Pharmacology, Biochemistry and Behavior 186 (2019) 172765

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The effects of the prodrug Vyvanse on spatial working memory and adiposity in rats

Ethan Ekstrand*, Helen M. Murphy, Cyrilla H. Wideman

Neuroscience Program, John Carroll University, 1 John Carroll Boulevard, University Heights, OH 44118, United States of America

ARTICLE INFO

Keywords:
Prodrug
Morris water maze
Working memory
Adiposity
Rat
Vyvanse

ABSTRACT

The present study investigated the influence of Vyvanse (lisdexamfetamine), a psychomotor stimulant, on spatial working memory, body weight, and adiposity in rats. Control and experimental rats were placed in individual cages equipped with a running wheel, and food and water were provided ad-libitum. The study was divided into three periods: 1) habituation, 2) experimental, and 3) withdrawal. Control rats received a placebo in periods 1, 2 and 3, while experimental rats received a placebo in periods 1 and 3. Experimental rats received a treatment of Vyvanse in place of the placebo during period 2. Spatial working memory was examined by utilizing the methodology of the Morris Water Maze. Rats were evaluated by performance in the maze each day during the experimental and withdrawal periods. Each assessment consisted of two trials. The first was a sample trial in which an escape platform was discovered by trial and error. The second was a test trial in which the platform location was recalled using working memory. Platform placement and start location of the rats were changed every session. It was hypothesized that Vyvanse would effectively enhance spatial working memory, and significantly decrease body weight and adiposity without side effects on activity level and anxiety in rats. Results supported the hypothesis. Compared to control rats, Vyvanse treated rats had significant improvement in working memory and significantly lowered body weight, as well as significantly decreased mesenteric, renal, and epididymal adiposity. No significant effects on activity level and task specific anxiety were noted in experimental animals. When compared to placebo treatment, Vyvanse treatment produced no significant influence on food and water intake. It was concluded that Vyvanse treatment in rats can enhance spatial working memory, and decrease adiposity without suppressing normal appetite.

1. Introduction

Cognitive enhancers are drugs that improve higher-order mental processes including memory, creativity, focus, and motivation in healthy individuals (Frati et al., 2015). Psychomotor stimulants are a major class of drugs utilized as cognitive enhancers. Most of these drugs are controlled substances, and are abused illegally (Wood et al., 2014a). The psychomotor stimulants that are most abused as cognitive enhancers are amphetamines, with Adderall (dextroamphetamine) being the most prevalent (Arria and DuPont, 2010). If abused, these drugs are harmful to the brain and recreational doses tend to create dependency and adverse side effects (Advokat et al., 2008). Dextroamphetamine, classified as schedule II by the Food and Drug Administration (FDA), has a high risk for abuse and adverse effects since the drug activates reward centers in the brain (Dackis and Gold, 1990). One way to lower the risk of abuse with amphetamines is to couple the drug with another compound and create a prodrug, which is a compound that must be metabolized in some way before the body has access to the physiologically active component (Krishnan and Montcrief, 2007).

Vyvanse (lisdexamfetamine) is a prodrug of dextroamphetamine (Food and Administration, 2017). The drug consists of a dextroamphetamine molecule bonded to L-Lysine. Once the drug is bloodborne, the Lysine group is cleaved off through an enzymatic reaction liberating the active component. Vyvanse is approved by the FDA to treat Attention-Deficit/Hyperactivity Disorder (ADHD), and Binge Eating Disorder (Food and Administration, 2017). Dextroamphetamine, the active form of the drug, is a serotonin, dopamine, and norepinephrine agonist (Heal et al., 2013) which explains why Vyvanse may be abused as a cognitive enhancer and as a means to achieve a euphoric high. However, lysine coupled with the amphetamine molecule, makes the risk much lower than other drugs such as Adderall due to the delayed release of the active form. This occurs because the blood levels of dextroamphetamine are limited by the rate of the enzymatic cleavage of lisdexamfetamine (Pennick, 2010). Thus, Vyvanse may

* Corresponding author.
E-mail address: eekstrand18@jcu.edu (E. Ekstrand).

https://doi.org/10.1016/j.pbb.2019.172765
Received 29 November 2018; Received in revised form 24 August 2019; Accepted 26 August 2019
Available online 27 August 2019
0091-3057/

prove to be a more therapeutic option for ADHD disorders and a safer alternative than Adderall. Designing drugs in such a way as to create a delayed release into the blood stream promotes hormesis, a process in a cell or organism that produces a biphasic response to exposure from increasing amounts of a substance or condition, with high levels of a substance being toxic, but low levels being beneficial. Hormesis is achieved when positive effects of the drug are maintained while as many negative side effects as possible are eliminated (Mattson and Calabrese, 2010).

There is still conflicting discourse as to whether or not amphetamines can truly boost cognitive performance in healthy individuals. There are, however, studies that show that these substances do improve cognitive performance (Spencer et al., 2015; Ilieva et al., 2015). One study demonstrated that, at lower doses, amphetamines can boost prefrontal cortex function in human subjects (Spencer et al., 2015) and another study found modest improvement in working memory and long-term memory with therapeutic doses of amphetamines (Ilieva et al., 2015). Because Vyvanse is a prodrug of amphetamine, it is a viable option for producing these improved cognitive functions given by amphetamines in healthy individuals without the usual adverse effects of amphetamines such as anxiety and hyperactivity (Arria and DuPont, 2010).

Vyvanse and other amphetamine drugs are believed to improve cognition through the ability of both isomers of amphetamine to act as dopamine agonists (Miller, 2011). Dextroamphetamine and levoamphetamine are trace amine associated receptor 1 (TAAR1) agonists. TAAR1 activation causes the efflux of monoamine neurotransmitters, particularly dopamine, from storage sites on the presynaptic neuron (Miller, 2011). A consequence of this is increased dopaminergic transmission. Dextroamphetamine can also bind to vesicular monoamine transporter 2 (VMAT2), being taken up by presynaptic vesicle stores of dopamine. The influx of dextroamphetamine causes the efflux of dopamine out into the neuronal cytoplasm, eliciting a similar effect as TAAR1 activation and enhancing the effect of TAAR1 activation (Eiden and Weihe, 2011). Augmented dopaminergic transmission would impact the mesocortical and mesolimbic tracts, which are two major pathways in cognition and memory. Both of these pathways are major branching points that connect various parts of the brain implicated in memory and executive function (McKim and Hancock, 2013). The nucleus accumbens, which receives information from the environment, projects dopaminergic fibers to the ventral tegmental area which then has many projections to other areas of the brain involved in the learning and memory system (McKim and Hancock, 2013). Since dextroamphetamine, a metabolite of Vyvanse, acts as a dopamine reuptake inhibitor in the brain (Miller, 2011), the drug causes amplification of dopaminergic transmission in the mesolimbic and mesocortical pathways. A recent study demonstrated that cognitive-enhancing effects of amphetamine occur through this indirect activation which, in turn, affects both D1 dopamine receptors and α2 adrenoceptors in the prefrontal cortex (Spencer et al., 2015). Studies have also shown that the effect of increased dopamine efflux in the nucleus accumbens increases task saliency, and performance on boring or difficult tasks (Malenka et al., 2009; Wood et al., 2014a, 2014b). ADHD patients generally have low levels of dopamine and lower task specific focus, and studies have demonstrated that amphetamine drugs can correct this cognitive deficit (Bidwell et al., 2011).

There has been a sparse amount of cognitive enhancement studies involving healthy subjects conducted on Vyvanse. Most studies, particularly those with rats, have been focused on the pharmacological aspects of the drug. One of these studies found Vyvanse to have about a 5 times higher lethal dose than that of dextroamphetamine (Krishnan and Montcrief, 2007). Another study on the behavioral effects of the drug demonstrated that Vyvanse was effective in reducing binge eating in rats by 71% (Vickers et al., 2015). Because there have been few studies done on Vyvanse as a cognitive enhancer, the main focus of the present study was to examine the effects of Vyvanse on spatial working memory in rats. Spatial working memory was compared between control and treatment groups using the Morris Water Maze (MWM). The MWM test was chosen because it has high reliability, experimental versatility, and specificity for spatial learning and memory (Vorhees and Williams, 2006). In addition, the effects of the drug and placebo on body weight, activity, food and water intake, anxiety, and adiposity were studied. Studying food intake, adiposity, and body weight of Vyvanse treated rats helps confirm or refute drug effects in healthy subjects, and gauges accordance of results with the study done by Vickers et al. on binge eating in rats (Vickers et al., 2015). It also helps confirm viability of an amphetamine prodrug depending on the effect drug treatment has on appetite. Little to no effect on appetite should be expected if the prodrug truly promotes hormesis, as an adverse effect of dextroamphetamine is appetite suppression. This also applies to animal locomotion and anxiety, where adverse effects associated with dextroamphetamine treatment should be diminished with treatment of its respective prodrug instead. The study of water intake can implicate if there any drug interactions with the endocrine system of the rats. It was hypothesized that Vyvanse would effectively enhance spatial working memory and would promote lower body weight and adiposity without producing side effects on activity level and anxiety in healthy rats.

2. Methods and materials

2.1. Animal care and housing

All procedures were approved by the local Institutional Animal Care and Use Committee and followed NIH guidelines. Particular emphasis was placed on implementation of the 3Rs. Twelve Long-Evans male rats (Envigo, Indianapolis, IN), weighing between 90 and 100 g, were placed in individual cages equipped with a running wheel attached to a device that recorded activity using VitalView software (Starr Life Sciences Corp., Oakmont, PA). Rats were approximately five weeks old at the start of the study. The running wheel sensor recorded activity every 5 min for the duration of the experiment. Each rat was weighed upon arrival, and the rats were assigned to control and treatment groups equally based on weight, with six rats in each group. Food (Purina Rodent Chow #5001, Nestle Purina PetCare Co., St. Louis, MO) and tap water were provided ad-libitum. Body weight was recorded every three days, while food and water consumption were monitored every day. Each day was divided into 12 h of light and 12 h of dark, making up a circadian cycle. Room temperature was held constant at 21–23 °C.

2.2. Habituation period

Days 1–16 of the study constituted the habituation period. A 250 μL condensed milk treat was presented in a shallow glass dish every day at the start of the dark cycle following the protocol of Murphy et al. (2015). The treat was completely consumed within 5 min by the end of the third day. Following removal of the dish, rats were provided with food and water ad-libitum throughout the 24-h cycle.

2.3. Experimental period

Days 17–37 constituted the experimental period. During this time, the treatment group received the milk treat with a solution of Vyvanse (Shire, Lexington, MA) dissolved in deionized water at a dose of 1.5 mg/kg as utilized by Vickers et al. (2015). During this period the experimenters were not blind to treatment. The dose utilized by Vickers et al. elicited therapeutic behavioral effects in rats, and therefore we elected to use the same dose in the current study. The control group received the condensed milk treat with deionized water which acted as a placebo. Administration of the drug and placebo to the respective groups was carried out at the start of the dark cycle every day throughout the experimental period.

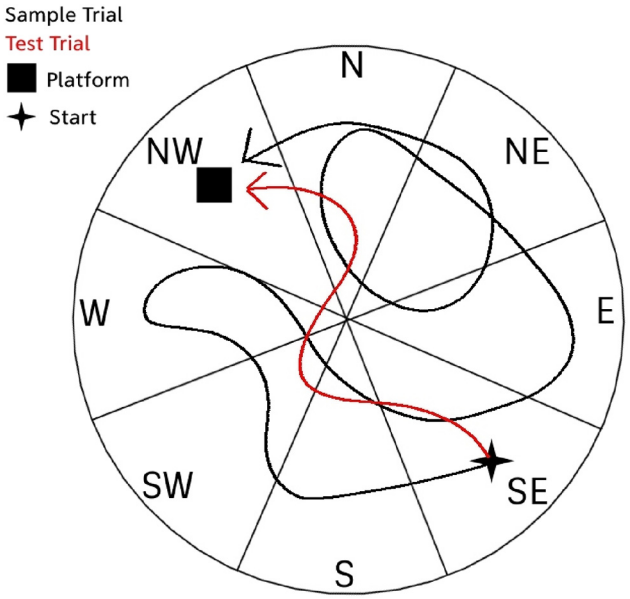

Fig. 1. A Schematic of MWM utilized in the study with typical pathing a rat would take from the start location to the escape platform.

2.4. Withdrawal period

Days 38–47 constituted the withdrawal period. During this period, both groups received the milk treat with deionized water at the start of the dark cycle.

2.5. Morris water maze test

During the experimental and withdrawal periods, spatial working memory for both groups of rats was assessed by using the MWM. Ten to twenty minutes were allotted for drug absorption before both groups were assessed by the MWM. Time allotted for drug absorption was based on a T_{max} of 0.17 h found for Vyvanse in rats by Pennick (2010). This was done to ensure that testing coincided with maximum drug concentration as close as possible. After the allotted absorption time, the animals were tested in the MWM. The maze was a circular pool that was 1.5 m in diameter and 60 cm deep. It was partitioned into 8 segments and three colored towels, which served as cues for the rats, were draped over the side in permanent locations throughout the experiment. A rough schematic of the maze is provided in Fig. 1 along with typical pathing of rats in the maze. The towels being in permanent locations kept the rat from associating platform placement with the cue only. Such an association could have introduced bias into the results because, instead of learning the location of the platform itself, the rat could have learned to associate the location of the cues with the platform placement.

Water in the pool was kept at room temperature, and monitored by a thermometer. All maze assessments were held during the dark phase of the circadian cycle utilizing a red light bulb of 5 lx. At the end of the allotted absorption time, at the start of the dark cycle each day, the protocol for spatial working memory in rats proposed by Vorhees and Williams (2006) was employed for both treatment and control groups. The assessment of each rat included 2 trials. The first trial was a sample trial which consisted of the rat discovering the location of the platform by trial and error. The second trial was a test trial which consisted of the rat recalling the location of the platform. During the sample trial, each rat was given 90 s to find the platform. Once the platform was discovered by the rat, it was allowed to rest on the platform for 15 s. If the rat did not find the platform after the allotted 90 s, it was guided to it. After the 15 s of rest, the animal was placed back in the start location of the maze. During the test trial, 90 s were allotted for the rat to find the platform. If the rat recalled the location of the platform, it swam faster to the platform during the test trial. After the maze assessment, each rat was patted gently with a paper towel to remove excess water and returned to its home cage. As shown in Table 1, platform location was varied every day of the study by following a chart proposed by Vorhees and Williams (2006).

Table 1
Alternation of platform and start configurations as proposed by Vorhees and Williams (2006).

Day	Platform placement	Rat placement
1	SE	N
2	NE	E
3	SW	S
4	SE	W
5	NE	S
6	NW	N
7	NE	W
8	SE	E
9	NW	W
10	SE	S
11	SW	E
12	SW	N
13	NW	E
14	NE	W
15	SE	N
16	SW	S
17	NE	N
18	NW	S
19	NW	E
20	SW	W
21	SE	N

Due to platform relocation every day, no learning of the platform position from the previous day could be transferred to the test trial of the next day; hence, recall on each day during the test trial was dependent on the sample trial of that day and measured only working memory.

2.6. Anxiety measurement

Anxiety was assessed at the start of all MWM test trials when the rat was placed back into the water after having discovered the platform. This method of anxiety assessment was elected to minimize experimenter handling of the animals as much as possible. The MWM task induced anxiety was rated by assessing rat behavior that was anxious in character (squirming, squealing, and aggression) on a scale from 0 to 3, with 0 showing no signs of anxiety, and 3 being extremely anxious.

2.7. Adiposity measurement

At the conclusion of the experiment, all rats were sacrificed and differences in renal, and mesenteric adiposity between the treatment and control groups were assessed by scoring following the procedures of Wideman and Murphy (2009). Epididymal fat was dissected from the body of each rat and weighed.

2.8. Statistical analysis

All results were statistically analyzed with SPSS 23 (IBM Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). Unless noted otherwise, the threshold of significance was set at $\alpha = 0.05$. For maze data, weekly sample and test trial times of each rat were averaged for the period for within group analysis. Test trial times of each group were averaged daily for between treatment group analysis. Body weight data was averaged by period for between group analysis. Body weight, running wheel revolutions, food intake, and water intake data were averaged by period for between group analysis. Mean sample and test trial times within each group were analyzed

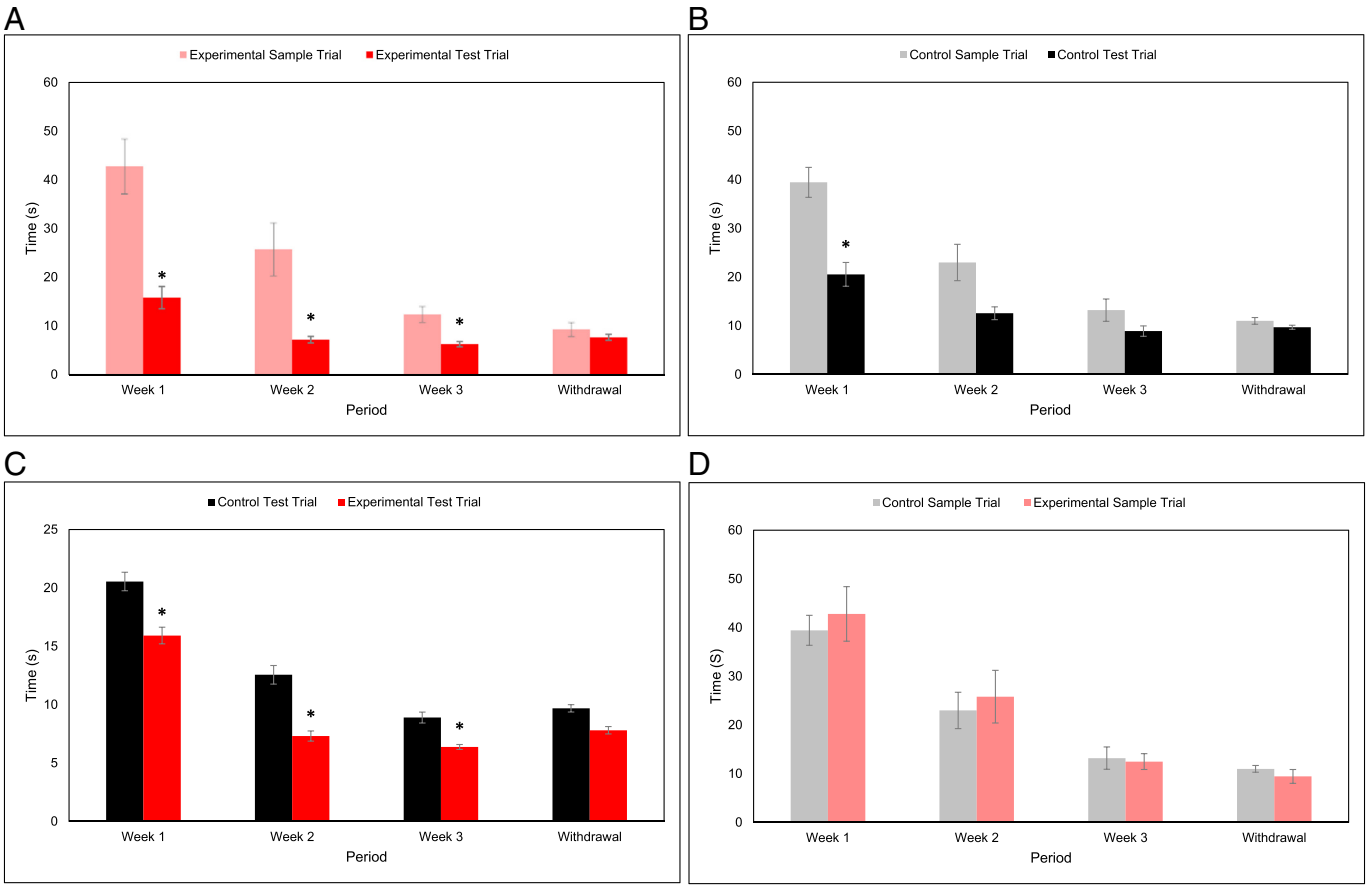

Fig. 2. A. Mean MWM times (± SEM) of the sample and test trials for the experimental group across weeks 1, 2, and 3 of the experimental period, and the withdrawal period. An * indicates a significant difference ($p < .05$) between trials.
B. Mean MWM times (± SEM) of the sample and test trials for the control group across weeks 1, 2, and 3 of the experimental period, and the withdrawal period. An * indicates a significant difference ($p < .05$) between trials.
C. Mean MWM times (± SEM) for test trials of both treatment groups across weeks 1, 2, and 3 of the experimental period, and the withdrawal period. An * indicates a significant difference ($p < .05$) between treatment groups.
D. Mean MWM times (± SEM) for sample trials of both treatment groups across weeks 1, 2, and 3 of the experimental period.

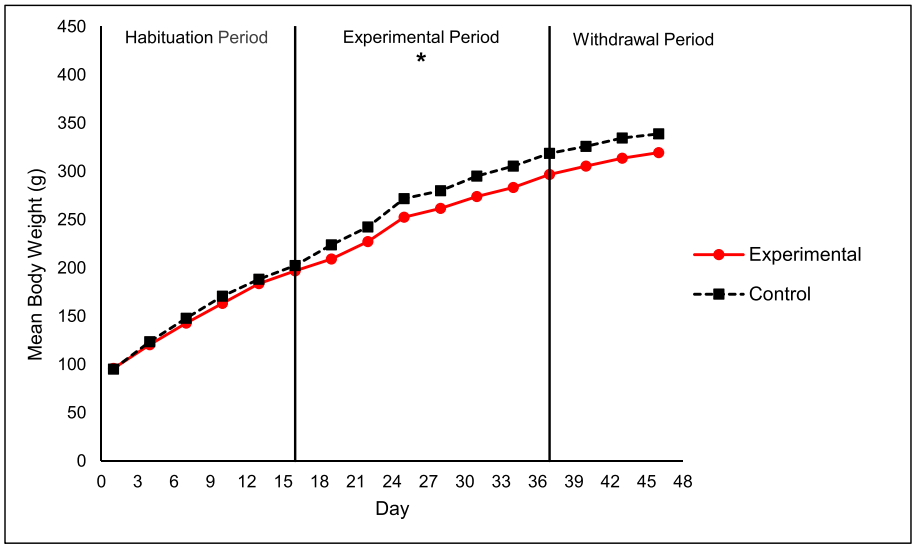

Fig. 3. A comparison of mean body weight between control and experimental rats during the entire study. An * indicates a significant difference in body weight between the experimental and control groups for the period ($p < .05$).

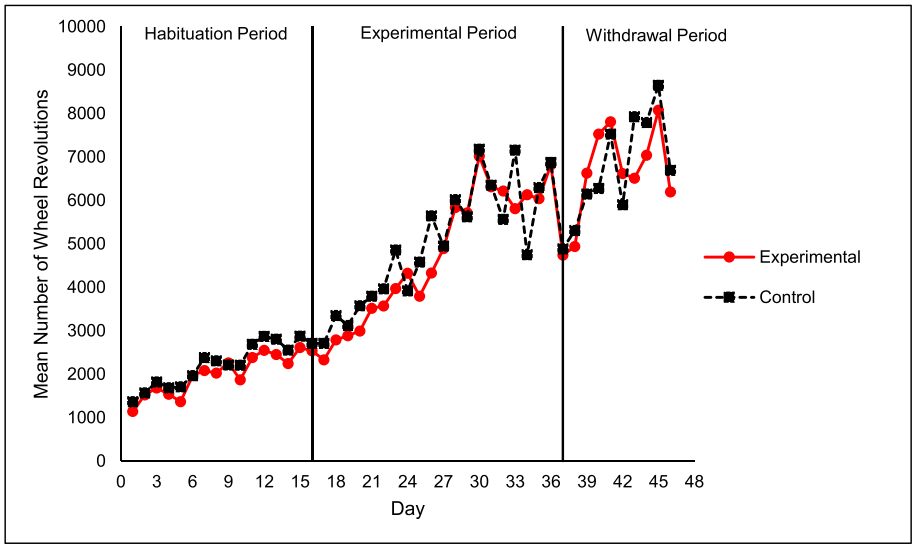

Fig. 4. A comparison of mean running wheel activity between experimental and control groups across all periods of the study.

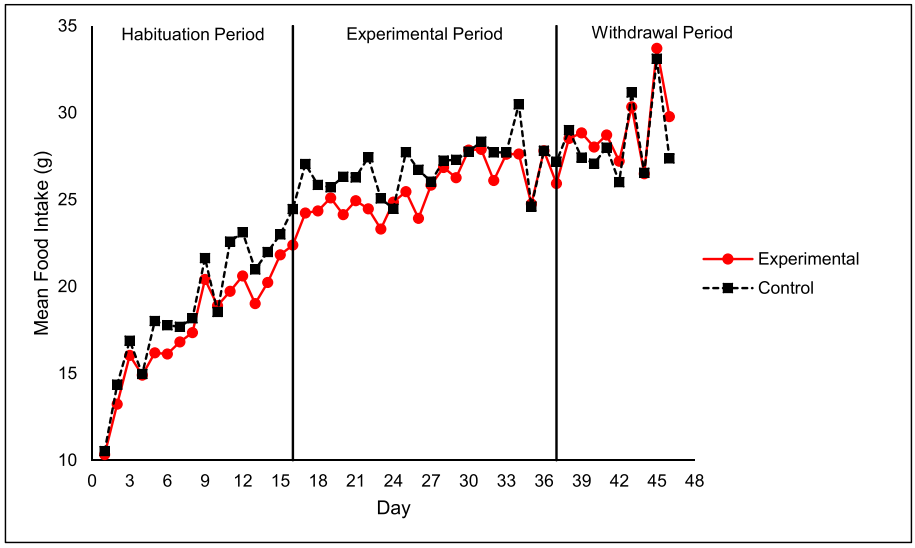

Fig. 5. A comparison of mean food intake of experimental and control groups during the entire study.

separately, using a 2 × 4 (trial × period) repeated measures ANOVA across weeks 1–3 of the experimental period and the withdrawal period. Mean sample and test trial times were compared between groups using a 2 × 4 (treatment × period) ANOVA. Body weight, running wheel activity, food intake, and water intake were analyzed using a 2 × 3 (treatment × period) repeated measures ANOVA. Anxiety scores were compared between treatment groups using a Mann-Whitney *U* Test across weeks 1–3 of experimental period and the withdrawal period. Renal and mesenteric adiposity scores were compared using a Mann-Whitney U Test. Epididymal adiposity was compared between treatment groups using an independent samples *t*-test. When necessary, post hoc Bonferroni tests were carried out for ANOVA tests. Effects of period on any independent variable are omitted due to the rats maturing through the study.

3. Results

Fig. 2A shows the comparison between mean sample and test trial maze times across weeks 1, 2, and 3 of the experimental period, and the withdrawal period within the experimental group of rats. All data distributions were normal as assessed by Shapiro-Wilk's test [$p > .05$], and Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction [$\chi^2(2) = 9.756$, $p > .05$]. The results of the ANOVA indicated that there was a statistically significant interaction between trial and period [$F(3,15) = 7.780$, $p < .01$], and a main effect for trial [$F(1,5) = 46.642$, $p < .01$]. Post hoc tests were run between sample and test trials of each week and it was found that the mean maze time of the sample trial was higher than that of the test trial for the experimental group during weeks 1, 2, and 3 of the experimental period. All differences were statistically significant [$F(1,5) = 18.749$, $p < .01$], [$F(1,5) = 13.677$, $p < .05$], and [$F(1,5) = 19.758$, $p < .01$] for weeks 1, 2, and 3 of the experimental period respectively. Post hoc tests showed that test trial times were not significantly different from sample trial times during the withdrawal period [$F(1,5) = 2.573$, $p > .05$]. Thus, Vyvanse treated rats performed the Morris water maze significantly faster during the test trial than the sample trial across weeks 1, 2, and 3 of the experimental period. During the withdrawal period the rats did not perform the maze significantly faster in the test trial compared to the sample trial.

Fig. 2B presents the comparison between mean sample and test trial maze times within the control group across weeks 1, 2, and 3 of the experimental period, and the withdrawal period. All data distributions

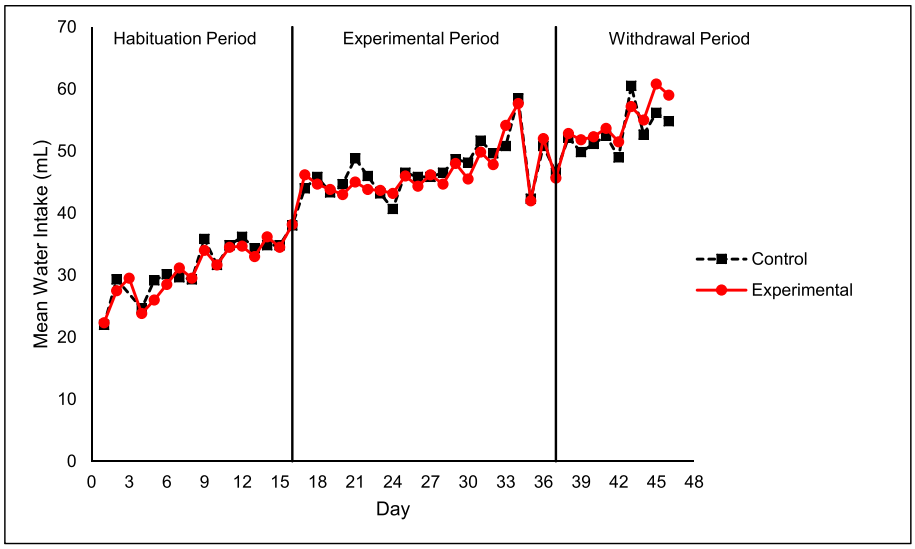

Fig. 6. A comparison of mean water intake of experimental and control groups during the entire study.

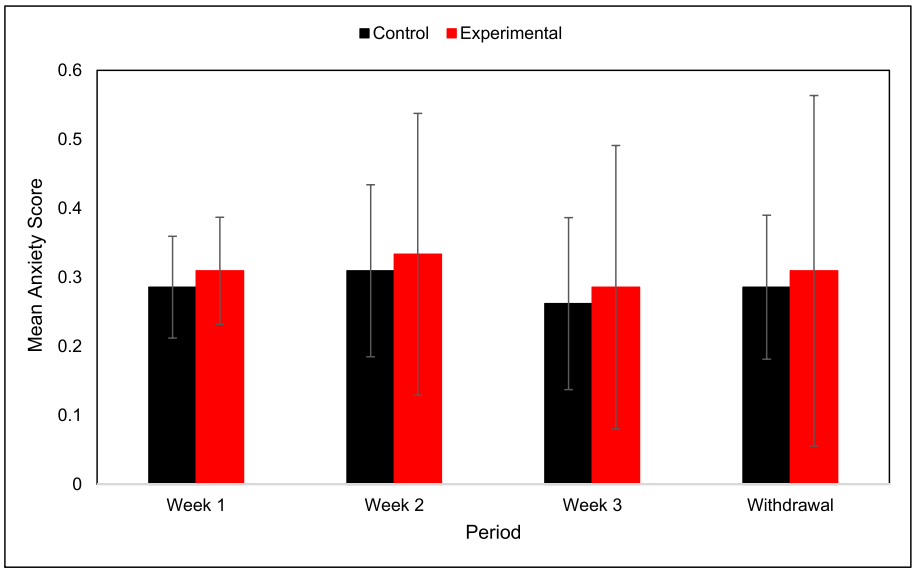

Fig. 7. A comparison of mean anxiety scores (± SEM) for experimental and control groups across weeks 1, 2, and 3 of the experimental period, and the withdrawal period.

were normal as assessed by Shapiro-Wilk's test [$p > .05$] except for test trial times during week 2 of the experimental period [$p = .010$]. This was not considered a severe enough violation to warrant transformation of the data. Mauchly's test of sphericity indicated that the assumption of sphericity was met for the two-way interaction [$\chi^2(2) = 5.731$, $p > .05$]. The ANOVA results indicated that there was a statistically significant interaction between trial and period [$F(3,15) = 3.967$, $p < .05$] with a main effect for trial [$F(1,5) = 28.225$, $p < .01$]. Therefore, post hoc tests were conducted to determine which weeks had the significant difference between trials. Mean sample maze times were significantly higher than that of trial maze times during week 1 of the experimental period [$F(1,5) = 11.824$, $p < .05$]. Sample trial times, however, were not significantly higher than test trial times during weeks 2 and 3 of the experimental period, and the withdrawal period [$F(1,5) = 4.959$, $p > .05$], [$F(1,5) = 4.586$, $p > .05$], and [$F(1,5) = 6.245$, $p > .05$] respectively. Thus, control rats performed the Morris water maze significantly better during the test trial than in the sample trial only during week 1 of the experimental period, and did not perform the maze significantly better during the test trial when compared to the sample trial during weeks 2 and 3 of the experimental period, and the withdrawal period.

Fig. 2C illustrates the mean MWM times for test trials between treatment groups across weeks 1, 2, and 3 of the experimental period, and the withdrawal period. All distributions were normal as assessed by Shapiro-Wilk's Test [$p > .05$] expect for maze times during week 2 of the experimental period in the experimental group [$p = .037$]. This was not considered a severe enough of a violation to warrant transformation of the data. The assumption of homogeneity of variances was violated, as assessed by Levene's test for equality of variances [$p = .013$]. Due to this violation, the threshold for alpha was changed from 0.05 to.005 for this test. ANOVA results showed that there was a statistically significant interaction between treatment and period [$F(3,52) = 12.676$, $p < .001$] with a main effect for treatment [$F(1, 52) = 78.139$, $p < .001$]. Post hoc tests determined that during weeks 1, 2, and 3 of the experimental period Vyvanse treated rats had significantly lower test trial times than that of control rats with statistics of [$F(1,52) = 38.304$, $p < .001$], [$F(1,52) = 51.207$, $p < .001$], and [$F(1,52) = 11.811$, $p = .001$] for weeks 1–3 respectively. During the

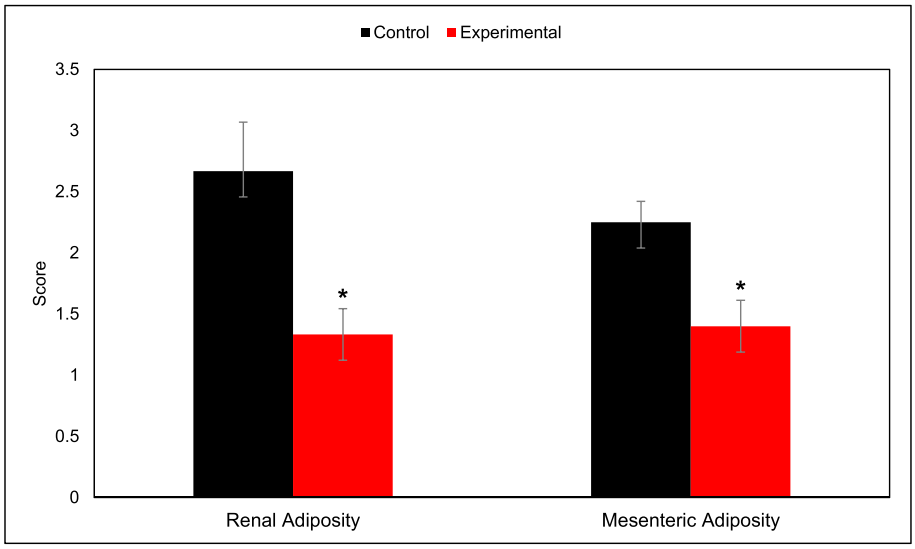

Fig. 8. A comparison of mean renal and mesenteric adiposity scores (± SEM) between experimental and control groups. An * indicates a significant difference between treatment groups ($p < .05$).

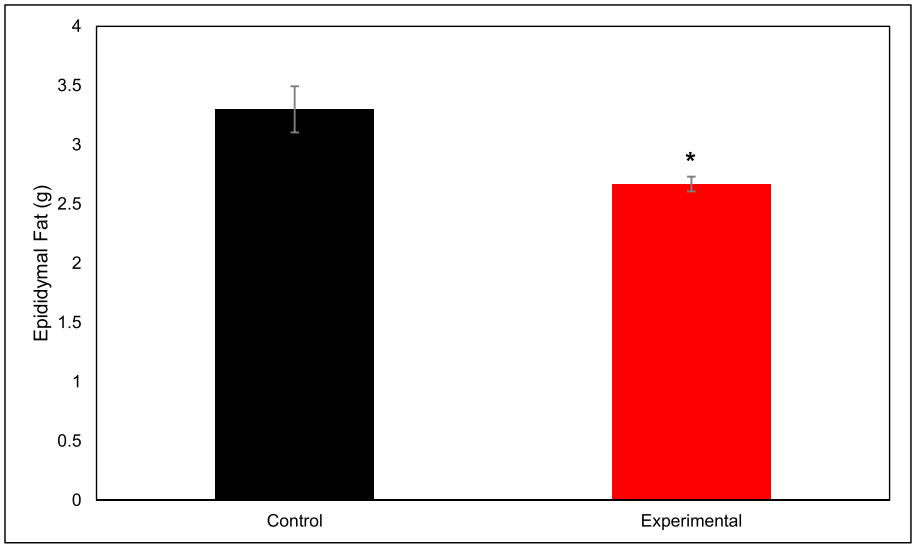

Fig. 9. A comparison of mean epididymal fat mass (± SEM) between experimental and control groups. An * indicates a significant difference between treatment groups ($p < .05$).

withdrawal period, mean test trial times of Vyvanse treated and control rats were not significantly different from one another [$F(1,52) = 0.016$, $p > .05$].

Fig. 2D illustrates the mean MWM times for sample trials between treatment groups across weeks 1, 2, and 3 of the experimental period. All distributions were normal as assessed by Shapiro-Wilk's Test [$p > .05$] except for the control group during the withdrawal period [$p < .001$] and the experimental group during week 1 of the experimental period [$p = .041$]. This was not considered a severe enough of a violation to warrant transformation of the data. The assumption of homogeneity of variances was violated, as assessed by Levene's test for equality of variances [$p < .001$]. Due to this violation, the threshold for alpha was changed from 0.05 to 0.005 for this test. ANOVA results showed that there was no statistically significant interaction between treatment and period [$F(2,12) = 0.372$, $p > .05$]. Average sample trial times for both groups were not significantly different across weeks 1–3 of the experimental period, and the withdrawal period.

Fig. 3 displays the average body weight comparison between control and experimental rats during the entire study. It was found from the ANOVA results that there was no statistically significant interaction between treatment and period [$F(2,186) = 1.3$, $p > .05$]; however, there was a main effect for treatment [$F(1,186) = 7.337$, $p < .01$]. Post hoc tests indicated that Vyvanse treated rats weighed significantly less than control rats during the experimental period [$F(1,186) = 6.1$, $p < .05$]. It should be noted that, although not significant, the withdrawal period showed a noticeable difference in weight [$p = .06$]. Difference in weight was not statistically significant during the habituation period.

Fig. 4 shows the comparison of average running wheel activity between experimental and control groups across all periods of the study. The ANOVA results showed that there was no statistically significant interaction between treatment and period [$F(2,86) = 1.068$, $p > .05$], and there was no main effect for treatment [$F(1,86) = 0.600$, $p > .05$].

Fig. 5 presents the comparison between experimental and control group mean food intake across the entire study. The ANOVA results showed that there was no statistically significant interaction between treatment and period [$F(2,86) = 0.03$, $p > .05$], and there was no main effect for treatment [$F(1,86) = 1.244$, $p > .05$].

Fig. 6 illustrates the mean daily water intake of control and experimental rats across the entire study. The ANOVA results showed that there was no statistically significant interaction between treatment and period [$F(2,86) = 0.972$, $p > .05$], and there was no main effect for treatment [$F(1,86) = 0.089$, $p > .05$].

Fig. 7 displays the comparison of mean anxiety scores between the experimental and control group across the experimental and withdrawal period. A Mann-Whitney U test was run to determine if there were differences in anxiety scores between the control and experimental groups. Distributions of the anxiety scores for both groups were similar, as assessed by visual inspection. Median anxiety scores were not significantly different between control and experimental groups across weeks 1–3 of the experimental period [$U = 901$, $z = 0.074$, $p > .05$], [$U = 899$, $z = 0.074$, $p > .05$], and [$U = 833$, $z = 0.730$, $p > .05$] respectively, and the withdrawal period [$U = 1713.5$, $z = 0.484$, $p > .05$].

Fig. 8 shows the comparison of mesenteric and renal adiposity scores between treatment groups. A Mann-Whitney U test was run to determine if there were differences in renal and mesenteric adiposity scores between the control and experimental groups. Distributions of the adiposity scores for both groups were not similar, as assessed by visual inspection. There was a significant difference in renal and mesenteric adiposity scores between control and experimental groups [$U = 5$, $z = -2.183$, $p < .05$], and [$U = 4$, $z = -2.442$, $p < .05$] respectively. Vyvanse treated rats had significantly lower renal and mesenteric adiposity scores than control rats.

Fig. 9 presents the comparison of mesenteric fat mass between treatment groups. It was found that control rats had significantly more epididymal fat mass than that of Vyvanse treated rats at the conclusion of the study [$t(10) = 3.070$, $p < .05$].

4. Discussion

The current study demonstrated that Vyvanse had significant positive effects on spatial working memory in healthy male rats when compared to a placebo. Possible mechanisms for the cognitive enhancement of Vyvanse can be postulated for the results of the present study in reference to other studies that have been done on amphetamines. Vyvanse possibly enhances working memory through the ability of its active form, dextroamphetamine, to increase dopaminergic transmission in areas of the brain dealing with cognition and memory (McKim and Hancock, 2013). Dopamine agonism causes efflux of the neurotransmitter in critical areas of the central nervous system that are essential to various cognitive processes (McKim and Hancock, 2013). One possible route for cognitive enhancing capabilities of Vyvanse can involve motivation and object salience based on classical operant models. Although not quantified in any way in the present study, it was observed that Vyvanse treated rats were more prone than control rats to stand up on their hind legs and look around the maze when discovering the platform at the end of the sample trial. This observation may explain the better overall performance of Vyvanse treated rats during the experimental period compared to control rats. The escape platform has salience on its own because it is a means for the rat to escape the water. An increase in dopamine in the mesolimbic pathway could possibly increase the salience of the location of cues in the maze with respect to the platform; thus, increasing the motivation to remember this spatial relationship. The cues in the case of the present study were the colored hand towels, which were placed in permanent locations around the maze throughout the study. In a sense, cognitive enhancement achieved through modification of object salience would be an indirect consequence of increased motivation in the rats (McKim and Hancock, 2013; Bidwell et al., 2011). Another route could include the mesocortical projections to higher areas of the brain, particularly the prefrontal cortex, which plays a role in executive functions (Diamond, 2013). The executive functions that pertain to the current study involve memory, and problem solving. Vyvanse treated rats had significantly lower test trial times in the MWM when compared to control rats as shown in Fig. 2C, but the sample trial times between the two groups showed no significant difference as shown in Fig. 2D. Therefore, maze performance can't be attributed to possible drug induced hyperlocomotion. Upon withdrawal of the drug, the test trial times of the experimental group decreased and were not significantly different from the sample trial (Fig. 2A).

The current study and the study carried out by Murphy et al. (2015) show a clear model for studying cognitive enhancement in rats for prospective studies utilizing the methodology of the MWM. That study involved the drug, Modafinil, a psychostimulant commonly abused for cognitive enhancement that also effects dopaminergic transmission (Murphy et al., 2015). The studies involved two different drugs, yet yielded similar results with respect to whether or not the drug had an impact on cognitive enhancement. There was a significant difference between sample trial and test trial times in the experimental groups, and there was a clear loss of cognitive enhancement during the withdrawal period in both studies.

Side effects of Vyvanse on rats that were monitored were not observed in the current study. There was no significant difference in wheel activity, water intake, and water maze induced anxiety between Vyvanse treated rats when compared to rats given a placebo. In addition, food intake was not significantly suppressed in Vyvanse treated rats when compared to rats given a placebo (Fig. 5). Body weight, however, showed a distinct difference between the two groups when the experimental rats were administered Vyvanse. Every weight measurement from the experimental period was significantly different between the experimental and control groups, with the mean body weight of control animals being higher than that of the experimental animals (Fig. 3). The withdrawal period showed a difference in mean weight of the two groups that was noticeable [$p = .06$], however, not significant based on the current study's value of α (Fig. 3). Therefore, it can be postulated that drug effects on body weight ceased with withdrawal, but body weight was slower to change afterwards. Mesenteric, renal, and epididymal adiposity were significantly lower in Vyvanse treated rats when compared to control rats (Figs. 8, and 9). The results of food intake did not agree with the results obtained for body weight and adiposity. It would be expected that the drug would have had some effect on appetite in light of observed effect on body weight and adiposity. Therefore, it can be concluded that the effect of a therapeutic dose of Vyvanse on body weight was not achieved through food intake suppression in the current study. However, amphetamine is known to affect fat metabolism. A study done by Pinter and Pattee (1968) demonstrated that the administration of amphetamine and methamphetamine led to an increase in the plasma free fatty acid concentration in human subjects. It was concluded in that study that amphetamine is an adipokinetic agent that causes an increase in the plasma free fatty acid concentration to a lesser extent, but of longer duration than equipressor doses of epinephrine. The effects on weight caused by Vyvanse in the current study may be due to a change in fat metabolism caused by dextroamphetamine, the active form of Vyvanse. A change in dopamine transmission in the mesolimbic pathway can have an impact on appetite as demonstrated by Pennick's (2010) study; however, food intake was no different when compared to the placebo in the current study. Perhaps, the therapeutic dose used in this study only prevents overeating in the rats and has no impact on normal appetite of the animal. This would suggest that the effect of the drug on body weight was only attributable to changes in fat metabolism in the current study. Noting the minimal drug effects on appetite, water maze induced anxiety, wheel activity, and water intake in the current study may suggest the viability of prodrugs. This is because Vyvanse treatment produced cognitive enhancing effects without severely suppressing appetite, increasing water maze induced anxiety, and causing hyperactivity in healthy rats.

A major limitation of the current study was the assessment of anxiety in the rats. Anxiety was only assessed while the rats were performing the MWM task. Therefore, it is debatable whether other forms

of anxiety in the rats were affected, and it can only be concluded that drug treatment did not impact task specific associated anxiety. In this case the stressor was the MWM task and the behavior being assessed was aggression and behavioral cues of fear in rats such as squirming, and squealing. However, the other parameters that were measured such as food intake, and activity can implicate other anxiety-like behaviors in rats. Studies have found links between overeating and hyperactivity in rats exposed to stressful stimuli (Levine, 1981; Kwak et al., 2009). And in this study, there was no difference between Vyvanse treatment and placebo for food intake nor activity. It should be noted that high variability for anxiety scores in the experimental group shown in Fig. 7 can be attributed to an outlier that was not removed. Subject 4 in the experimental group showed disproportionate amounts of aggression, and was the only subject to score a 3 on an established scale of 0–3 throughout the duration of the study. Another limitation of the current study was that a single dose was used. Although results showed cognitive enhancement with the dose that was chosen, the degree of that effect could have possibly been found with testing multiple doses. There was also a limitation of small sample size, with 6 rats in each group. However, each week represented multiple replicates of data for each rat, and statistics were analyzed conservatively where assumptions for the respective statistical test were violated. A stronger conclusion could be reached with an increased sample size in future studies. One mitigating factor related to the MWM was the absence of a habituation period. Thus, week 1 of the experimental period was the first time the animals were introduced to the maze. As a consequence, the control group and the treatment group had similar performance in the maze during week 1 of the experimental period; however, not during weeks 2 and 3.

In conclusion, Vyvanse treatment had significant positive effects on cognition in healthy rats, without negative side effects on activity level or task specific anxiety. Drug treatment also significantly reduced peritoneal adiposity without impacting normal appetite in healthy rats. This provides evidence that prodrugs promote hormesis due to the ability of prodrugs to release the active form of drugs in more of a controlled manner, as opposed to drugs causing their effects immediately. The implications of prodrugs are promising for the treatment of diseases involving the nervous system, without inducing adverse side effects. Particularly, with the treatment of ADHD, the results of the current study suggest that Vyvanse is possibly a safer alternative to correcting cognitive deficits caused by the disease than alternatives such as Adderall.

References

Advokat, C.D., Guildry, D., Martino, L., 2008. Licit and illicit use of medications for attention-deficit hyperactivity disorder in undergraduate college students. J. Am. Coll. Heal. 56 (6), 601–606. https://doi.org/10.3200/JACH.56.6.601-606.

Arria, A.M., DuPont, R.L., 2010. Nonmedical prescription stimulant use among college students: why we need to do something and what we need to do. J. Addict. Dis. 29 (4), 417–426. https://doi.org/10.1080/10550887.2010.509273.

Bidwell, L.C., McClernon, F.J., Kollins, S.H., 2011. Cognitive enhancers for the treatment of ADHD. Pharmacol. Biochem. Behav. 99 (2), 262–274. https://doi.org/10.1016/j.pbb.2011.05.002.

Dackis, C.A., Gold, M.S., 1990. Addictiveness of central stimulants. Adv. Alcohol Subst. Abuse 9 (1–2), 9–26. https://doi.org/10.1300/J251v09n01_02.

Diamond, A., 2013. Executive functions. Annu. Rev. Psychol. 64, 135–168. https://doi.org/10.1146/annurev-psych-113011-143750.

Eiden, L.E., Weihe, E., 2011. VMAT2: a dynamic regulator of brain monoaminergic neuronal function interacting with drugs of abuse. Ann. N. Y. Acad. Sci. 1216, 86–98. https://doi.org/10.1111/j.1749-6632.2010.05906.x.

Frati, P., Kyriakou, C., Rio, A., Marinelli, E., Vergallo, G., Zaami, S., Busardo, F., 2015. Smart drugs and synthetic androgens for cognitive and physical enhancement: revolving doors of cosmetic neurology. Curr. Neuropharmacol. 13 (1), 5–11. https://doi.org/10.2174/1570159x13666141210221750.

Heal, D.J., Smith, S.L., Gosden, J., Nutt, D.J., 2013. Amphetamine, past and present – a pharmacological and clinical perspective. J. Psychopharmacol. (Oxford, England) 27 (6), 479–496. https://doi.org/10.1177/0269881113482532.

Ilieva, I.P., Hook, C.J., Farah, M.J., 2015. Prescription stimulants' effects on healthy inhibitory control, working memory, and episodic memory: a meta-analysis. J. Cogn. Neurosci. 27 (6), 1069–1089. https://doi.org/10.1162/jocn_a_00776.

Krishnan, S., Montcrief, S., 2007. Toxicity profile of lisdexamfetamine dimesylate in three independent rat toxicology studies. Basic Clin. Pharmacol. Toxicol. 101, 231–240. https://doi.org/10.1111/j.1742-7843.2007.00093.x.

Kwak, H.R., Lee, J.W., Kwon, K.J., et al., 2009. Maternal social separation of adolescent rats induces hyperactivity and anxiolytic behavior. Korean J. Physiol. Pharmacol. 13 (2), 79–83. https://doi.org/10.4196/kjpp.2009.13.2.79.

Levine, A.S., 1981. Stress-induced eating in rats. Am. J. Phys. 241, R72–R76. https://doi.org/10.1152/ajpregu.1981.241.1.R72.

Malenka, R.C., Nestler, E.J., Hyman, S.E., 2009. Chapter 13: higher cognitive function and behavioral control. In: Sydor, A., Brown, R.Y. (Eds.), Molecular Neuropharmacology: A Foundation for Clinical Neuroscience, 2nd ed. McGraw-Hill Medical, New York, USA, pp. 318–321.

Mattson, M.P., Calabrese, E.J., 2010. Hormesis: A Revolution in Biology, Toxicology, and Medicine. Springer, New York.

McKim, W.A., Hancock, S.D., 2013. Chapter 5: substance use and addictive disorders. In: Drugs and Behavior: An Introduction to Behavioral Pharmacology, 7th ed. Pearson, Boston.

Miller, G.M., 2011. The emerging role of trace amine associated receptor 1 in the functional regulation of monoamine transporters and dopaminergic activity. J. Neurochem. 116 (2), 164–176. https://doi.org/10.1111/j.1471-4159.2010.07109.x.

Murphy, H.M., Ekstrand, D., Tarchick, M., Wideman, C.H., 2015. Modafinil as a cognitive enhancer of spatial working memory in rats. Physiol. Behav. 142, 126–130. https://doi.org/10.1016/j.physbeh.2015.02.003.

Pennick, M., 2010. Absorption of lisdexamfetamine dimesylate and its enzymatic conversion to d-amphetamine. Neuropsychiatr. Dis. Treat. 6, 317–327.

Pinter, E.J., Pattee, C.J., 1968. Fat-mobilizing action of amphetamine. J. Clin. Investig. 47 (2), 394–402.

Spencer, R.C., Devilbiss, D.M., Berridge, C.W., 2015. The cognition-enhancing effects of psychostimulants involve direct action in the prefrontal cortex. Biol. Psychiatry 77 (11), 940–950. https://doi.org/10.1016/j.biopsych.2014.09.013.

U.S. Food and Drug Administration, 2017. VYVANSE ® (lisdexamfetamine dimesylate) capsules, for oral use, CII. Retrieved August 28, 2018, from. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/208510lbl.pdf.

Vickers, S.P., Hackett, D., Murray, F., Hutson, P.H., Heal, D.J., 2015. Effects of lisdexamfetamine in a rat model of binge-eating. J. Psychopharmacol. 29 (12), 1290–1307. https://doi.org/10.1177/0269881115615107.

Vorhees, C.V., Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 1 (2), 848–858. https://doi.org/10.1038/nprot.2006.116.

Wideman, C.H., Murphy, H.M., 2009. Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. Nutr. Neurosci. 12 (5), 233–240. https://doi.org/10.1179/147683009x423436.

Wood, S., Sage, J.R., Shuman, T., Anagnostaras, S.G., 2014a. Psychostimulants and cognition: a continuum of behavioral and cognitive activation. Pharmacol. Rev. 66 (1), 193–221. https://doi.org/10.1124/pr.112.007054.

Wood, S., Sage, J.R., Shuman, T., Anagnostaras, S.G., 2014b. Psychostimulants and cognition: a continuum of behavioral and cognitive activation. Pharmacol. Rev. 66 (1), 193–221. https://doi.org/10.1124/pr.112.007054.