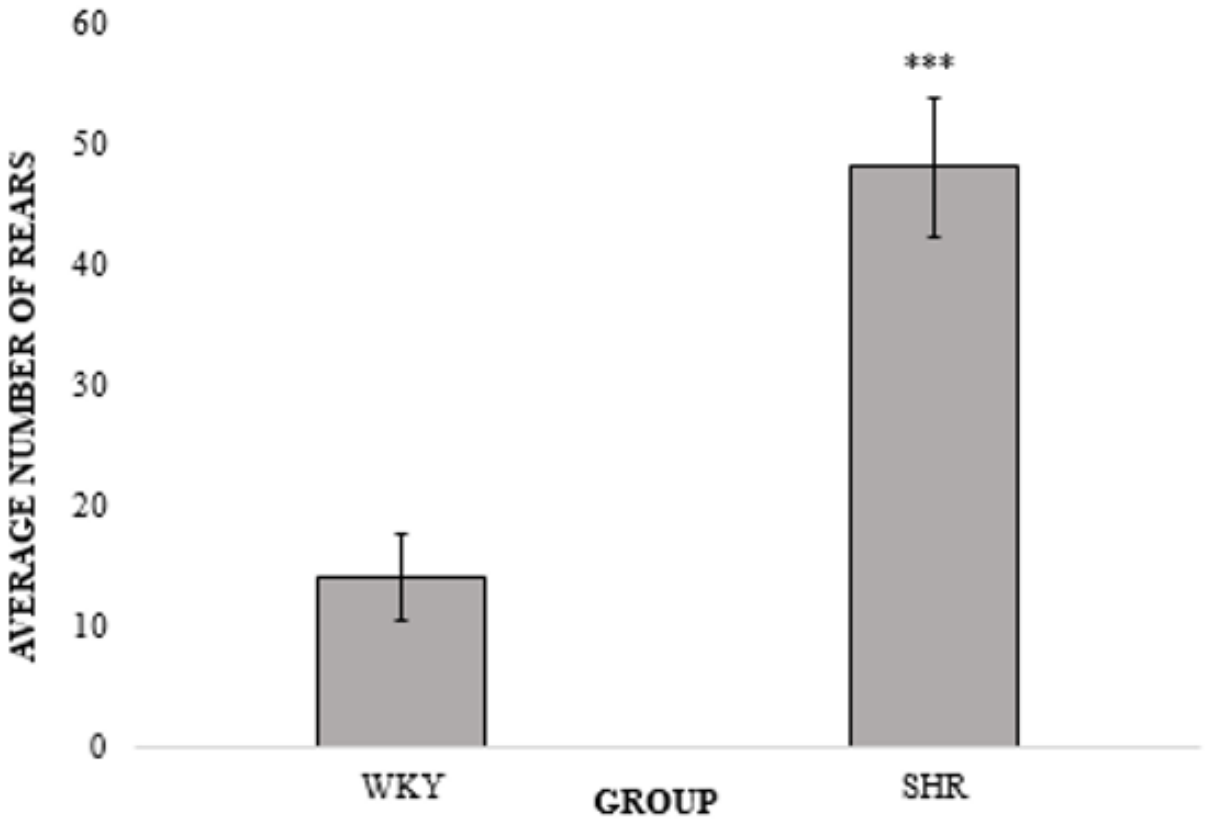

***Figure 18*. Open Field- Number of rears**. The SHR animals reared significantly more in the open field compared to the WKY ($p<.001$).

Elevated Plus Maze

Twenty-four animals were subjected to the Elevated Plus Maze and all were included in data analysis. Data presented here from the EPM is from one 10 min video-taped session. All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) Version 25. We used the EPM as a measure of anxiety. We had hypothesized that there would be no differences between the groups in the number of open arm entries. However, our results indicated that the SHR rats (M= 6.667, SEM=4.942) entered open arms more frequently than the WKY rats (M=1.917, SEM=.633),. Figure 19 depicts the mean number of open arm entries by

group. An independent samples t-test confirmed that the observed difference was significant ($t(22)= -3.043$, $p = .006$).

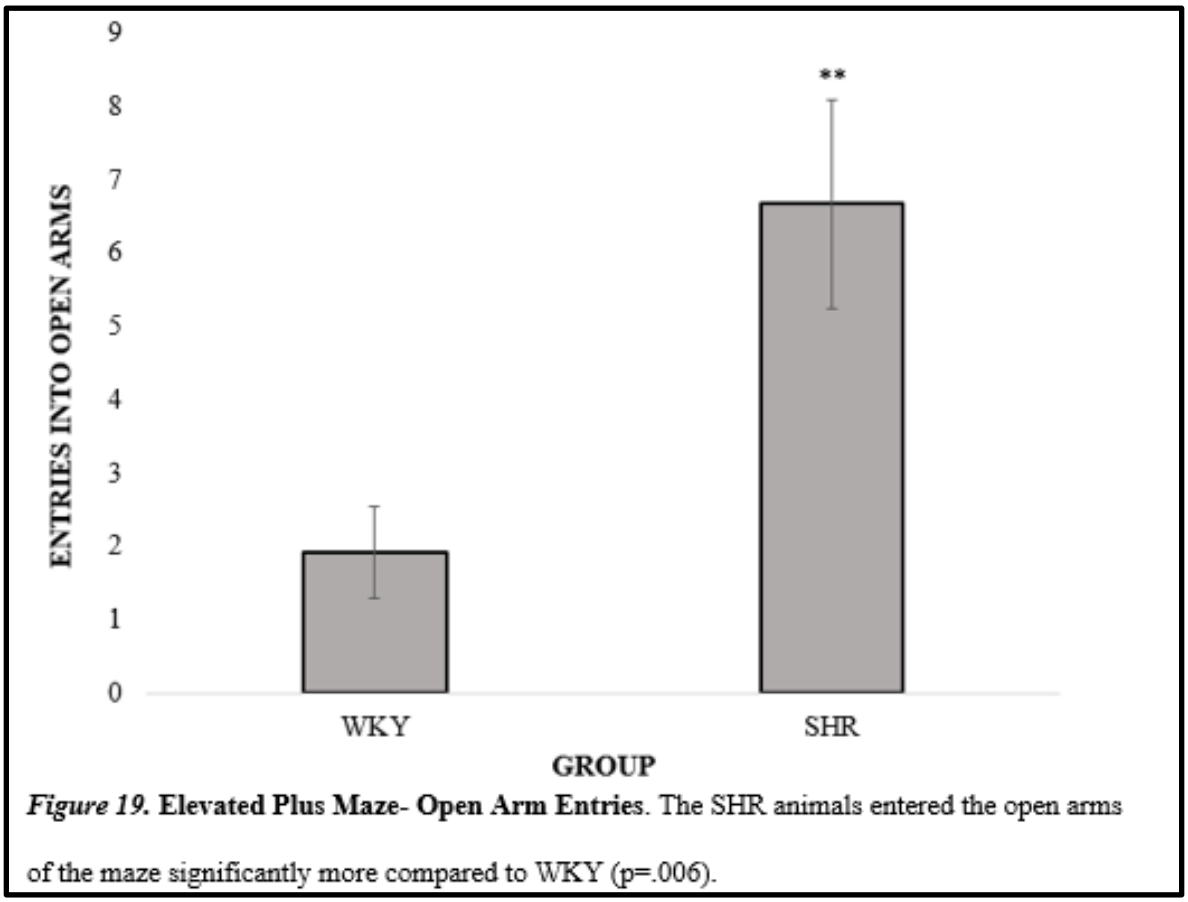

Figure 19. **Elevated Plus Maze- Open Arm Entries**. The SHR animals entered the open arms of the maze significantly more compared to WKY (p=.006).

As a secondary measure of anxiety on the EPM, we hypothesized that there would no difference in the amount of time the animals spent in the open and closed arms of the maze. The time spent in the open and closed arms of the maze were analyzed using independent samples t-test. Results did not support our hypothesis. The SHR animals (M=43.942, SEM=12.685) spent significantly more time in the open arms of the maze compared to the WKY ((M=19.933, SEM=5.908), $t(22)$=-2.963, p=.007). Figure 20 displays the mean time spent in the open arms by group. However, there was no significant difference in the amount of time each group spent in

the closed arms of the maze (SHR: *M*=401.917, *SEM*=20.834; WKY: *M*=456.250, *SEM*=17.677, *t*(22)=1.989, *p*=.059).

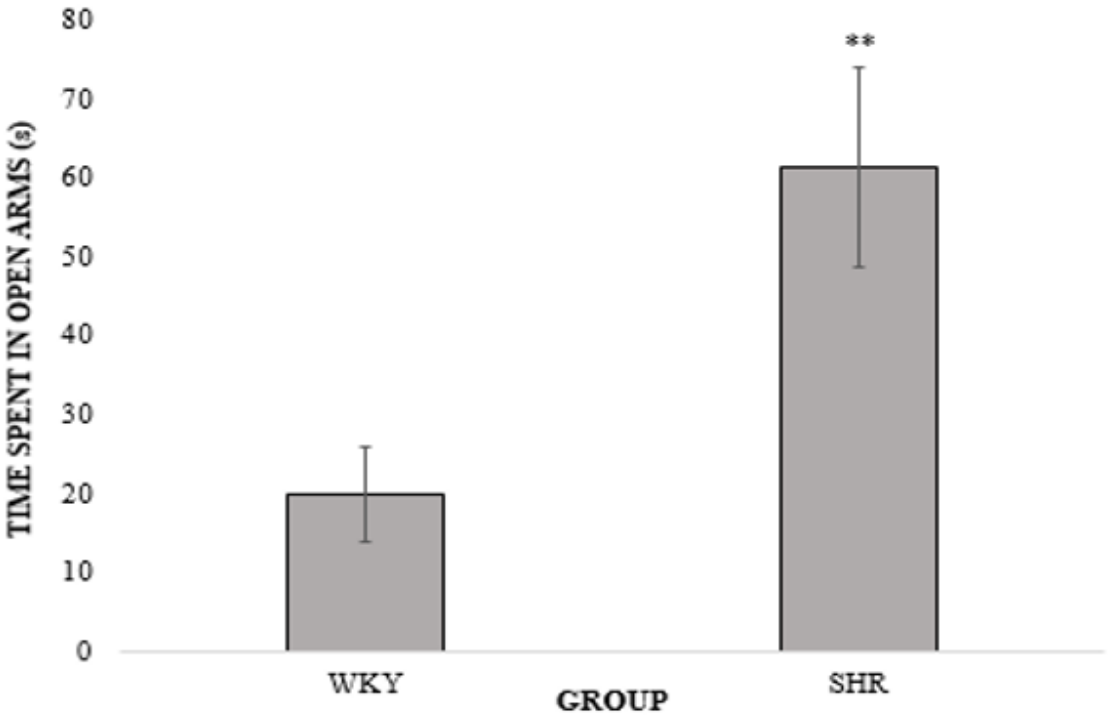

Figure 20. **Elevated Plus Maze- Time spent in open arms.** The SHR animals spent significantly more time in the open arms of the elevated plus maze compared to WKY (p=.007).

In addition, we predicted that if the rats entered the open arms, there would be no difference in the amount of time the SHR and WKY spent in the distal portion of the arm (defined as the 3" at the distal end of the open arms). Our data support our hypothesis as there was no significant difference in the amount of time spent in the distal portion of the open arms of the EPM between the SHR (*M*=19.775, *SEM*=8.268) and WKY (*M*=3.200, *SEM*=2.179) as indicated by an independent samples t-test, *t*(22) = -1.939, *p*= .065. Furthermore, we anticipated no difference between the groups in the amount of time they spent in the center of the maze. The

data support this hypothesis as the independent samples t-test showed no statistical difference between the SHR (*M* =56.908, *SEM*=5.301) and the WKY (*M*=60.683, *SEM*=5.301) in the amount of time they spent in the center of the maze between the open and closed arms, *t*(22)= .292, *p*=.773.

As a measure of hyperactivity, we predicted that the SHR animals would exhibit higher locomotion in the EPM by entering the closed arms of the maze more frequently than the WKY animals. Our data did not support this hypothesis as we found no significance difference in the amount of closed arm entries between the SHR (*M*=12.583, *SEM*=1.667) and WKY animals ((*M*=8.750, *SEM*= 1.498), *t* (22) = -1.710, *p* = .101).

Histology Results

For the histology portion of this experiment, 48 animals were used in the analysis (24 trained animals and 24 untrained, naive animals). All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) Version 25.

To analyze the primary histology hypothesis of the study, the volume of the dentate nucleus in both hemispheres of the brain, a two-way MANOVA was conducted. The independent variables were the Group (SHR vs WKY) and Condition (EXP vs CTL) and the dependent variables were the volume in microns of the dentate nucleus in both the Right hemisphere and the Left hemisphere. Results indicated a significant effect of Group, $F(2,43)=8.364$, $p=.001$; Wilks' $\lambda=.720$, partial $\eta=.280$. Follow-up between-subjects ANOVAs supported our original hypothesis and revealed that the SHR animals ($M=1.817\text{x}10^{12}$, $SD=2.240\text{x}10^{11}$) had a significant smaller volume of the dentate nucleus in the Right hemisphere compared to the WKY animals (($M=2.097\text{x}10^{12}$, $SD=3.312\text{x}10^{11}$), $F(1,44)=11.884$, $p=.001$). In addition, there was also a significant difference in the dentate nucleus volume in the Left

hemisphere as the SHR (M= 1.677x10^{12}, SD=3.162x10^{11}) animals had a smaller volume compared to the WKY animals ((M=2.036x10^{12}, SD=3.352x10^{11}), F (1,44) =14.362, p <.001). Figure 21 displays the mean dentate nucleus volume by condition and group. However, the results of the two-way MANOVA results did not provide support for our hypothesis that the trained animals, regardless of group, would have a larger dentate nucleus volume compared to the untrained, naïve animals, F (2,43) =.179, p=.837; Wilks' λ=.992, partial ή=.008. There was also no significant interaction between Group and Condition (F (2,43) = 1.346, p=.271; Wilks' λ=.941, partial ή=.059).

To see what was driving the volumetric difference that we found, the large neuron area fraction of the dentate nucleus was also analyzed with a two-way MANOVA. The data do not support our hypothesis that the SHR animals would exhibit fewer neurons in the dentate nucleus. We found no significant effect of Group (F (2,43) =.268, p=.766; Wilks' λ=.988, partial ή=.012). The SHR animal did not show fewer neurons in the dentate nucleus. We also saw no significant effect of Condition (F (2,43) =.123, p =.885; Wilks' λ=.994, partial ή=.006). This means that there was no difference between the trained and untrained animals in the number of neurons in the dentate nucleus. Finally, there was no significant interaction between Group and Condition (F (2,43) =.738, p=.484; Wilks' λ=.967, partial ή=.033).

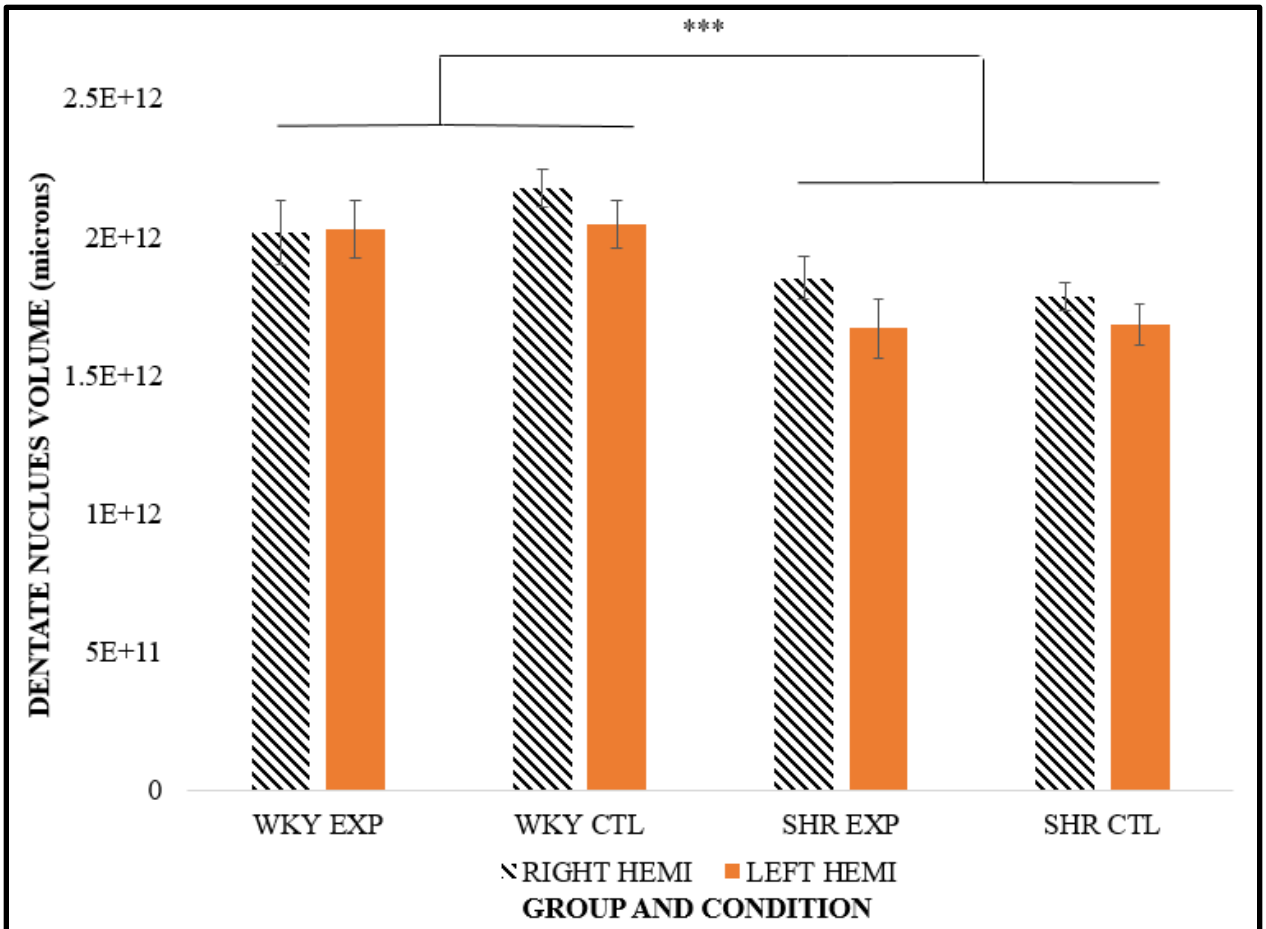

Figure 21. **Dentate Nucleus volume**. The dentate nucleus is smaller in the SHR animals compared to the WKY, regardless of training condition or which hemisphere (HEMI) is measured ($p < .001$).

In addition, we examined whole brain and cerebellum weight. All whole brains were weighed in grams and were analyzed with a one-way analysis of variance (ANOVA). The ANOVA revealed a significant main effect for Group ($F(1,44)=5.893$, $p = .019$). SHR animals ($M=1.547$, $SD=.123$) had significantly smaller whole brain weights compared to the WKY animals ($M=1.634$, $SD=.140$) and this is depicted in Figure 22a. Furthermore, there was a significant main effect for Condition ($F(1, 44) = 7.072$, $p=.011$). Animals who were subjected to behavioral training ($M=1.639$, $SD=.166$) had significantly larger whole brain weights compared

to the animals who were naïve controls (M=1.543, SD=.079) and this is displayed in Figure 22b. There was no significant interaction between Group and Condition (F (1,44)=.108, p=.744).

We then separated the cerebellum, weighed it, and analyzed via a one-way ANOVA. No statistical differences between cerebellar weights were found between the SHR (M=.590, SD=.077) and WKY ((M=.648, SD=.129), F(1,44)=3.012, p=.090) or between the trained (M=.648, SD=.138) and untrained, naive animals ((M=.595, SD=.060), F(1,44)=3.022, p=.089). There was no significant interaction between Group and Condition (F(1,44)=.336, p=.565).

We aimed to replicate the findings that the cerebellum vermis is smaller in the SHR animals. The results of a one-way ANOVA indicate a significant main effect for Group (F (1,44) =17.924, p < .001). The SHR animals (M=1.668x10^{14}, SD=3.495x10^{13}) had a significantly smaller vermis volume compared to the WKY (M=2.038x10^{14}, SD=2.734x10^{13}). Visual representation of the mean vermis volume by group is shown in Figure 23a. Furthermore, and interestingly, there was a significant main effect of Condition (F (1, 44) = 4.307, p =.044). Figure 23b shows that the trained animals (M=1.944x10^{14}, SD=3.790x10^{13}) had a significantly larger vermis volume compared to the untrained, naïve animals (M=1.763x10^{14}, SD=3.277x10^{13}). There was no significant interaction between Group and Condition (F (1,44) = 1.223, p=.275).

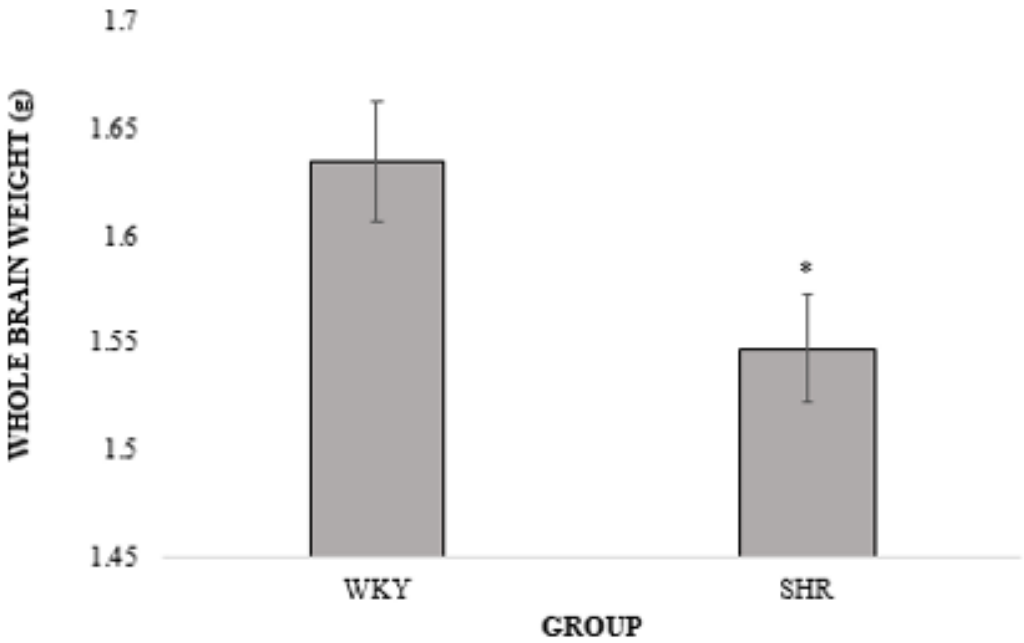

Figure 22a. **Whole brain weight by group.** The SHR animals had a significantly smaller whole brain weight (g) compared to the WKY animals (p=.019).

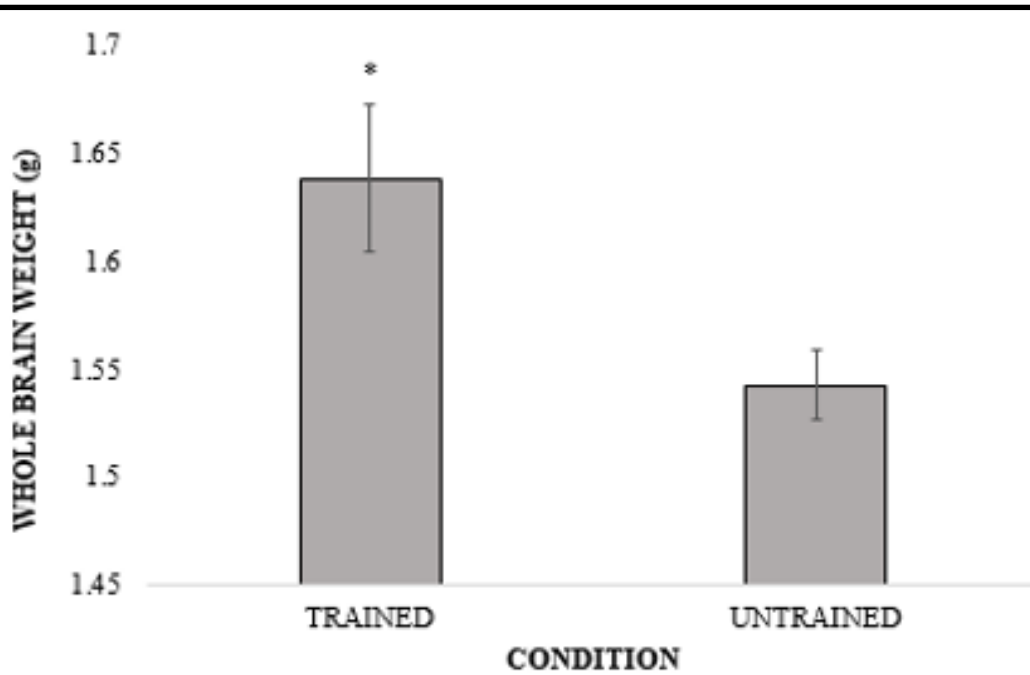

Figure 22b. **Whole brain weight by condition.** The trained animals had a significantly larger whole brain weight (g) compared to the untrained, naïve animals (p=.011)

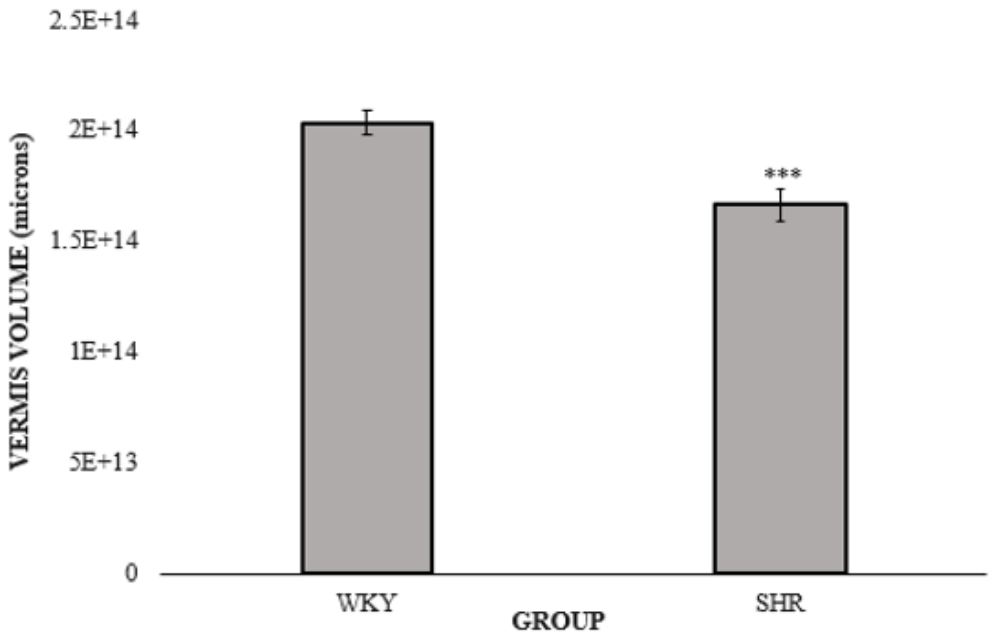

Figure 23a. **Vermis volume by group**. The SHR animals had a significantly smaller cerebellum vermis volume compared to the WKY ($p < .001$).

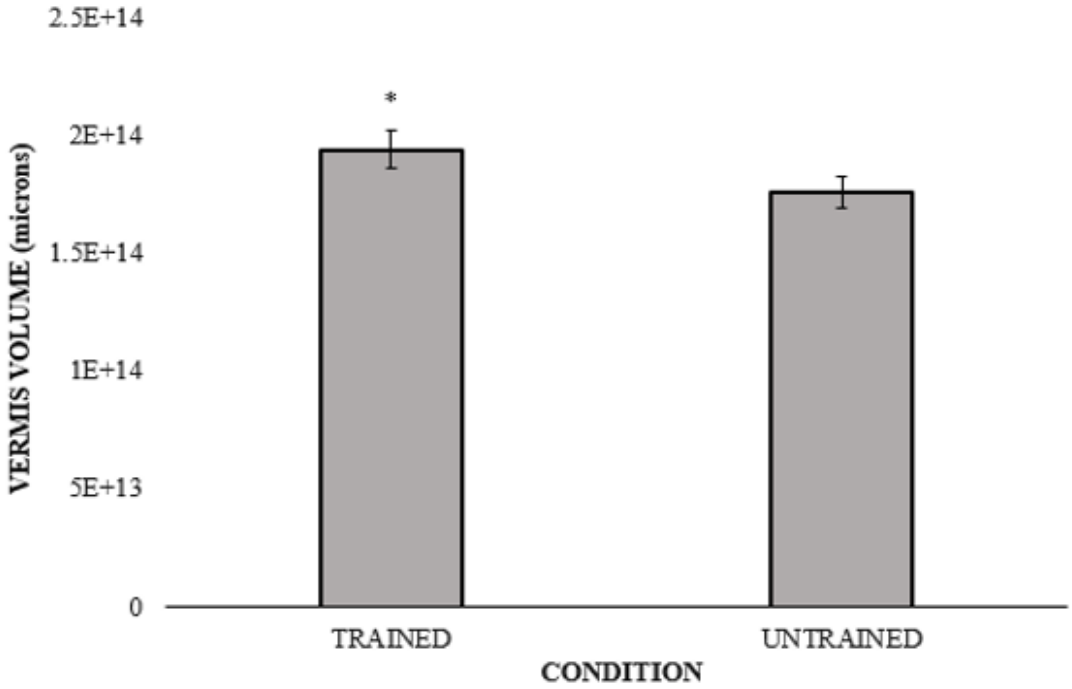

Figure 23b. **Vermis volume by condition**. The Trained animals had a significantly larger vermis volume compared to the untrained, naïve animals ($p = .044$).

Finally, we aimed to replicate a previous finding that the SHR animals exhibit fewer Purkinje cells in Lobule 8 of the cerebellar vermis**.** A one-way ANOVA confirmed this finding by showing a significant main effect of Group ($F(1,44)= 134.651$, $p < .001$). There are fewer Purkinje cells in Lobule 8 in the SHR (M=9.925, SD= .707) than there are in the WKY (M=12.953, SD=1.093) animals. Average Purkinje cell counts by group are displayed in Figure 24. There were no statistically significant differences between the trained (M=11.639, SD=1.908) and untrained animals ((M=11.239, SD=1.660), $F(1,44) = 2.349$, p= .133). There was no significant interaction between Group and Condition, $F(1,44)=1.362$, p=.249.

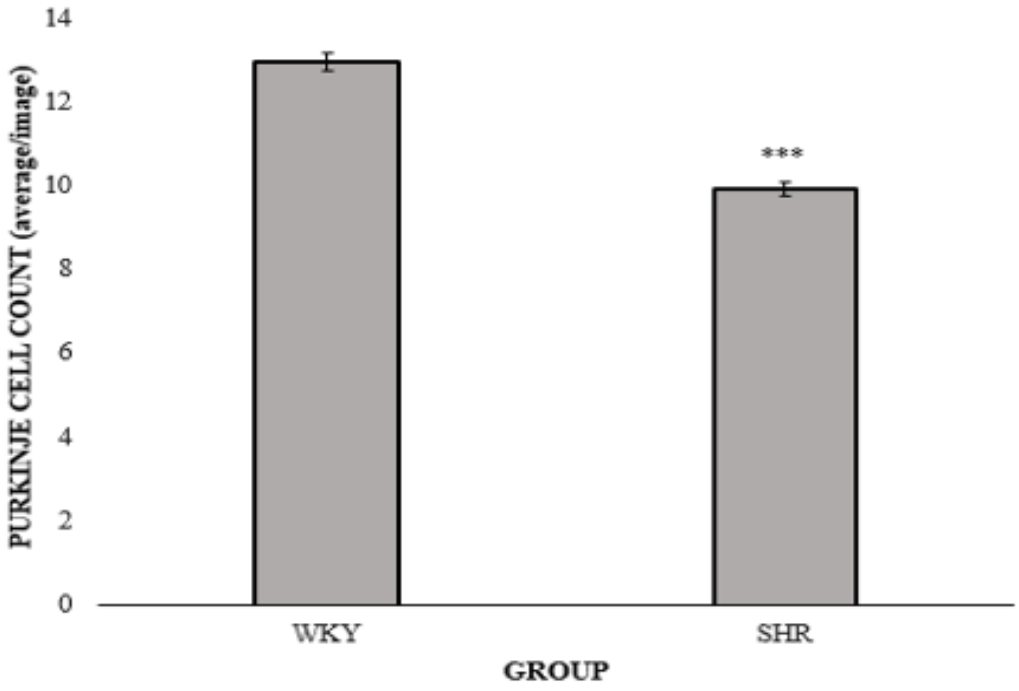

Figure 24. **Purkinje cell count in Lobule 8 of the cerebellum vermis**. SHR animals had significantly fewer Purkinje cells compared to the WKY animals (p<.001).

Discussion

In the current study we used an animal model of Attention-Deficit Hyperactivity Disorder (ADHD), defining an animal model as "*an animal sufficiently similar to a human target group in its physiology or behavior, based on a natural, bred, or experimentally induced characteristic in the animal, and which purpose is to generate knowledge that may be extrapolated to the human target group"* (Sjoberg, 2017, pg. 3). While we cannot infer that the SHR animal has ADHD, we can use the rat's behavior as an approximation of the behavioral symptoms seen in humans with ADHD clinical diagnoses.

Operant Chamber

Motivation

The primary behavioral aim of this study was to determine if SHR animals display reduced breakpoints in a progressive ratio operant task, a task that has historically been used to measure motivation. We predicted that SHR rats would cease operant responding at a lower ratio than their WKY controls. Our result supported this hypothesis. On average, SHR animals stopped responding at a breakpoint ratio average of 114 while WKY rats stopped at a ratio average of 205. It is possible that the earlier breaking point in the SHR rats could be attributed to strain size, strain taste preferences, stress, frequency of off-task behaviors, or failure to learn the task. Our results suggest that none of these are likely.

First, a reduction of motivation, defined as a lower breakpoint ratio, could be in part due to a strain-specific size difference of body weight in grams. As part of the monitoring of animals' health, all animals in this study were handled and weighed daily. Previous SHR literature has indicated a size difference between the SHR animals and WKY controls, especially a significant body weight difference in the young, developing animals (Buchanan, Youn, Campese, & Sipos,

1992; Han, Chen, Wu, Zhu, & Gao, 2010; Kurtz & Morris, 1987; Rostron et al., 2017; Swislocki & Tsuzuki, 1993)). A size difference would be particularly troubling for the interpretation of the results if the SHR animals were smaller than the control animals. A decreased body size may result in a decreased appetite or the number of pellets the animals are able to consume, therefore resulting in reduction of lever pressing due to the diminished interest in eating. In this study we used juvenile rats and it was important to consider their expected growth as they age, especially in a food-deprivation study. We analyzed the average body weights for both the WKY and SHR animals during the three days of consistency on the PR20 schedule. We found no significant difference in body weight between the two groups of animals for these three days of consistency, indicating that the difference in breakpoint ratio cannot be attributed to the size difference of the animals.

Second, it is also possible that the strains of animals (SHR and WKY) were differentially motivated to strain-specific preferences of flavor. Evidence suggests that different strains of rats show distinct flavor preferences (Tordoff, Larcon, & Lawler, 2008). However, all animals were acclimated to the food pellets prior to training and all animals consumed equal numbers of pellets in the acclimation, shaping, and FR training periods. We used standard rodent lab chow pellets rather than sweet or salty pellets used in other studies (Bourjeili, Turner, Stinner, & Ely, 1995; Rostron et al., 2017). Interestingly, it has been found that SHR animals actually show a high preference for sodium chloride in both food and drinking water (Catalanotto, Schechter, & Henkin, 1972). This would be an interesting avenue to peruse to see if the motivational deficit we observed in these animals could be rescued using reinforcers that the animals preferred. In addition, the difference in the number of food pellets consumed was not apparent until animals reached the PR20 schedule when it required the animals to expend substantially more work in to

receive a single food pellet. We found no striking aversion to the food pellets we used by either strain of rat, providing evidence that the difference in the breakpoint ratio cannot be due to strain-differences in flavor preference.

Third, the decreased breakpoint that we see in this study may have arisen as a result a greater stress response on the part of the SHR rats to being placed in the operant chamber. It has been proposed that the SHR animals do exhibit an increased and altered stress response (Wickens, Macfarlane, Booker, & McNaughton, 2004) to novel environments and that might explain why the SHR had reduced breakpoints. A recent study reported that the SHRs exhibited lower response rates on a non-delayed food reinforcement paradigm and proposed that this may be due to the stress the SHR animals experience while in the chamber (Rostron, Gaeta, Brace, & Dommett, 2017). The authors suggested that the altered stress response in the SHR animals would most likely result in either the animals exhibiting freezing behavior or to have increased escape-like behavior while in the chamber (Fox, Caramia, Haskell, Ramey, & Singha, 2017), but they could not make that conclusion given that the chamber sessions were not recorded or watched live. We find, in our task, that it is not the case that the SHR animals freeze or sit inactive due to a stress response to a novel environment. In the current study, the animals were acclimated to the chamber for multiple days prior to the beginning of testing. In the operant chamber, the SHR animals engaged in more exploratory behaviors such as smelling, rearing, and walking around the operant chamber, indicating these animals were more interested in their environment, as if the chamber captured their attention more readily, rather than the task at hand. In addition, the breakpoint ratio was calculated from the last three days that the animals spent in the chamber. Most animals spent, on average, 2-3 weeks in the operant chamber reducing the possibility that the reduced breakpoint ratio in the SHR animals is due to a stress response to a

novel environment. Furthermore, we followed up the operant chamber with the open field and EPM and found that the SHR animals display a reduction of anxiety/stress-like behaviors in both of these paradigms.

Fourth, the reduction in breakpoint ratio that we see in the SHR animals could have been caused by their engagement in off-task behaviors. We report that the SHR animals engaged in significantly more instances off-task behavior in addition to spending significantly more time off-task. In fact, the SHR animals spent more than half the behavioral session engaged in off-task behaviors. Therefore, it could be that their engagement in these off-task behaviors is the reason that their breakpoint is lower. We defined breakpoint as the value of the ratio (the number of lever presses required to receive a food pellet) toward which a rat was working but failed to achieve to do a 15 min period of inactivity (absence of lever pressing) or by reaching the end of a 1 hr training session. An absence of lever pressing could be due to the animals sitting inactive or engaging in off-task behaviors long enough to meet the breakpoint criteria. None of the animals, particularly the SHR animals, timed out due to the absence of lever pressing. This infers that the SHRs animals remained active throughout the session. While the SHR animal did engage in more instances and spent more time off task, none of those instances were long enough to reach a breakpoint. Instead, SHRs engaged in short bursts of off-task behavior.

Furthermore, we had initially used pre-ratio pause as a measure of impulsivity in these animals. While there was no difference in the time of the pre-ratio pause between the two groups, failing to support our hypothesis of impulsivity, the pre-ratio pause can be re-examined in this situation. The pre-ratio pause was defined as the amount of time after receipt of a reward and prior to the start of a ratio for subsequent rewards (the first lever depression in a ratio). While it is true that the SHR animals engaged in more off-task behaviors and spent more time off-task,

the pre-ratio pause measure provides support that SHR animals are returning to the lever in the same amount of time as the WKY animals do. Taken together, the fact that engagement in off-task behaviors did not cause the animals to reach a breakpoint during the PR20 schedule and that the SHR animals still returned to the lever to begin pressing again for a subsequent ratio refutes the idea that the off-task behaviors are the reason for the reduction in the breakpoint ratio we see in the SHRs.

Finally, it is possible that the SHR animals never learned the lever-pressing task and this consequently would explain why performance was significantly lower on the PR20 schedule. We would argue that the SHR animals did in fact learn the task. We presented data that showed there was no significant difference between the animals groups in the number of days to shape in the operant chamber, indicating that both groups of animals were able to learn and acquire the lever pressing task. We then moved through various FR schedules to train the rats on schedules that got progressively more difficult before switching to a PR20 schedule. During the FR schedules, all rats either worked for the entire hour or achieved their 50 pellet max, providing evidence in favor that the rats learned and continued to perform. It was on the PR20 schedules that we see the differences in the number of ratios completed. It is also important to note that none of the animals timed out in the PR20 sessions due to inactivity. Animals remained alert and active during the entire session, but the SHR animals engaged in other types of behavior besides lever-pressing. Given our inability to ascribe the reduction in breakpoint to any of these other factors, the most parsimonious explanation is that the rats ceased responding because their drive state (motivation) was insufficient to prompt more effortful behavior for the size of the given reinforcer.

Inattention

While the main behavioral aim of the current study was to provide evidence that the SHR animals display lower levels of motivation, we had several secondary aims. We wanted to provide additional evidence that the SHR animal, which has been previously validated numerous times, displayed the core diagnostic symptoms of inattention, hyperactivity, and impulsivity while in the operant chamber during a PR20 schedule. Regarding inattention, we assessed the amount of time the animals spent engaged in off-task behaviors. Off-task behaviors were defined as any behavior that was not pressing the lever, seeking pellet in the food cup, or eating a food pellet. The findings from the current study show that the SHR animals spent significantly more time off-task compared to the WKY controls. On average, during the 1 hr training session, the SHR animals spent around 34 min engaged in off-task behavior while the WKY animals engaged in 23 min of off-task behavior. The SHR animals spent over half of their time in the chamber engaged in off-task activity. We interpret the failure for them to remain on task as evidence that the SHR animals display problems with attention. In contrary to the Rostron et al. (2017) study which inferred the rats were off task if they were not pressing the lever, our study design allowed us to watch that the animals and ascertain whether animals were on or off task. Another measure used as evidence in support of inattention in the SHR animals was the examination of the average number of consecutive lever presses before walking away from the lever. We approximated this to be similar to attention span. Using this data, we found that the WKY animals, on average, pressed the lever 22 times in a row prior to taking a break (defined as 10 s or more) compared to the SHR animals who pressed, on average, 8 consecutive times. The break length was also analyzed and there were no differences in the amount of time spent on a break between the two strains. On the PR20 schedule, particularly on the three days of breakpoint

consistency, the SHR animals spent significantly more time off task and participated in the task in short bursts as evidenced by a lower consecutive lever presses, both of which provide evidence for altered attention to participate in the on-task behavior for lengthy periods of time.

Hyperactivity

To assess hyperactivity in the operant chamber, we used the number of instances of off-task behaviors as a measure of the animals' activity levels. The behavioral sessions were scored live, and experimenters tallied every type and instance of off-task behavior occurring. Some of these behaviors included biting the floor, sniffing the ceiling, licking the hopper, grooming, etc. Our data support the hypothesis that the SHR animals would exhibit hyperactive-like behavior by engaging in significantly more instances off off-task behaviors compared to the WKY animals. On average, the SHR animals engaged in 168 instances of off-task behavior while the WKY engaged in an average of 106. This finding of higher activity by the SHRs in an operant paradigm has been reported before (Berger & Sagvolden, 1998; Natsheh & Shiflett, 2015; Sagvolden, 2000; Sagvolden et al., 1992; Sagvolden, Pettersen, & Larsen, 1993). Interestingly, Fasmer & Johansen (2016) video-recoded SHR animals while they were in the chamber during a variable interval 180s schedule of reinforcement. They report that not only do the SHR animal engage in higher levels of activity, they also move around the cage with a higher velocity.

Furthermore, because we scored behavior live, we were able to categorize the types of behaviors the animals were engaged in. We grouped behavior into the following categories: bite, groom, rear, sit inactive, smell, and walk around. Our study showed that the SHR animals engaged in significantly more instances of rearing, smelling, and walking around, which are considered to be exploratory-like behaviors in rodents (Belzung, 1999; Glickman & Hartz, 1964; Sarafino, 1978). Engaging in exploratory behaviors rather than lever presses could be interpreted

as increased distractibility, which is prevalent in humans diagnosed with ADHD as evidenced by the diagnostic criteria of the DSM-V and previous human studies (American Psychiatric Association, 2013; Mourik, Oosterlaan, Heslenfeld, Konig, & Sergeant, 2007). For example, children with ADHD often pay more attention to events happening in and outside the classroom and less attention to schoolwork compared to their non-ADHD peers. Furthermore, children who are diagnosed with ADHD tend to have greater difficulty with inhibiting conflicting stimuli which results in distraction from the relevant task (Scheres et al., 2004). Further research should be conducted assessing the distractibility in the SHR animal model.

As a secondary measure of hyperactivity in the operant chamber, we examined run rate. Based of previous studies that have reported that SHR animals' response rates increased as the delay of reinforcement increased due to the hyperactive nature of these animals (Fox, Hand, & Reilly, 2008; Hill, Herbst, & Sanabria, 2012; Natsheh & Shiflett, 2015;Sanabria & Killeen, 2008), it was initially hypothesized that run rates would be higher in the SHR animals compared to WKY while in the operant chamber. Run rate, or the number of lever presses per min, was used as a measure of activity (Bauer, Kerr, & Swain, 2011). However, results from this study found the opposite pattern of what we predicted as the WKY animals had a significantly higher run rate compared to the SHRs. We examined why this would occur a little further. The animals in this study were restricted to a 1 hr training period. To calculate the run rate, we took the 60 min session, subtracted out the amount of time the animals spent off task and the pre-ratio pauses between each ratio, which left us with the time the rat spent actually pressing the lever. We then took the number of lever presses and divided by the number of minutes the rats pressed the lever. This would give us an indication of activity. We assumed, given previous reports of hyperactivity in the SHR rats, that they would press the lever more in the given time frame.

Logically speaking, because the WKY had a higher average breakpoint ratio, it would only make sense that the WKY animals must physically press the lever more times in the given time period in order to obtain the higher breakpoint ratio, therefore lending to the higher run rate value. Our initial hypothesis was not logically sound.

Impulsivity

We wanted to provide evidence that the SHR animals displayed the impulsivity symptomology of ADHD while in the operant chamber. To assess this, we used pre-ratio pause as a measure of impulsivity. If the animals were impulsive, the pre-ratio time would be shorter as they would be quick to start a subsequent ratio. This hypothesis was based on findings of previous studies that had also used an operant training paradigm and found that the SHR animals exhibit the inability to inhibit responses when a delay is introduced, which is a defining feature of ADHD (Boix, Quio, Kolpus, & Sagvolden, 1998; Orduña & Mercado, 2017; Sanabria & Killeen, 2008). Interestingly, our study did not support the hypothesis and there was no significant difference between the two animal groups in the length of the pre-ratio pause. Although not measured, it appeared that during the pre-ratio pauses, the WKY and SHR animals chose different behaviors to engage in. The researchers noticed that after receiving a pellet upon the completion of the ratio, the SHR animals tended to walk away from the hopper to engage in off-task behaviors. It is important to note that if the animals was visibly chewing, they were considered to be still “on-task” and the time and number of off-task instances they engaged in while chewing were not recorded. Conversely, it seemed like the WKY remained near the hopper and took some time to chew each pellet. Both animals returned back to the lever in the same amount of time to begin the next ratio. This could be due in part to the training paradigm that we used. Other studies examining the impulsivity of the SHR animals have not used PR schedules of

reinforcement, but have rather mostly used interval, variable, and fixed ratios. We had no defined inter-stimulus delay as the amount of time between ratios was up to the rat. Therefore, the PR20 schedule may not be sensitive enough to detect a difference in the pre-ratio pause, or impulsivity, between the two groups of animals.

Open Field

We used the open field apparatus to assess hyperactivity and anxiety-like features in our animal groups. The animals were all acclimated for two days prior to the testing session, so this was not used a measure of a response to a novel environment. We first assessed hyperactivity by measuring how many total squares animals crossed during a 10 min session in the open field maze. The results from the current study support the initial hypothesis that the SHR animals would display hyperactive-like behavior in the open field apparatus by crossing significantly more squares, during a 10 min session. This is not a novel finding as multiple SHR studies have reported similar findings (Knardahl & Sagvoldent, 1981; Qian, Lei, Castellanos, Forssberg, & Heijtz, 2010; Sagvolden, Hendley, & Knardahl, 1992; Sagvolden, Pettersen, & Larsen, 1993; Schaefer, Brackett, Gunn, & Wilson, 1978). Not surprisingly, increased locomotion via behaviors such as fidgeting, or the inability to "sit still", is prominent and heavily reported in humans with the disorder (American Psychiatric Association, 2013; DuPaul, Weyandt, & Janusis, 2011; Verret, Guay, Berthiaume, Gardiner, & Béliveau, 2010).

It was initially proposed that the SHR animals would exhibit non-purposeful explorative behavior in the open field and therefore, would spend less time in the inner zone of the open field. While we predicted hyperactivity through increased number of crossed squares, we assumed the animals would stay on the outside edges of the open field. Data from this study did not support this hypothesis as the SHR animals spent significantly more time on the inside of the

field. Rodents have a natural inclination to avoid wide, open spaces and so in the open field, animals tend to stay near the walls of the box. However, the SHR animals defied this common finding. We followed up with the EPM to assess anxiety-like behaviors in this strain. We recorded the number of rears, grooms, defecations, and urinations by each animal while in the open field. We grouped these behaviors together and found that the SHR animals reared and defecated more than the WKY. These are slightly confounding as rearing is typically viewed as an exploratory behavior and defections as an anxiety-like behavior. While we measured these types of behaviors, we did not anticipate them to inform us much about the SHR animal as a model of ADHD.

Elevated Plus Maze

Our open field data hinted at the idea that the SHR animals may exhibit less anxiety-like behavior in the open field. We followed up with the EPM. The EPM relies upon rodents' proclivity toward dark and enclosed spaces opposed to those that are elevated and open (Montgomery, 1958) and would allow us to parse out the anxiety-like behaviors we see in in the SHRs.

The elevated plus maze was used as an assessment of anxiety-related behaviors. It was hypothesized that there would be no differences in the number of open arm entries or time spent in open arms between the SHR and WKY animals. However, this study found evidence in opposition of our hypothesis. The SHR animals not only entered the open arms more frequently, they also spent significantly more time in them. A careful examination revealed that there was no difference in the amount of time the SHR and WKY spent in the distal portion of the open arms or in the center of the maze. In addition to assessing anxiety, we hoped to use the number of entries into closed the closed arms as a measure of locomotion or hyperactivity. It was

hypothesized that SHRs would exhibit more entries into closed arms due to the increased hyperactivity of these animals. The data of the current study failed to support our hypothesis.

Taken together, our EPM results indicate that the SHR animals displayed reduced anxiety-like behavior compared to the WKY controls. While we were surprised to see this finding, we have replicated what previous studies have shown regarding the SHR performance on the EPM (Ferguson & Gray, 2005; Goto, Conceição, Riberio, Frussa-Filho, 1993; Sterley, Howells, & Russell, 2011). However, these findings of reduced anxiety-like behavior in the SHR animal is at odds with the human literature that ADHD is typically comorbid with anxiety disorders in up to one fourth or one third of the patient population (D'Agati, Curotolo, & Mazzone, 2019; Pliszka, 2019; Schatz & Rostain, 2006). Further research is needed to examine the decreased anxiety-like behaviors in the SHR model.

Histology

The primary histological aim of this study was to determine if the SHR animals had a smaller dentate nucleus volume, a region of the brain previously shown to be involved in motivation, compared to the WKY controls. Our result supported this hypothesis. On average, in the right hemisphere, the SHR animals had a significantly lower average dentate nucleus volume of 1.817×10^{12} microns compared to the WKY average of 2.097×10^{12} microns. In the left hemisphere, we report that the SHR animals had a significantly lower average dentate nucleus volume of 1.677×10^{12} microns compared to the WKY average of 2.036×10^{12} microns.

Previous studies have found that lesioning or temporarily inactivating the dentate nucleus resulted in a marked decrease in the average breakpoint ratio in a standard laboratory rat signifying a role for the dentate nucleus in motivation-like behavior (Bauer, Kerr, & Swain, 2011; Peterson et al., 2012). As such, because of the decreased motivation we see in the operant

chamber, we were interested in investigating whether the dentate nucleus was fundamentally different compared to the WKY controls. The dentate nucleus projects to various areas of the brain, including the motor cortex and the PFC. The dentate nucleus is further subdivided into the dorsal and ventral regions, which have distinct and non-overlapping projections to various areas of the brain. The ventral portion of the dentate nucleus has been shown to be highly connected to areas of the PFC that have are involved in exploration, social behavior, motivation, and other complex behaviors (Bauer et al., 2011; D'Agata et al., 1993; Joyal et al., 2001; Middleton & Strick, 2000; Noblett & Swain, 2003). In the current study, we did not subdivide the dentate nucleus into the dorsal and ventral regions, which have been shown to have motor or cognitive connections, respectively. Therefore, an overall reduction in total volume of the dentate nucleus, as we see in the SHR animals, would most likely result in fewer output connections to the various PFC regions, as well as motor cortex. While we did not rule out specific motoric deficits, the data from the study provides evidence that the volume difference of the dentate nucleus is largely affecting the motivational behavior. We are not implying that the dentate nucleus is the locus of motivation, but rather that the dentate nucleus is part of the CTC pathway, and that reduction of output from the dentate nucleus hinders the overall performance of the system subsequently affecting motivation.

Given the lower SHR dentate nucleus volume, we measured cell number to determine if a reduction in neuron number was driving the reduction in dentate nucleus volume. The cells in the dentate nucleus consist of large neurons, small neurons, and neuroglia. In this study, we focused on the large neurons, of which there are two primary types. The first type consists of large cells with a smooth perikaraya and these account for 40% of the large neuron profile; whereas, the second type has a more irregular outline and accounts for 60% of profile (Chan-Palay,1977). The

neurons within the dentate nucleus project to and terminate in various regions of the brain including the midbrain, subthalamic regions, and the thalamus (Chan-Palay, 1977). The dentate nuclei terminations in the thalamus therefore make up the cerebello-thalamic portion of the CTC pathway. We acquired estimates of large neurons, without distinguishing between type, in the dentate nucleus using unbiased stereology. It was predicted that the SHR animals would exhibit a lower area fraction of large neurons in the dentate nucleus, therefore driving the volumetric difference. Evidence from this study does not support this hypothesis as we found no difference in mean area fraction of the large neurons between the SHR and WKY animals. While we found no difference in the estimates of large neuron number, there could be a variety of other factors underlying the volumetric difference.

Some of the other factors that could underlie the volumetric difference of the dentate nucleus between the SHR and WKY include differences in large neurons type, size of cells, other cells in the dentate nucleus, and abnormalities in cortical structures. Although there was not a difference in large neuron estimate, regardless of type of large neuron, it could be the case that one of the types of large neurons is fewer than average. Because the two types of large neurons of the dentate nucleus can be identified by their shape, further research should parse out and conduct neuron estimates of both the smooth type of large neuron and the irregular type of large neuron to determine if one of these types are differentially affected. Another possibility that could be driving the volumetric difference in the SHR animals is that the large neurons, regardless of type of large neurons, are smaller than normal. It may not be the case that there are fewer of them, but that the surface area of the large neurons in the SHR animals could be smaller than the WKY contributing to the smaller volume. Future studies should examine and measure large neuron cell size in the SHR animal dentate nucleus. For the most part, we have been

focusing on abnormalities of the large neurons of the dentate nucleus to be the driving reason for the volume difference. However, it could very well be possible that it is the other types of cells, such as the small neurons and neuroglial cells, that are located within the dentate nucleus that are abnormal in terms of number or size. Finally, the reduced dentate nucleus volume that we have reported in the SHR animal could have nothing to do with the actual cells contained within the structure. It is feasible that the cerebellar cortical structures that have projections to the dentate nucleus, such as various lateral hemisphere lobules, could be abnormal. Abnormalities in the cortical structures may result in fewer connections to the dentate nucleus, which ultimately results in fewer synaptic connections within the dentate nucleus contributing to the smaller volume.

We also analyzed the weight of the whole brain and cerebellum in the SHR and WKY animals, although we did not initially make any hypotheses regarding the whole brain and cerebellum weight. Upon extraction of the brain, we weighed each of them. The SHR animals had a significantly lower whole brain weight compared to the WKY animals, and this has been reported previously (Li et al., 2007; Nelson, Coulson, Myers, & Browning, 1993; Tajima et al., 1993). However, we report a significant difference of whole brain weight in the trained animals, who were exposed to the operant chamber, open field, and elevated plus maze, compared to the untrained, naïve animals. The trained animals had a significantly greater whole brain weight compared the controls. To the best of our knowledge, we are the first to report this in the SHR model. This supports the idea that the brain is plastic, especially at a young age, and can be modified by engaging in various forms of motor activity (Cotman, Berchtold, & Christie, 2007; Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013). We used young, juvenile SHR rats in attempts to approximate human children with ADHD. This is important to note for future

treatment and therapy designs for ADHD that motor activity can increase the whole brain weight, regardless of condition.

We then separated the cerebellum from the cerebrum to conduct weight measurements on just the cerebellum. Here we found no difference between the SHR and WKY animals or between the trained and untrained, naïve animals. The fact that we did not see a weight difference (g) in the SHR animals somewhat conflicts with the human literature which reports that cerebellum volumes are significantly smaller in those diagnosed with ADHD (Berquin et al., 1998; Castellanos et al., 1996; Castellanos et al., 2001; Durston et al, 2004; Valera, Faraone, Murray, & Seidman, 2007; Wyciszkiewicz, Pawlak, & Krawieck, 2017). We cannot directly compare weight to volume, so future research on the SHR cerebellum is needed.

These measurements of whole brain and cerebellum should not be taken at face value and should be interpreted lightly. While we did attempt to maintain similarity amongst all brains, it difficult to be certain without proper measurement tools. We obtained brain weights in a crude method. During brain extraction, the length of the spinal cord remaining on the brain varied on which experimenter was removing it. In addition, the olfactory bulbs and paraflocculi are often damaged during extraction. In attempts to standardize the brains prior to weight measurements, we measured and kept a specific length of spinal cord on each brain. We also removed all olfactory bulbs and paraflocculi. In the future, attempts at removing the complete spinal cord from the cerebellum, efforts to reduce damage to the cerebellum, and a more precise scale would result in more accurate whole brain and cerebellum weight measurements.

To conclude our study, we aimed to include two other replications regarding the cerebellum anatomy. First, the volume of the cerebellum vermis has been reported to be significant smaller in humans diagnosed with ADHD and in the SHR animals (Berquin et al.,

1998; Bussing, Grudnik, Mason, Wasiak, & Leonard, 2002; Castellanos et al., 2001; Hill et al., 2003; Li et al., 2007; Mostofsky, Reiss, Lockhart, & Denckla, 1998). Therefore, the cerebellar vermis was also examined in this study. It was hypothesized that the finding would remain consistent with previous literature and that the volume of the cerebellar vermis in the SHR animals would be significantly smaller than the WKY control animals. In this study, we replicated the finding that the cerebellar vermis is significantly smaller in the SHR animals compared to controls. In addition, we present a novel finding that the trained animals, regardless of animal strain, had a significantly higher vermis volume compared to the untrained, naïve controls again lending to the evidence that motor activity at a young age has positive, restorative effects on the brain (Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013).

Bledsoe, Semrud-Clikeman, & Pliszka (2009) reported that chronically-medicated children with ADHD had cerebellar vermis sizes comparable to the healthy controls, suggesting that chronic stimulant treatments helps to normalize the development of the cerebellar vermis in ADHD. Furthermore, it has been reported that an inverse relationship exists between symptom severity and regional vermis volume differences in the human ADHD population (Ivanov, Murrough, Bansal, Hao, & Peterson, 2014). Our study provides novel evidence that simple motor activity, i.e. the act of pressing a lever, increases the volume of the cerebellar vermis in both the control and SHR animals at a young age. It would be interesting to examine if the proper motor training protocol could rescue the vermis volume deficit and aid in the reduction of ADHD symptomology. Furthermore, because our finding was in juvenile rats it would be critical to follow up to see how long the vermis volume difference remained after the motor activity (lever pressing) subsided.

Purkinje cells are large neurons, that have many branching extensions, and are found in the cortex of the cerebellum between the granule and molecular layer. Purkinje cells in the cerebellum obviously play a fundamental role in movement and coordination, but also contribute to cognitive functioning, including motor learning (Ito, 2000; Lee et al., 2007). Dysfunction or lack of Purkinje cells has been shown to play a role in various neurodevelopmental and neurodegenerative disorders, including ADHD (Fatemi et al., 2002; Fukutani, Cairns, Rosser, & Lantos, 1996; Xia et al., 2013). We hypothesized that if the cerebellum vermis volume was smaller in the SHR animals, the difference could possibly be explained by a fewer number of Purkinje cells within the vermis. We proposed to do Purkinje cell counts throughout the entire vermis, but Yun and colleagues (2014) had shown that the SHR animals have fewer Purkinje cells in specifically Lobule 8 of the cerebellum vermis compared to control animals. Therefore, we aimed to and found support for the replication of this study's finding. The SHR animals display fewer numbers of Purkinje cells in the Lobule 8 of the cerebellar vermis. Lobule 8 has been shown to primarily play a role the motor control of the body, rather than one of a cognitive nature (Stoodley & Schmahmann, 2010). Interestingly, Yun and colleagues (2010), who originally reported a reduction of Purkinje cells in Lobule 8, found that animals who ran on a treadmill had significantly more Purkinje cells compared to the controls. The authors inferred that the motor activity of running substantially retarded the death of Purkinje cells in lobule 8. This result supports the possibility that regular exercise might be an effective interventional program to inhibit Purkinje cell loss in the cerebellum, and therefore potentially reduce problematic symptomology.

Future Directions

This study only scratches the surface on the SHR as a model of ADHD. Because the cerebellum is largely understudied in this animal model, the literature could benefit from a comprehensive anatomical analysis of the dentate, fastigial, and interpositus nuclei. In addition, we report a lower in dentate nucleus volume in the SHR animals. Future studies should examine the size of the cells, the types of cells, and cortical structures that project to the dentate nucleus for anatomical anomalies. From there, one could examine if the cerebellar connections to the PFC are altered in this model, as seen in humans with ADHD. In the current study, we report that the SHR animals display a low breakpoint ratio, which served as a measure of motivational level, in comparison to the control strain. The SHR animal model responds well to stimulant medication and therefore, it would be interesting to examine if pharmacological intervention in these rats would reverse the motivational deficits. In addition, our study restricted the animals' training session to a 1 hr time period. Future studies should extend the length of the behavioral sessions until the animals cease to respond. Finally, we report a higher total brain weight and a higher cerebellar vermis volume in the trained animals compared to the untrained, naïve animals. Future studies should first train rats on different motor activities, such as exercise or the acrobatic task. Following a motor regimen, animals could then be assessed on the operant breakpoint paradigm. Brain weights and volumes would then be analyzed to inform us as to how motor activity could differentially affect the brain, and if motor activity could potentially rescue motivational deficits.

Conclusions and Implications.

To the best of our knowledge, this is the first study to have used the operant chamber breakpoint paradigm with the SHR animal to model motivational deficits. We have shown that the SHR model displays lower levels of motivation compared to the control strain and that this is not due any other confounding factor that we have examined. The motivational behavioral deficit maps well on to the human literature and what we observe in humans with clinical diagnosis of ADHD. Furthermore, we have provided evidence that the dentate nucleus, one of the three cerebellar deep nuclei, a structure previously shown to play a role in motivation, is anatomically smaller in this model. In sum, we have added novel behavioral and histological results to the face and construct validity of the SHR as a model of ADHD, respectively.

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