Spaced Training in the 5CSRTT Proves Beneficial in Early Levels for SHRs

Rebecca R. Warren and Amy Jo Stavnezer
College of Wooster, Wooster, Ohio 44691

ADHD is a common neurodevelopmental disorder characterized by inattentiveness, impulsivity, and hyperactivity. Due to these characteristics, children with ADHD typically exhibit lower academic performance compared to neurotypical children. However, a mechanism known as spaced training has been found to increase academic performance in neurotypical children. Even with its benefits, this mechanism has yet to be studied in an ADHD population. Spaced training involves studying over short periods for multiple days rather than multiple hours in one day. Using a rat model of ADHD, known as the SHR strain, the goal of the present study was to assess how spacing training from 90-trials a day to 45-trials a day in the 5-Choice Serial Reaction Time Task (5CSRTT) would improve performance abilities. The 5CSRTT is an attention and reward-based task for rodents. It was found that spacing was beneficial during earlier levels of 5CSRTT training that required less focused attention. Furthermore, in the earliest levels of training, it took only four averaged days to reach optimal performance in some of the spaced rats. In addition, during the earliest level of training, males in the spaced group benefited more than females, closing the previously reported sex gap that found female SHRs performed better than male SHRs in the 5CSRTT. Due to the hyperexploratory nature of the SHR, spaced training in 5CSRTT did not impact hyperactivity in the open field but did validate the hyperactive nature of this strain. These findings imply that by simply spacing out training, the SHRs demonstrate a significant improvement in some training levels of the 5CSRTT. Future studies should assess the effect of spacing on academic work and attentiveness training in children with ADHD.

Abbreviations: ADHD – Attention Deficit Hyperactivity Disorder; SHR– Spontaneously Hypertensive Rat; 5CSRTT – 5-Choice Serial Reaction Time Task; TR – Training Level

Keywords: ADHD; Massed Training; Open Field; Spaced Learning; Attention; Neurodevelopment

---

Introduction

When studying for a test, a student has two options; they can either study for five hours the night before the exam, or they can study an hour a day for five days before the exam. The first method is called massed practice, better known as cramming, and the second method is called spaced training (Carey, 2015). Spaced training is a learning strategy that involves studying a certain amount of material over multiple days or sessions versus all at once (Rohrer & Taylor 2006). We will use the term "spaced training," "spacing out," and "spaced out" to describe this phenomenon. Spaced training is extremely beneficial for memory of new vocabulary, math problems, and working memory in general (Kornmeier et al., 2014; Rohrer & Taylor, 2006; Wang et al., 2014). Its beneficial effects can be seen across populations from animals, to neurotypical and neurodivergent people (Commins et al., 2003; Grassi, 1971; Kerfoot et al., 2007). Even though spaced training is a popular method in the cognitive and behavioral neuroscientific field, it has yet to be assessed in a population with Attention Deficit Hyperactivity Disorder (ADHD) or its animal model, the Spontaneously Hypertensive Rat (SHR). Further, the effects of spaced training have been limited to memory, and there is potential to explore other areas of this training such as attention, as giving individuals a break between trials could heighten their focus and attention. The aim of the current study is to assess if spaced training will improve acquisition of a task and if spaced training can reduce hyperactivity, which may impair performance, in the SHR strain.

ADHD, is a neurodevelopmental disorder that impairs many children's overall well-being and performance in school. The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) describes ADHD as being characterized primarily by inattention, impulsivity, and hyperactivity (American Psychiatric Association, 2013). Patients can have a primarily inattentive type, meaning that their level of inattention is greater than their hyperactivity, or a combined type, meaning that there is a presence of both hyperactivity and inattention in the patient (Epstein & Loren 2013).

The current prevalence of ADHD in the United States is about 10.84% and has recently increased due to an expansion in the age-of-onset criterion in the DSM-V (Vande Voort et al., 2014). Though prevalence rates were increasing since the release of the DSM-IV, there were similar prevalence rates of the disorder in non-US countries as well (Faraone et al., 2003). This would indicate that ADHD is less the result of cultural practices in the United States and is more a valid behavioral and neurodevelopmental disorder that affects many children from differing races, ethnicities, and cultures. Furthering this claim, there are also many neurological differences associated with ADHD.

Brain Areas and Neurotransmitters Associated with ADHD

ADHD's relation to the temporo-parietal network is an important aspect of the attention problems seen in patients. When a visual attention task was given to medication naïve children with ADHD and their brains were scanned, the temporo-parietal regions were under-activated (Rubia et al., 2007). Specifically, this network has been found to play a role in detecting novel stimuli in a sensory environment which requires high amounts of attention and focus (Downar et al., 2000). Therefore, under-activation in this network indicates a deficit in allocating attention to higher-order visual processes. Children with ADHD may have difficulty focusing on certain stimuli because of the interaction between these two lobes. It has also been observed that both medication naïve children and medicated children with ADHD were more likely to have low activation in their temporo-parietal network when performing a cognitive flexibility task, specifically task-switching (Smith et al., 2006). This visual-spatial task uncovered that they had lower response inhibition—the ability to not respond when it is inappropriate— than controls and therefore lower attention to change in detail.

A deficit in the fronto-parietal network often indicates a deficit in visuospatial attention (Waldie & Hausmann, 2010) as well as prolonged attention (Schneider et al., 2010). An fMRI was used during a continuous performance test to assess the prolonged attention of adults with ADHD and emphasized that symptom severity was highly correlated with decreased activity in the parietal cortical regions (Schneider et al., 2010). Essentially, the worse the person's attention, the smaller the activation in their fronto-parietal network. Therefore, ADHD can be understood to be highly attention-based and there are neurological correlates to these behavioral differences. The lower activation in these areas and networks are partially due to the neurotransmitters, dopamine and norepinephrine.

Dopamine is an important neurotransmitter in the reward pathway, nigrostriatal pathway, and prefrontal areas (Levy & Swanson, 2001). Fewer D2/D3 receptors were noted in the reward pathway of those with ADHD who had less motivation to work hard (Volkow et al.; 2011). This partially explains why maintaining focus on non-rewarding tasks for those with ADHD can be difficult.

Since ADHD typically indicates high inattention and impulsivity, its relationship with norepinephrine is unsurprising. Norepinephrine is a neurotransmitter that is largely involved in response-inhibition and learning from feedback (Aston-Jones & Gold, 2009; Chamberlain et al., 2006). Norepinephrine binding in the ADHD brain has been found to be influenced by a single nucleotide polymorphism (SNP) in the norepinephrine transporter gene (NET), a transporter that recycles extracellular norepinephrine. A study analyzed the level of hyperactivity in children with ADHD and found, due to the presence of an SNP in the NET, that their hyperactivity strongly correlated with lower binding of norepinephrine in the cerebellum (Sigurdardottir et al., 2016). This lower binding indicates that the NET is overactive when this SNP is present and thus removes too much norepinephrine from the synapse possibly contributing to poorer response inhibition (Kollins et al., 2008). The involvement of dopamine and norepinephrine in ADHD-related symptoms have led to the creation of medications that influence these neurotransmitters in the synapse. Yet the idea of giving stimulants, such as methylphenidate, to children can be fearful for a parent so it is natural to consider alternative treatments.

ADHD Learning Difficulties and Associated Rodent Model

Those diagnosed with ADHD often perform worse on memory tests and have poorer learning skills than the average individual. These difficulties are mainly due to poor executive function which can negatively affect one's ability to study, plan, and memorize new information. This is partly why children and adolescents with ADHD perform more poorly in school (Ek et al., 2011).

Even though better time management (Reaser et al., 2007) and breaks between studying (Brand et al., 2002) are assumed to be helpful for those with ADHD, no study on spaced training and its effect on those with ADHD have been published. There have also been no published studies that look at the effects of spaced training on SHRs.

Spontaneously Hypertensive Rats, or SHRs, are a common animal model used to assess ADHD due to the similar attributes they share with patients with ADHD. Specifically, this strain, derived from Wistar Kyoto Rats (WKY rats), has been found to have inattentiveness, impulsivity, and hyperactivity on similar levels to children with ADHD (Sagvolden et al., 1992; 2009). SHRs also show similar performance on delayed reinforcement tasks to children with ADHD. In particular, SHRs responded less once a delayed reinforcement was introduced, but had higher responses when the reinforcement was consistent (Johansen et al., 2005). This means that SHRs gave up on responding and could not stay consistently focused and interested in the task if there was a delay between rewards. Children who had hyperactive tendencies were also more likely to go for a small reward than wait a period of time for a big reward similar to the SHRs (Sonuga□Barke et al., 1992). This highlights how the SHR is a good model to observe the effects of spacing because it may be easier for SHRs to learn under conditions where focus does not need to be extended. Since spaced training is a widely studied topic in the behavioral neuroscience field, it should also be studied in SHRs.

Spaced Memory Training in Humans and Rodents

Spaced and massed training are two types of training that differ in the amount of rest between training intervals. Massed training involves training continuously without significant rest while spaced training involves rest between training intervals (Lindsay, 2012). While massed training has been found to be more helpful for perfecting motor skills (Ahmadvand et al., 2016; Lakshmanan et al., 2010), spaced training results in a higher retention of information and therefore, higher performance on memory tasks (Roediger & Pyc, 2012). Many human studies have found memory to benefit from spaced training.

In a neurotypical population, spaced training has been shown to have incredible benefits for learning. Math is often a difficult subject to learn, so Rohrer & Taylor (2006) had college students practice a math problem. The group performing spaced training, practiced five problems and then had a one-week interval before the next five problems while the massed group practiced ten problems all in one session. There was either a one-week or four-week interval before the final test. Though there was no difference on the test between the spaced and massed group at the one-week interval, there was at the four-week interval. Therefore, since the spaced group was more likely to successfully retrieve the skill after a longer period of time there is emphasis for spaced training leading to higher consolidation and memory for the task. Other studies on neurotypical populations have found similar results from spaced training for clinical knowledge and retention of words in German and Japanese (Kerfoot et al., 2007; Kornmeier et al., 2014) as well as on a task of fluid intelligence (Wang et al., 2014).

Studies on non-neurotypical individuals have found memory results similar to those done on neurotypical people. Spaced training helped with verb-learning in children with specific language impairment (SLI) at five to six years old (Riches et al., 2005). Specifically, children with SLI were able to recall more verbs after spacing their practice than children who did not. Children with SLI have similarities to children with ADHD in language, social skills, and executive functions such as working memory (Cohen et al., 2000; Bruce et al., 2006), therefore, the benefits seen in spaced training for children with SLI could be achieved in children with ADHD. Further, spacing out training in cognitive training could create a stronger carry-over effect.

The carry-over effect seen when attention and working memory are cognitively trained is well-researched. Carry-over is defined as the ability to take skills gained from one task and apply those skills to a new task or harder version of the same task. Cognitive training involves performing tasks that exercise and stimulate the brain. Those with ADHD who received memory and attention training through activities like cognitive video games showed improvement in working memory and attention. Multiple studies have found that after training occurred, a reduction in ADHD symptoms were seen (Gray et al., 2012; Halperin et al., 2013; Lim et al., 2012). Training attention has also been shown to reduce inattentiveness in those with ADHD, but these effects were only seen in the case of an eight-week intensive training and no spaced training was performed (Lim et al., 2012). Thus, it is possible that improving attention spans could be even more possible if training was spaced out. This is why we plan to space behavioral training in a task that measures ADHD-like symptoms and, thus, observe whether limiting required attention decreases these impairing symptoms. Animal studies may be a good way to test the potential benefits of spaced training in an ADHD population.

Multiple studies have observed the spacing effect in rat populations when spreading training out over multiple days. Recent spatial memory tests have involved the Morris water maze, a test in which a rat learns the location of a platform submerged in the water. Rats in the spaced condition (16 trials with a 20-minute inter-trial interval) swam in the platform area longer than those in the massed condition (16 consecutive trials), thus highlighting improved spatial memory performance (Commins et al., 2003). Similar results were also found during spaced training in the object displacement task (Bello-Medina et al., 2013; Commins et al., 2003) and in desensitization to fearful stimuli (Baum & Myran, 1971). A review article observed that spaced training over longer periods of time, specifically over 24 hours, led to the highest performance on reinforcement tasks (Smolen et al., 2016).

People with ADHD have lower attention spans which can inhibit their ability to learn. During both passive and active listening tasks, children with ADHD had lower arousal, and therefore a lower attention span, than controls did (Shibagaki et al., 1993). Thus, focusing on improving attention spans is essential to helping those with ADHD. Many studies have attempted to improve attention spans in neurotypical children and children with ADHD by giving them “brain breaks” or moments where they do not have to focus (Chaney, 2005; McGinley, 2011; Reiber & McLaughlin, 2004; Weslake & Christian, 2015). Thus, breaks, especially ones with low physical activity, are beneficial for the attention span (McGinely, 2011; Weslake & Christian, 2015). Often, teachers will institute more breaks for children with ADHD to help increase attention span and also set realistic goals the child feels they can meet. Ultimately, if the child has to focus for a shorter period of time, they will become more encouraged to continue later because less is being asked of them in each unit of time (Busch, 1993; Reiber & McLaughlin, 2004).

Tasks to Measure ADHD-like Symptoms in Rats

The Five-Choice Serial Reaction Time Task (5CSRTT) conditions a rat to poke their nose in a slot with a light over it to receive a reward. It is often used with SHR rats to measure their symptoms and general performance on attention-based tasks (Hunziker et al., 1996). There are different training levels for the task that involve the light being on for shorter intervals (Asinof & Paine, 2014). This task specifically tests for impulse control and attentional ability by necessitating focus from the rat when the light comes on so they can receive the reward. Sex differences have been found during this task. When SHRs' baseline performance in this task was compared to WKYs', an SHR control strain, male SHRs were less attentive than female SHRs, even though they were equally impulsive (Bayless et al., 2015). However, spaced training could alleviate this gender gap by allowing the male SHRs to pay more attention and thus score higher in attentiveness. Females may not benefit from the spaced training as much as the males, but they may still benefit more than the females receiving massed training.

Essentially, spaced training this out would allow for the SHRs to sustain their attention for a shorter period of time, and an increase in performance could be seen. Asinof and Paine (2014) mention in their paper that the rat can take months to properly learn the 5CSRTT, but they do not mention the duration of training levels, which are typically thirty minutes long, as a possible hindrance to their performance. If an SHR rat already has a low focus and attentional ability, it makes logical sense to make the task shorter, so they do not have to pay attention for an extended period of time. Since the 5CSRTT is a great way to test for ADHD-like symptoms such as inattention and impulsivity, it is possible to measure if spreading out trials over multiple days has an effect on ADHD-like symptoms. Impulsive responses are seen as faulty inhibitory responses or premature responses that involve responding before the light comes on (Fox et al., 2008; Asinof & Paine, 2014). Inattentive responses are categorized as a low number of correct responses. Attentiveness overall is categorized as a high number of correct responses and a low number of premature responses. (Asinof & Paine, 2014). Thus, these measures can help researchers determine whether the subject is displaying ADHD-like symptoms while also assessing if the rat is becoming more focused and less impulsive as training goes on. However, the ADHD symptom this task does not directly measure is hyperactivity.

SHRs demonstrate hyperactive behavior in the open field. The open field is a task in which a rodent is placed in an empty box and their activity levels are measured. The SHR strain travels a much greater distance indicating a tendency toward hyperactivity in comparison to control strains (Knardahl & Sagvolden, 1979). Female SHRs typically traveled further distances than male SHRs in the open field (Chelaru et al., 2012; Cierpial et al., 1989). In the present study this sex difference was predicted to occur, and the open field was used to see if training in the 5CSRTT would have an effect on both male and female activity levels.

In a previous experiment in our lab that used both the 5CSRTT and the open field in SHRs (with massed testing only), a secondary data analysis was run to compare activity levels to performance on the 5CSRTT. There was a significant correlation in the SHRs with high activity levels and lower performance on the 5CSRTT, specifically for a middle-difficulty training schedule. Therefore, it is important to look at whether spaced training could have an indirect effect on hyperactivity in the open field through training in the 5CSRTT. Another way to measure activity in the open field is to record the SHR's rearing activity. Rearing is characterized by the rat standing on its hind legs at any angle, supported or unsupported by a wall (Sturman et al., 2018). Rearing has been found to highly correlate with activity levels in the open field and is therefore a good secondary measure to observe when looking at SHR activity levels (Díaz Morán et al., 2014). Thus, by measuring ADHD-like symptoms with the 5CSRTT and the open field, a potential carry-over effect of spaced training on activity level can be measured.

Hypotheses

The general aim of the present study was to discover if spacing attentiveness training affected ADHD symptoms including inattention, impulsivity, and hyperactivity. 1) An overall improvement in performance on the 5CSRTT and an overall decrease in hyperactivity was hypothesized for both groups due to the positive benefits of cognitive training in humans with ADHD (Gray et al., 2012; Halperin et al., 2013; Lim et al., 2012). 3) 2) An increase in performance in the 5CSRTT was hypothesized to be related to lower activity levels and rearing in the open field. This is due to decreases in hyperactivity also being affected by simply training memory or attention (Gray et al., 2012; Lim et al., 2012). 3) Females were hypothesized to exhibit higher activity and rearing based on previous findings (Chelaru et al., 2012; Cierpial et al., 1989). 4) Improvements in attention, impulsivity, and hyperactivity were hypothesized to be more significant in the spaced group because of spaced training's well-known effect on memory and attention in both rodents and humans (Commins et al., 2003; Grassi, 1971; Kerfoot et al., 2007; Kornmeier et al., 2014; Riches et al., 2005; Wang et al., 2014). 5) The spaced group was also hypothesized to have a greater carry-over effect between training levels. For example, if a rat had a high attentiveness score one week, they would be able to maintain that score when they moved to a harder level. There was evidence this carry over effect would occur because training in cognitive video games has led to higher attentiveness in other tasks (Gray et al., 2012; Halperin et al., 2013; Lim et al., 2012). 6) Lastly, males in the spaced group were hypothesized to experience a larger improvement in their 5CSRTT attention scores. Bayless et al. (2015) found a strain difference in attentiveness scores for SHR males, but not for females in baseline measures. Spaced training was predicted to therefore close this gap by improving attentiveness.

Materials and Methods

All procedures were approved by the IACUC Board at the College of Wooster; approval number S2019-05. No pain was involved in the testing.

Subjects

There were twenty-four SHRs (12 females, 12 males) in this experiment. The SHRs were 2 months old at the beginning of testing. The SHRs were placed at random into one of two groups: massed (M) or spaced (S) training. These groups were equal in sex and number and were balanced across litters. All SHRs were same-sex-paired housed throughout the duration of the study. No WKY rats, control strain, were used in this experiment because unpublished data from our lab found that WKYs performed significantly worse in the 5CSRTT than SHRs under massed conditions, and because this study was specifically focused on alleviating ADHD-like symptoms in SHRs.

5CSRTT Materials

Three Med Associates 5CSRTT chambers and adapted MedPC code were used to train the rats. Twenty-four hours before the task was conducted, rats were food deprived to 85% of their body weight (Asinof & Paine, 2014). During the task, the rats were rewarded with sugar pellets. Each chamber contained five infra-red activated slots for the rats to poke their noses in and the interiors of the chambers were all 23.5" x 22" x 14" (Figure 1). Before each training session, chambers were wiped down with 70% ethyl alcohol and paper towels were placed in the droppings pan.

5CSRTT Procedure

All SHRs received Continuous Reinforcement (CRF) training before they began the open field and received regular training, this involved teaching them how to receive the reward by having all lightbulbs remain on. The massed group was trained 90 trials per day for four days on each TR. The spaced group was trained with 45 trials per day for eight days on each TR. For data analysis purposes, two complete 45 trial sessions were totaled together to match the 90 trial sessions of the massed group. Testing lasted eight weeks. Rats progressed through the training levels in increasing order (Table 1). Once the training for each TR schedule (4 x 90 or 8 x 45) was completed, all rats moved to the next training level. The day before testing began, rats were food deprived to ensure motivation to complete the food-based task. To perform the task correctly and get a reward, the rat had to poke their nose into the correct slot. The correct slot was indicated with a lightbulb. If the rat picked the correct slot, they received a reward. Performance on the task was recorded by the number of correct responses and improvement on the task over time. Impulsivity was recorded by the number of premature responses made by the rat (Figure 2).

TR1 is the first training level the SHRs were taught after the CRF training. This training level is considered the easiest, with the stimulus duration being 30 seconds and the amount of time given to respond, or limited hold, being 30 seconds (Table 1). Attention is necessary to do well on this level, but it is also the least demanding of all the training levels.

TR3 is the second training level the SHRs were taught after completion of TR1. It is also considered a fairly simple training level with the stimulus duration being 15 seconds and the limited hold also being 15 seconds (Table 1). Yet there is a significant decrease in the amount of time the rat sees the light and can respond, so this training level puts a higher tax on attention than TR1.

TR4 is considered much harder than TR3 with the stimulus duration only being five seconds and the limited hold being 10 seconds (Table 1). Given that the rat had very little time to focus on the light and a much shorter time to respond, high attentiveness was essential to performing well during this training level.

TR5 was the last training level taught to the SHRs. It is not the hardest training level that can be implemented, but it is the most difficult one in this study. It has a stimulus duration of two seconds and a limited hold of five seconds. This training level requires more attentiveness than all the training levels prior given that the rat has very little time to focus on the light and very little time to respond.

Animals were also immediately fed after they completed the task, for one hour during training weeks, or ad lib if training was finished for the week or TR.

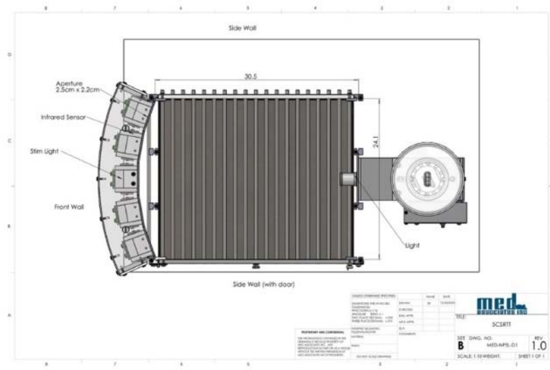

Figure 1: Schematic shows the interior and exterior dimensions of the 5CSRTT chamber. The SHR would respond to the stimulus light by poking their nose in the hole under the illuminated light during a trial and then gain their reward from the magazine area. The infrared sensors recorded all pertinent behaviors and created all associated measures (courtesy of Zachary Beebe, Mechanical Engineer, MedAssociates).

Table 1. Training Levels

Training Stage	Trials/Session	Stimulus Duration (SD)	ITI Duration	Limited Hold (LH)	Time Out (TO)
TR-1	Massed (90 trials or 30 min) Spaced (45 trials or 15 min)	30	2	30	2
TR-3	Massed (90 trials or 30 min) Spaced (45 trials or 15 min)	15	3	15	3
TR-4	Massed (90 trials or 30 min) Spaced (45 trials or 15 min)	5	3	10	3
TR-5	Massed (90 trials or 30 min) Spaced (45 trials or 15 min)	2	3	5	3

Note: The stimulus duration (SD) indicates how long the lightbulb was on in seconds. The ITI or the intertrial interval was the amount of time between each trial. Limited hold (LH) was the amount of time the SHR had to respond. Time out (TO) was the amount of time the SHR had to wait after giving an incorrect response. SD, ITI, LH, and TO were all recorded in seconds. TR2 was not included in this study due to high similarity to TR3.

Open Field Materials

The SHRs performed the open field task in a 3'x 3'x 18" wooden open field. It contained a painted black bottom and painted white sides. Activity levels, such as distance traveled and rearing, were recorded with an overhead video camera. Nature's Miracle was used to wipe down the open field and remove any odor between each animal. A start box was placed in the open field for the initial 10 seconds of the task. This allowed for the SHR to start the trial in the same location.

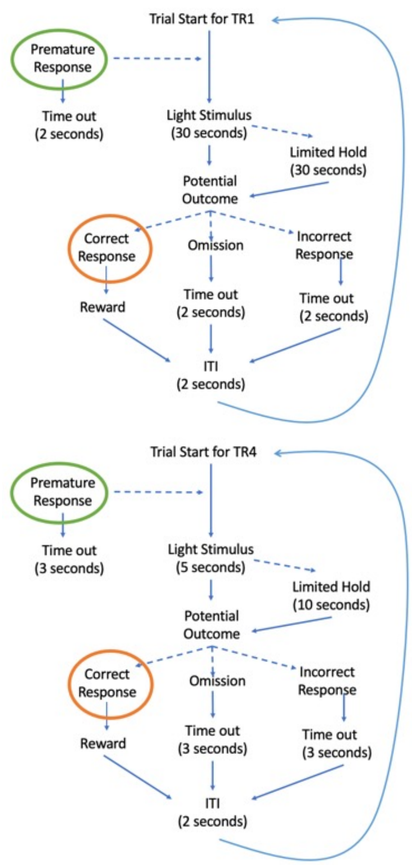

Figure 2: These flow charts represent the order of trial progression for TR1 and TR4. Premature response, circled in green, was used as the impulsivity measure. Correct response, circle in orange, was used as the attention and accuracy measure. Note the decreased presentation of the illuminated light stimulus and limited hold duration and the increase in time out duration between the two training sessions (adapted from Asinof and Paine, 2014).

Open Field Procedure

At the end of each training session (four or eight days), the rats were placed in the open field to measure their activity levels. Indirect, incandescent lighting was used to not stress the animals (Katz et al., 1981). The start block was removed after 10 seconds and the camera began recording the SHR's activity. Rearing was recorded by tallying every time the researcher observed it occur. Both supported and unsupported rearing was recorded, but it was not differentiated between for statistical analysis. After five minutes elapsed the recording was stopped, and the rat was returned to their home cage. The field was cleaned before the next subject began the task. Distance traveled was then analyzed with the Noldus Ethovision 12 software program.

Results

The main purpose of this study was to determine whether spaced training would improve performance by increasing attentiveness and lowering hyperactivity and impulsivity scores in the SHR strain of rats. To the authors' knowledge spaced training in SHRs has not been reported in any published research.

5CSRTT Training Level 1

5CSRTT data were analyzed using a 2x2x4 ANOVA, with treatment and sex as between subject variables and days as within subjects, unless otherwise noted. As expected, the spaced group exhibited higher percent correct scores than the massed group in TR1, $F(1, 20) = 9.443$, $p = 0.006$, $\eta2 = 0.321$. There was no main effect of sex in TR1, $F(1, 20) = 1.980$, $p = 0.175$. There was, however, a significant interaction between group and sex, $F(1, 20) = 5.592$, $p = 0.028$, $\eta2 = 0.219$. A secondary independent t-test was run and males in the spaced group performed significantly better than males in the massed group, $t(10) = 3.923$, $p = 0.003$, $d = 0.975$, while females groups did not differ (Figure 3). There was also a significant within subjects effect of days for percent correct scores, $F(3, 60) = 63.835$, $p < 0.001$, $\eta2 = 0.761$ meaning that both groups improved significantly over the days they trained. There was no significant interaction between days and group, days and sex, or between sex, group, and days (all ps > 0.084) (Figure 4).

A 2x4 ANOVA for premature responses, found no effect of training $F(1, 22) = 0.197$, $p = 0.662$, indicating that spaced training did not alter impulsivity. A day effect was seen in premature responses, but instead of significantly decreasing their premature responses over training days, indicating lower impulsivity, an increase in premature responses was observed for both groups, $F(3, 66) = 37.686$, $p < 0.001$, $\eta 2 = 0.631$. There was no significant interaction between days and group, $F(3, 66) = 0.479$, $p = 0.698$.

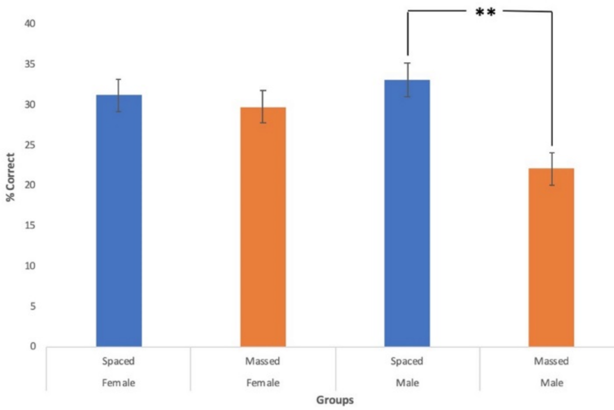

Figure 3: Males in TR1, specifically in the massed group, performed significantly worse than males in the spaced group while females in both groups did not significantly differ. Thus, spaced training benefited males more than females. (** = p < 0.01)

5CSRTT Training Level 3

The spaced group performed significantly better than the massed group in percent correct responses, $F(1, 20) = 19.219$, $p < 0.001$, $\eta 2 = 0.490$. There was no main effect of sex or interaction between treatment and sex in this training level (all ps > 0.05). A significant within subjects effect of days was seen for their percent correct scores, indicating that both groups improved significantly over the days they trained, $F(3, 60) = 41.250$, $p < 0.001$, $\eta 2 = 0.673$. There was also a significant interaction between days and group, $F(3, 60) = 4.345$, $p = 0.008$, $\eta 2 = 0.178$ (Figure 4). A paired sample t-test was run to compare the massed and spaced groups' improvement during TR3. When day one versus day four was looked at, both the spaced, $t(11) = -5.051$, $p < 0.001$, $d = 0.933$ and massed group, $t(11) = 10.045$, $p < 0.001$, $d = 0.997$ had a significant difference. Yet when scores were compared between day two and day four, the massed group still significantly differed in their percent correct scores, $t(11) = -5.856$, $p < 0.001$, $d = 0.470$ while the spaced group did not ($p = 0.163$). These data indicate that the spaced group hit their optimal performance on day two and did not improve further, while the massed group continued to improve (Figure 4). There was no significant interaction between days and sex or sex, days, and group (all ps > 0.05; Figure 4).

The spaced group also exhibited significantly lower premature responses than the massed group, $F(1, 20) = 16.661$, $p = 0.001$, $\eta 2 = 0.454$. There was a significant decrease in premature responses over the days they trained, $F(3, 60) = 12.736$, $p < 0.001$, $\eta 2 = 0.389$ unlike in TR1. There was a group by day effect for premature responses as well, $F(3, 60) = 4.792$, $p = 0.005$, $\eta 2 = 0.193$. A paired sample t-test was run to compare the slopes of premature responses between the massed and spaced groups. Between day one and day four, both the massed, $t(11) = 4.440$, $p = 0.001$, $d = 0.847$ and spaced groups, $t(11) = 3.201$, $p = 0.008$, $d = 0.858$ significantly decreased in their premature responses.

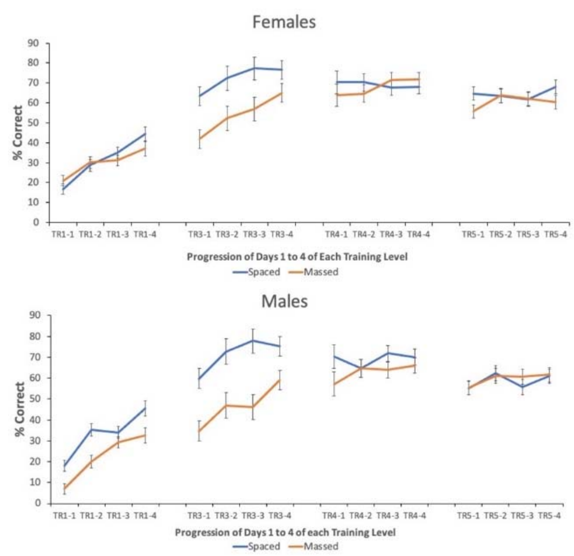

Figure 4: This demonstrates that the spaced group performed significantly better than the massed group during TR1 (p = 0.006) and TR3 (p < 0.001) in their percent correct scores. It also demonstrates that both groups substantially improved over days during TR1 (p < 0.001), TR3 (p < 0.001), and TR5 (p = 0.023), but not during TR4 (p = 0.181). It is also seen in TR3 that the spaced group reached their optimal level of performance by day two while the massed group was still improving (p = 0.008).

Between day two ($M = 76.27$, $SD = 49.58$) and day four ($M = 56.36$, $SD = 42.97$) though, the massed group significantly decreased in their premature response, $t(10) = 2.440$, p = 0.035, d = 0.182, while the spaced group did not significantly decrease (p = 0.520). Therefore, similar to their percent correct responses, the spaced group had already hit their optimal level of performance for premature responses by day two and did not decrease much further. The massed group, on the other hand, still had room to decrease.

5CSRTT Training Level 4 & 5

For percent correct and premature responses, there were no main effects of spacing, sex, or an interaction for TR4 or TR5 (all ps > 0.05). There was no significant effect of days for either measure for TR4 and no interactions with days either. There was a significant effect of days with percent correct on TR5 $F(3, 60) = 3.395$, p = 0.023, $\eta 2 = 0.145$, but the increase was small, with only a 5% improvement between days 1 and 4. There were no interactions with percent correct scores over days, and no effect of premature responses on days during TR5.

Carry Over Effects

It was hypothesized that carry-over effects, the ability to take skills gained from one task and being able to apply those skills to a new task or harder version of the same task, would occur more in the spaced group as complexity was increased. Carry-over was considered to have occurred if a group, after completing one TR, performed just as well on the next TR's first day of training.

Paired sample t-tests were used to measure carry-over effects by comparing day four scores to the first day scores between each TR level. When comparing percent correct responses on day four of TR1 (M = 45.00, SD = 9.26) to the first day of TR3 (M = 61.57, SD = 12.31), the spaced group had a significant increase in their scores, $t(11) = -7.700$, p < 0.001, d = 0.961 while the massed group did not, $t(11)$ = -1.279, p = 0.227.

For premature responses, when comparing day four of TR1 (M = 55.17, SD = 12.02) to the first day of TR3 (M = 112.50, SD = 46.75), premature responses significantly increased in the massed group, $t(11) = -4.080$, p < 0.001, d = 0.984. In the spaced group however, premature responses on day four of TR1 (M = 51.58, SD = 26.52) and the first day of TR3 (M = 51.67, SD = 25.77) did not significantly differ, $t(11) = -0.016$, p = 0.988. This indicates that the spaced group carried over a higher level of attentiveness.

When comparing day four of TR3 (M = 75.93, SD = 7.45) to the first day of TR4 (M = 70.28, SD = 11.87), the spaced group had an approaching significant decrease in their percent correct scores, $t(11) = 2.001$, p = 0.071. In the massed group however, the percent correct responses on day four of TR3 (M = 62.04, SD = 13.98) and the first day of TR4 (M = 60.56, SD = 14.83), did not significantly differ, $t(11) = 0.457$, p = 0.657.

When comparing day four of TR4 (M = 69.09, SD = 6.17) to the first day of TR5 (M = 59.91, SD = 7.59), the spaced group significantly decreased in their percent correct scores, $t(11)$= 4.510, p = 0.001, d = 0.902. The massed group also had a significant decrease in percent correct responses on day four of TR4 (M = 68.85, SD = 11.28) versus the first day of TR5 (M =55.55, SD = 9.00), $t(11) = 5.544$, p < 0.001, d = 0.891.

For day four of TR3 (M = 25.58, SD = 15.00) and the first day of TR4 (M = 28.00, SD = 25.45), the spaced group did not significantly differ in their premature responses, $t(11)$ = -0.448, p = 0.663. For the massed group, day four of TR3 (M = 58.58, SD = 41.68) and the first day of TR4 (M = 34.92, SD = 25.40) significantly decreased, $t(11) = 2.750$, p = 0.019, d = 0.389. Therefore, though the massed group decreased their premature responses significantly, the spaced group did not experience a significant increase in premature responses. Therefore, it can be suggested that the spaced group, when switching levels, maintained their low premature responses into TR4. The massed group, on the other hand, may have been catching up to the spaced group in training through carry-over, emphasizing the decrease in their premature responses.

Finally, for day four of TR4 (M = 31.17, SD = 12.97) and the first day of TR5 (M = 24.50, SD = 13.24), premature responses did not significantly differ for the spaced group, $t(11)$ = 1.604, p = 0.137. Day four of TR4 (M = 22.27, SD = 13.80) and the first day of TR5 (M = 28.73, *SD* = 16.27) also did not significantly differ for the massed group, $t(10) = -1.462$, $p = 0.175$.

Open Field Rearing & Distance Traveled

There was no significant main effect of group on distance traveled (all $ps > 0.492$) in the open field indicating that spaced training in the 5CSRTT did not affect hyperactivity through carry over. As expected, there was a significant effect of sex. Females traveled a greater distance, $F(1, 20) = 57.665$, $p < 0.001$, $\eta 2 = 0.742$ and reared more, $F(1, 20) = 45.127$, $p < 0.001$, $\eta 2 = 0.693$ than males in the open field. There were no sex interactions for either rearing or distance traveled (all $ps > 0.05$). There was a statistically significant increase in distance traveled over days, $F(4, 80) = 4.583$, $p = 0.002$, $\eta 2 = 0.186$ (Figure 5). There was a significant positive correlation with rearing and distance traveled, $r = 0.720$, $p < 0.001$. This indicates that the SHRs who traveled the furthest distance also performed the most rearing.

To test whether hyperactivity in the open field and performance in the 5CSRTT were related and to replicate findings found in our lab, another correlation was run. This correlation compared the percent correct scores for day four of TR5 to the distance traveled in the open field after training in TR5. No significant correlation was found, $r = 0.169$, $p = 0.431$, which did not replicate earlier findings in our lab.

As spaced training benefitted learning and decreased impulsivity in the 5CSRTT in TR1 and TR3, we wanted to explore if spaced training would carry over to decrease activity in the OF. We conducted 2, 2 x 2 x 2 repeated measures ANOVAs comparing Day 1 to Day 2 to assess the impact of TR1, and Day 1 to Day 3 to assess the impact of TR3 training.

In both analyses, there was a statistically significant effect of days (both $ps <0.01$), but in the direction opposite the hypothesis. Activity increased following 5CSRTT training. There was no significant effect of spacing or interactions, but the sex difference was upheld with females traveling a significantly greater distance in both analyses ($ps <0.001$).

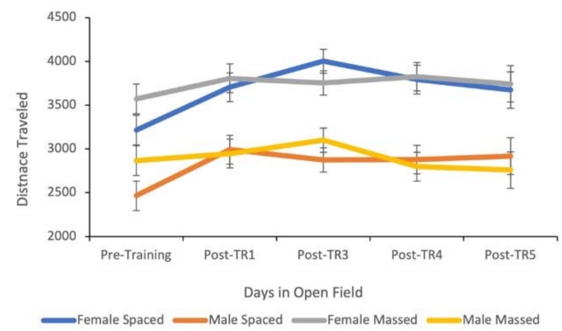

Figure 5: This figure indicates that over all days, there were no group differences in distance traveled. There was however a large difference in sex, with females traveling significantly more. This was not modified by group, days, or days and group though. A large increase can also be seen from day 1 to day 2 for both groups, with the spaced group increasing slightly more.

Discussion

5CSRTT

It was predicted that spacing out training in the 5CSRTT by having 45 trials over eight days instead of 90 trials over four days would increase percent correct scores and decrease premature responses, demonstrating improved learning. Human studies have shown that attentiveness training leads to better attentiveness (Gray et al., 2012; Halperin et al., 2013; Lim et al., 2012) and that spaced training in humans and rats is beneficial for long-term memory (Commins et al., 2003; Grassi, 1971; Kerfoot et al., 2007; Kornmeier et al., 2014; Riches et al., 2005; Wang et al., 2014). Overall, animals improved in percent correct responses for TR1, TR3, and TR5, and improved in impulsivity during TR3. In TR1 and TR3, spaced training did indeed lead to higher percent correct responses and during TR3, spaced training led to decreased impulsivity. Additionally, during TR3, spaced training allowed the animals to reach their optimum level of performance by day two while the massed group was still catching up (Figure 4).

The spaced group performing better in fewer days than the massed group supports our hypothesis on carry-over. It was hypothesized that if a group, after performing well in one TR, performed just as well on the next TR's first day of training, carry-over had occurred. Literature on carry-over effects has found that training attentiveness via cognitive video games has led to higher attentiveness in children with ADHD (Gray et al., 2012; Halperin et al., 2013; Lim et al., 2012). Our findings replicated that literature from TR1 to TR3 for percent correct responses and premature responses.

From the last day of TR1 to the first day of TR3, which were separated by one week, the spaced group performed significantly better in their percent correct scores. They also did not decrease in their premature responses, which were already quite low. The massed group on the other hand, performed the same in their percent correct scores and significantly increased in their premature responses. Therefore, the decreased impulsivity in the spaced group may have assisted in accuracy by carrying over from TR1 to TR3. The impact of spaced practice carry-over did not continue into TR4 and TR5, most likely because the task parameters were much more difficult and the SHRs needed more training.

Spaced practice did seem to benefit premature responses in the later stages of testing. This can be seen between the last day of TR3 and the first day of TR4 where the spaced group's premature responses were already at their optimum level, and therefore unchanging, while the massed group started at a relatively higher point and caught up to the spaced group. This highlights that SHRs were more attentive in the spaced group than in the massed group because they not only performed better, but they were also acting less impulsively and more accurately. Overall, spacing out attention in the 5CSRTT by having a fewer number of trials to complete daily created a higher ability to pay attention, specifically during the less complex levels. Thus, there is support for the idea that spacing out training does not only have to revolve around memory it can revolve around attention.

Spacing out attention should be instituted in future studies that use the 5CSRTT. If SHRs have the chance to increase their performance more quickly, the good portion of time typically spent on training the rats to optimal performance could be alleviated (Asinof & Paine, 2014). Typically, rats train for weeks before they are able to move on to the next TR, but some of our spaced trained rats reached this optimal level well within four averaged days (Figure 5) (Asinof & Paine, 2014). This implies that limiting the number of trials SHRs have to focus on may actually allow them to reach optimum performance faster. Finally, spacing out attentiveness training in children or adults with ADHD could prove to be extremely beneficial. Future research should conduct a replication of this study with humans by spacing out cognitive training and seeing if attentiveness increases more effectively. Other learning tasks with SHRs should also be spaced out if researchers want SHRs to learn tasks more efficiently and for a longer period of time.

Sex Differences During TR1

It was originally predicted that males' percent correct scores would benefit more from spaced training than females due to literature indicating that male SHRs typically perform lower in this area (Bayless et al., 2015) and that spaced training can lead to higher performance (Commins et al., 2003; Grassi, 1971; Kerfoot et al., 2007; Kornmeier et al., 2014; Riches et al., 2005; Wang et al., 2014). Supporting the literature, during TR1, spaced training led to males performing significantly better on their percent correct scores than males who were massed while females did not differ (Figure 4). Yet these findings were not significant across TRs, so sex only truly played a role in the beginning of training. Consequently, since this finding of sex differences were only observed when the rats were first introduced to the task, future studies should ask whether these sex and group interactions still occur after mastery of the task is attained, or if early spaced training can provide lasting effects. By instituting spaced training at the highest level, both sexes and groups can enter with mastery versus no experience.

TR4 and TR5: Where Spaced Training was Ineffective

Spaced training was expected to benefit performance throughout all conditions. Furthermore, since spaced training has been shown to increase retention of information after learning, we predicted that spacing out attention during simpler levels would increase attention during harder levels (Kerfoot et al., 2007). Yet once TR4 and TR5 were reached, the main effects of spaced training were lost. There was no improvement in performance during TR4 indicating that all groups needed more time to train. This is likely due to the large increase in complexity at TR4, with a much shorter stimulus time and limited hold (Table 1). As the animals did not learn in the TR4 conditions, when they moved to the even more difficult TR5, they demonstrated only slight improvement. Our testing parameters held each TR training to a total of 360 trials, however more typical testing procedures require that the animals achieve over 75% accuracy before moving to the next training condition. Future studies could implement these criteria and determine the number of trials required to reach criterion between spaced and massed training. Along with this, many reward-based tasks have used shorter intertrial intervals of about 10 minutes when doing spaced training in rats (Elmes et al., 1979; Lattal, 1999). Since the 5CSRTT is reward-based, it is possible these short intertrial intervals could be more beneficial than our chosen 24-hours. Additionally, short intertrial intervals are similar to giving a child with ADHD a break (Chaney, 2005; McGinley, 2011; Weslake & Christian, 2015; Reiber & McLaughlin, 2004).

Open Field

Females were expected to be more active in the open field (Chelaru et al., 2012; Cierpial et al., 1989). We replicated these findings with the females displaying higher activity in the open field than males in both rearing and distance traveled. If future studies plan to use both female and male SHRs, this significant sex difference in activity should be taken into account, as this increase in activity could alter measurements of learning or accuracy of performance.

There was also an expectation that cognitive training in the 5CSRTT would decrease hyperactivity because human studies that instituted cognitive training saw a decrease in hyperactivity and ADHD symptoms (Halperin et al., 2013; Lim et al., 2012). No published paper prior to this one had looked directly at whether hyperactivity symptoms decreased in SHRs after instituting training in the 5CSRTT. Our lab had previously seen that more hyperactive SHRs took longer to reach criterion levels of performance in the 5CSRTT. Fundamentally, if hyperactivity influences attention or impulsivity, it was predicted that attention may also influence hyperactivity. Unexpectedly, the distance traveled in the open field was found to significantly increase after the first round of training occurred in 5CSRTT. Though rearing did decrease over days of testing, it was essential for both to decrease to emphasize a decrease in hyperactivity. Most importantly, no group differences were found in rearing or distance traveled, meaning spaced training did not carry-over to influence hyperactivity levels as expected.

However, it is also possible that carry-over from attention to hyperactivity in SHRs is not relevant. When a follow up statistic was run to compare performance on the 5CSRTT to open field activity, no correlation was noted. The original data that correlated performance and activity in the open field looked at days to reach criterion against distance traveled. Our correlations used percent correct. It is also possible that hyperactivity does not influence learning scores in the 5CSRTT. On the other hand, we did replicate the literature that emphasized how the SHR's hyperexploratory nature in the open field leads to a lack of habituation and therefore, no decrease in activity (Knardahl & Sagvolden, 1979). If future studies wish to decrease hyperactivity or this hyperexploratory nature, then it would make sense to institute more intense cognitive training to see if this decrease occurs. However, future studies may also choose to not use the open field since hyperactivity in the SHR may not significantly influence cognitive performance.

Conclusions and Future Directions

Spaced training was beneficial during early 5CSRTT learning. Giving the spaced group fewer trials to focus on in each session improved their overall performance. Spaced training also led to lower impulsivity being presented in the 5CSRTT. It did not however, lower hyperactivity levels in the SHRs in the open field. ADHD is a disorder that pervades many children's academic lives because of their difficulties with maintaining focus (American Psychiatric Association, 2013; Ek et al., 2011; Schneider et al., 2010; Smith et al., 2006). Because SHRs are not the same as children with ADHD, future studies may decide to examine spacing out a child's attention to increase their academic performance. These studies could test if spaced training leads to an increase in academic performance. Furthermore, these studies could find out if spaced training leads to more effective attentiveness training, training that has been proven to reduce ADHD symptoms, but not long term (Gray et al., 2012, Halperin et al., 2013, Lim et al., 2012). Delving into the potential benefits of spaced training in humans with ADHD could possibly change systems that solely focus on ADHD medications as a cure-all and provide a simple and economically practical solution.

Acknowledgements

R. Warren would like to thank Amy Jo Stavnezer, her faculty mentor and co-author, in helping her write and edit this paper. R. Warren would also like to thank Julie Pringle, our rat lab technician, for assisting her in the caretaking of the rats. She also thanks Sadie Phillips for being her research assistant. Finally, R. Warren thanks the College of Wooster for funding this study.

Corresponding Author

Rebecca Warren
The College of Wooster
Warrenr475@gmail.com
11401 Hounds way, Rockville, MD 20852

Amy Jo Stavnezer
The College of Wooster
ajstavnezer@wooster.edu

References

Ahmadvand R, Kiani SMA, Shojae M (2016) The effect of mass & distributed practice on performance and learning of discrete simple and complex skills in volleyball. Turkish J Kines 2:49–55.

American Psychiatric Association (Ed.) (2013) Diagnostic and statistical manual of mental disorders: DSM-5 (5th ed). Washington, DC: American Psychiatric Association.

Asinof SK, Paine TA (2014) The 5-choice serial reaction time task: a task of attention and impulse control for rodents. J Vis Exp 90:515-74.

Aston-Jones G, Gold JI (2009) How we say no. Biol Psychiatry 65:548–549.

Baum M, Myran DD (1971) Response prevention (flooding) in rats: the effects of restricting exploration during flooding and of massed vs distributed flooding. Can J Psychol 25:138–146.

Bayless DW, Perez MC, Daniel JM (2015) Comparison of the validity of the use of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder in males and females. Behav Brain Res 286:85–92.

Bello-Medina PC, Sánchez-Carrasco L, González-Ornelas NR, Jeffery KJ, Ramírez Amaya V (2013) Differential effects of spaced vs. massed training in long-term object identity and object-location recognition memory. Behav Brain Res 250:102–113.

Brand S, Dunn R, Greb F (2002) Learning styles of students with attention deficit hyperactivity disorder: who are they and how can we teach them? The Clearing House: J Ed Strat Issues Ideas 75:268–273.

Bruce B, Thernlund G, Nettelbladt U (2006) ADHD and language impairment: A study of the parent questionnaire FTF (Five to Fifteen). Eur Child Adolesc Psychiatry 15:52–60.

Busch B (1993) Attention deficits: current concepts, controversies, management, and approaches to classroom instruction. Ann Dyslexia 43:5–25.

Carey B (2015) How we learn: the surprising truth about when, where, and why it happens (Reprint edition). Random House Trade Paperbacks.

Chamberlain SR, Müller U, Blackwell AD, Clark L, Robbins TW, Sahakian BJ (2006) Neurochemical modulation of response inhibition and probabilistic learning in humans. Science (NY) 311:861–863.

Chaney WR (2005) Top-of-hour break renews attention span. Teach Profess 19:1–8.

Chelaru MI, Yang P, Dafny N (2012) Sex differences in the behavioral response to methylphenidate in three adolescent rat strains

(WKY, SHR, SD). Behav Brain Res 226:8–17.

Cierpial MA, Shasby DE, Murphy CA, Borom AH, Stewart RE, Swithers SE, McCarty R (1989) Open-field behavior of spontaneously hypertensive and Wistar Kyoto normotensive rats: effects of reciprocal cross-fostering. Behav Neural Biol 51:203–210.

Cohen NJ, Vallance DD, Barwick M, Im N, Menna R, Horodezky NB, Isaacson L (2000) The Interface between ADHD and language impairment: an examination of language, achievement, and cognitive processing. J Child Psychol Psychiatry 41:353–362.

Commins S, Cunningham L, Harvey D, Walsh D (2003) Massed but not spaced training impairs spatial memory. Behav Brain Res 139: 215–223.

Díaz-Morán S, Estanislau C, Cañete T, Blázquez G, Ráez A, Tobeñ a A, Fernández Teruel A (2014) Relationships of open-field behaviour with anxiety in the elevated zero-maze test: Focus on freezing and grooming, World J Neurosci 4:1-11

Downar J, Crawley AP, Mikulis DJ, Davis KD (2000) A multimodal cortical network for the detection of changes in the sensory environment. Nat Neurosci 3:277–283.

Ek U, Westerlund J, Holmberg K, Fernell (2011) Academic performance of adolescents with ADHD and other behavioural and learning problems -a population based longitudinal study: Academic performance of adolescents with ADHD. Acta Paediatr 100:402–406.

Elmes DG, Willhite JC, Bauer GB (1979) Reference memory effects of distributed practice on radial maze learning by rats. Bull Psychon Soc 14:109–111.

Epstein JN, Loren REA (2013) Changes in the definition of ADHD in DSM-5: subtle but important. Neuropsychiatry, 3:455–458

Faraone SV, Sergeant J, Gillberg C, Biederman J (2003) The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2:104–113.

Fox AT, Hand DJ, Reilly MP (2008) Impulsive choice in a rodent model of attention-deficit/hyperactivity disorder. Behav Brain Res 187:146–152.

Grassi JR (1971) Effects of massed and spaced practice on learning in brain-damaged, behavior-disordered, and normal children. J Learn Disabil 4:237–242.

Gray Sa, Chaban P, Martinussen R, Goldberg R, Gotlieb H, Kronitz R … Tannock R (2012) Effects of a computerized working memory training program on working memory, attention, and academics in adolescents with severe LD and comorbid ADHD: a randomized controlled trial. J Child Psychol Psychiatry 53:1277–1284.

Halperin JM, Marks DJ, Bedard A-CV, Chacko A, Curchack JT, Yoon CA, Healey DM (2013) Training executive, attention, and motor skills: a proof-of concept study in preschool children with ADHD. J Atten Disord 17:711–721.

Hunziker MH, Saldana RL, Neuringer A (1996) Behavioral variability in SHR and WKY rats as a function of rearing environment and reinforcement contingency. J Exp Anal Behav 65:129–144.

Johansen EB, Sagvolden T, Kvande G (2005) Effects of delayed reinforcers on the behavior of an animal model of attention-deficit/hyperactivity disorder (ADHD). Behav Brain Res 162:47–61.

Katz RJ, Roth KA, Carroll BJ (1981) Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. Neurosci Biobehav Rev 5:247–251.

Kerfoot BP, DeWolf WC, Masser BA, Church PA, Federman DD (2007) Spaced education improves the retention of clinical knowledge by medical students: a randomised controlled trial. Med Educ 41:23–31.

Knardahl S, Sagvolden T (1979) Open-field behavior of spontaneously hypertensive rats. Behav Neural Biol 27:187–200.

Kollins Sh, Anastopoulos Ad, Lachiewicz Am, FitzGerald D, Morrissey-Kane E, Garrett Me … Ashley-Koch Ae (2008) SNPs in dopamine D2 receptor gene (DRD2) and norepinephrine transporter gene (NET) are associated with continuous performance task (CPT) phenotypes in ADHD children and their families. Am J of Med Genet B: Neuropsychiatr Genet 147B:1580–1588.

Kornmeier J, Spitzer M, Sosic-Vasic Z (2014) Very similar spacing-effect patterns in very different learning/practice domains. PLoS One, 9:e90656.

Lakshmanan A, Lindsey CD, Krishnan HS (2010) Practice makes perfect? When does massed learning improve product usage proficiency? J Consum Res 37:599–613.

Lattal KM (1999) Trial and intertrial durations in Pavlovian conditioning: issues of learning and performance. J Exp Psychol Anim Behav Process 25:433–450.

Levy F, Swanson JM (2001) Timing, space and ADHD: the dopamine theory revisited. Aust N Z J Psychiatry 35:504–511.

Lim CG, Lee TS, Guan C, Fung DSS, Zhao Y, Teng SSW … Krishnan KRR (2012) A brain-computer interface based attention training program for treating attention deficit hyperactivity disorder. PLoS One 7:e46692.

Lindsay S (2012) Student practice: spaced vs. massed practice materials. Retrieved September 6, 2019, from Missouri EduSAIL website: http://www.moedu-sail.org/spaced-vs-massed-practice-materials/

McGinley L (2011) Test performance and study breaks. Master's Theses, 153. https://scholars.fhsu.edu/theses/153

Reaser A, Prevatt F, Petscher Y, Proctor B (2007) The learning and study strategies of college students with ADHD. Psychol Sch 44:627–638.

Reiber C, McLaughlin TF (2004) Classroom interventions: methods to improve academic performance and classroom behavior for students with attention deficit/hyperactivity disorder. Int J Spec Educ 19:1–13.

Riches NG, Tomasello M, Conti-Ramsden Gina (2005) Verb learning in children with SLI. J Speech Lang Hear Res 48:1397–1411.

Roediger HL, Pyc MA (2012) Inexpensive techniques to improve education: Applying cognitive psychology to enhance educational practice. J Appl Res Mem Cogn 1:242–248.

Rohrer D, Taylor K (2006) The effects of overlearning and distributed practise on the retention of mathematics knowledge. Appl Cogn Psychol 20:1209–1224.

Rubia K, Smith AB, Brammer MJ, Taylor E (2007) Temporal lobe dysfunction in medication-naïve boys with attention-deficit/hyperactivity disorder during attention allocation and its relation to response variability. Biol Psychiatry 62:999–1006.

Sagvolden T, Johansen EB, Wøien G, Walaas SI, Storm-Mathisen J, Bergersen LH … Faraone SV (2009) The spontaneously hypertensive rat model of ADHD – the importance of selecting the appropriate reference strain. Neuropharmacology 57:619–626.

Sagvolden T, Metzger MA, Schiorbeck HK, Rugland A-L, Spinnangr I, Sagvolden G (1992) The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): changed reactivity to reinforcers and to psychomotor stimulants. Behav Neural Biol 58:103–112.

Schneider MF, Krick CM, Retz W, Hengesch G, Retz-Junginger P, Reith W, Rösler M (2010) Impairment of fronto-striatal and parietal cerebral networks correlates with attention deficit hyperactivity disorder (ADHD) psychopathology in adults — a functional magnetic resonance imaging (fMRI) study. Psychiatry Res 183:75–84.

Shibagaki M, Yamanaka T, Furuya T (1993) Attention state in electrodermal activity during auditory stimulation of children with attention-deficit hyperactivity disorder. Percept Mot Skills 77:331–338.

Sigurdardottir HL, Kranz GS, Rami□Mark C, James GM, Vanicek T, Gryglewski G … Lanzenberger R (2016) Effects of norepinephrine transporter gene variants on NET binding in ADHD and healthy controls investigated by PET. Hum Brain Mapp 37:884–895.

Smith AB, Taylor E, Brammer M, Toone B, Rubia K (2006) Task-specific hypoactivation in prefrontal and temporoparietal brain regions during motor inhibition and task switching in medication-naive children and adolescents with attention deficit hyperactivity disorder. American J Psychiatry 163:1044–1051.

Smolen P, Zhang Y, Byrne JH (2016) The right time to learn: mechanisms and optimization of spaced learning. Nat Rev Neurosci 17:77–88.

Sonuga□Barke EJS, Taylor E, Sembi S, Smith J (1992) Hyperactivity and delay aversion—I. The effect of delay on choice. J Child Psychol Psychiatry 33:387–398.

Sturman O, Germain P-L, Bohacek J (2018) Exploratory rearing: a context- and stress sensitive behavior recorded in the open-field test. Stress 21:443–452.

Vande Voort JL, He JP, Jameson ND, Merikangas KR (2014) Impact of the DSM-5 attention-deficit/hyperactivity disorder age-of-onset criterion in the US adolescent population. J Am Acad Child Adolesc Psychiatry, 53:736–744.

Volkow ND, Wang G.-J, Newcorn JH, Kollins SH, Wigal TL, Telang F … Swanson JM (2011) Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. Mol Psychiatry 16:1147–1154.

Waldie KE, Hausmann M (2010) Right fronto-parietal dysfunction in children with ADHD and developmental dyslexia as determined by line bisection judgements. Neuropsychologia, 48:3650-3656

Wang Z, Zhou R, Shah P (2014) Spaced cognitive training promotes training transfer. Front Hum Neurosci 8:217

Weslake A, Christian B (2015) Brain breaks: help or hindrance? Teach Collec Christian Ed, 1. Retrieved from https://research.avondale.edu.au/teachcollection/vol1/iss1/4