EFFECTS OF METHYLPHENIDATE ON ADDICTIVE BEHAVIOR AND SHORT TERM MEMORY RETRIEVAL IN STANDARD HOUSING AND ENRICHED ENVIRONMENT MICE

Sarah Barowka, Dr. Kimberly Gerecke Stephen Spainhour, Rachel Trout

2008 Rhodes Institute for Regional Studies

Attention deficit hyperactivity disorder (ADHD) is one of the most commonly diagnosed childhood disorders (Akay, 2006). Methylphenidate (Ritalin®) is the most commonly prescribed drug for this disorder. Though highly effective as an ADHD treatment, there is very little known about the possible negative effects of the drug. The objective of this experiment was to explore some potential negative effects of methylphenidate (MPH). It was hypothesized that chronic therapeutic i.p. administration of MPH would be addictive and cause deficits in short term memory formation. It was also hypothesized that those animals housed in an enriched environment (EE) would be more protected against these possibly detrimental long-term effects of the drug. In order to test these hypotheses, both a condition place preference (CPP) and an object recognition (OR) test were used. In addition half of the mice were housed in an EE while the other was housed in standard housing (SH). Those mice in the testing group of the CPP were administered of MPH (5mg/kg) every morning for six days and assigned to one side of the box. Those animals not receiving the drug were given saline injections. On the final day the mice were allowed to freely explore the box for fifteen minutes and the time spent on each side of the box was recorded. Those mice participating in the OR test were introduced to the OR box in a five minute habituation period. Testing began the following day where they were introduced to two sample objects. On the second testing day, they were exposed to both the sample and a novel object. The results of both the CPP and OR yielded no significance with respect to both drug condition (SAL or MPH) or housing (SH or EE). These results demonstrate that chronic therapeutic use of the drug does not exhibit the negative behavioral side effects of addiction or short term memory impairment. This suggests that treatment may be the best option for someone affected by ADHD as the behavioral consequences of the disorder may outweigh the possible risks. This research did not consider the possible negative effects that MPH may have on brain anatomy, therefore caution should still be used when considering large scale chronic use of MPH.

INTRODUCTION:

Attention deficit/hyperactivity disorder (ADHD) is one of the top diagnosed childhood disorders and is characterized by inattention, hyperactivity and impulsivity (Akay, 2006; DSM-IV, 1994). The prevalence of ADHD in the age span from age 2 through childhood is 2-5% with nearly a third of these individuals continuing to be affected into adolescence and adulthood (Angold et al., 2000). The etiology of the disorder is not well-understood, however there has been much research done on both the genetic and environmental influence of the disorder (Kahn et al., 2006).

Though researchers continue to investigate the causes of the disorder, research on the mechanism of ADHD focuses on dopamine (DA) dysfunction in the brain. One suggested mechanism for ADHD is that DA released into the synapse is taken up too quickly causing a decrease in DA activity in the reward pathway of affected individuals (Grund et al, 2006). The reward pathway is a DA dependant system consisting of the amygdala, hippocampus, prefrontal cortex, nucleus accumbens and ventral tegmental area. Low DA levels in the synapse causes less neurotransmission which depresses this pathway responsible for emotions, short term memory, spacial navigation, and behavioral moderation. These deficits may then lead to expression of the behavioral deficits seen with ADHD (Kandel, 2000).

There has been no cure found for ADHD, however, methylphenidate (MPH), the most commonly prescribed drug for the disorder, has been highly effective in relieving many of its symptoms (Grund et al., 2006). MPH is a DA agonist that binds with dopamine transport proteins on the pre-synaptic neuron slowing the reuptake of dopamine from the synapse (Gatley et al., 1997). Therefore, the amount of DA available to the post-synaptic neuron is increased and the reward pathway is stimulated.

This is the same mechanism by which certain drugs of abuse such amphetamine and cocaine act (Solanto, 2000). With similar pharmacological properties to these highly addictive and frequently abused drugs, it is possible that MPH is also an addictive drug. Prior research has suggested that when taken orally in doses ranging from 10mg/kg -15 mg/kg MPH is not addictive (Kollins et al, 2000; Dafny, 2005). Other research has indicated that treatment of ADHD with MPH reduces the risk for substance abuse (Anderson et al. 2002). Further investigation has also shown that when MPH is taken intravenously, intranasally or in dosages above therapeutic range it is addictive. However, this misuse of MPH causes blood plasma levels of the drug to be increased more rapidly causing a "high" effect which would lead to addiction (Grund et al., 2006; Kollins et al., 2000).

While the addictive properties of MPH are not well known, it is a known drug of abuse. It is frequently taken by those to whom it is not prescribed as well as incorrectly by those to whom it has been prescribed. Abuse of this drug ranges from taking more than the prescribed dose to taking it intravenously or intranasally. Shillington et al. (2006) performed a study in southwestern California in which they studied MPH abusers at an undergraduate college. They found that its use was relatively widespread for various reasons such as increasing academic productivity and producing a "high" (Teter et al., 2005). They found that between 4.7% and 5.7% of college students had abused MPH within the last year. However, other research has reported numbers as high as 10.3% of students who had abused MPH (Low and Gendaszek, 2002).

Another possible effect of MPH is on alterations in memory formation. Dopamine is highly active in regions such as the prefrontal cortex and hippocampus which are areas essential to learning and memory. As this is a drug prescribed to children as young as 4, there is little

research regarding the effects of chronic administration on neurodevelopment (See Grund et al., 2006 and Akay et al. 2006 for review.) As such, there have been many questions posed about the long term implications on brain activity, specifically memory formation (Volkow and Insel, 2003). High concentrations of DA can be detrimental to working memory which may also have effects on longer term memory storage (Arnsten, 2001; Chuhan, 2005). As such, while MPH is a highly effective drug for the treatment of ADHD, the potential side effects could outweigh the benefits.

There have been many studies done on both physical and cognitive activity as a method of addiction prevention and memory improvement (Perry, 2008). One experiment showed that an enriched environment (EE) has a number of beneficial physiological and neurological effects (Cotman and Berchtold, 2002). An EE for rodents is created by housing them in a cage consisting of running wheels, tunnels, toys and much open space. This research showed that an enriched environment increases the amount of neurotrophic growth factors causing higher synaptic density and therefore increased cognitive performance effects (Cotman and Berchtold, 2002). Xu et al. (2007) showed that an EE could lessen both a morphine-induced reward and drug seeking behavior in mice when undergoing a condition place preference test for addiction. Taken together, an enriched environment may provide protection against the possible deleterious effects MPH use may have on the brain.

While MPH is a highly effective drug for the treatment of ADHD, there are many possible long term detrimental effects that it may have on the developing brain. We may, however, also be able to protect against these negative effects through such changes in lifestyle as regular exercise and consistent cognitive activity. This experiment explored the effects of

MPH on both addiction and memory in mice. It was hypothesized that even at therapeutic dosages that addictive behavior and short-term memory impairment would be observed.

MATERIALS AND METHODS

Animals

Forty adult (eight-week old) female C57/B16J mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were maintained in a temperature-controlled environment with free access to food and water, which was monitored on a regular basis. The diet consisted of standard rat chow. Mice were kept on a regulated 12 hour light/dark cycle. Housing for all mice consisted of two groups: standard mouse housing cages or an enriched environment (EE). Standard housing cages consisted of four to five mice per cage, with free access to food and water. EE mice were housed in a large cage (91.4 cm X 91.4 cm) containing free access to food and water, running wheels, rubber balls, tunnels, other assorted toys, and standard nesting materials. All mice were maintained in accordance with the guidelines set fourth in the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and all protocols were approved by Rhodes' Institutional Animal Care and Use Committee.

Conditioned Place Preference

Apparatus

The testing was performed in a small testing room separate from the animal living area. The testing apparatus consisted of a divided wooden box with two chambers each measuring 38 x 24 x 30cm. One side was painted in alternating black and white horizontal stripes 35 cm in width. The other side was identical in pattern with the exception that the stripes were vertically oriented.

Condition Place Preference Testing

Prior to the conditioning phase, mice were placed on one side of the condition place preference (CPP) box and were allowed to freely explore the two separate sides of the box for twenty minutes. The total time spent on each side was recorded to determine if any initial preference existed. Any mouse which spent more than 60% of the twenty minutes on one side of the box was eliminated from the CPP test. Remaining animals were then randomly assigned to receive either a MPH (SH n=4, EE n=4) or SAL (SH n=4, EE n=4) i.p. injection. The conditioning phase, in which the animals received injections, began 24 hours following the last preference test.

In the conditioning phase, MPH (5 mg/kg) or saline was administered via i.p. injection to the testing and control groups respectively. All behavioral testing was completed by midday so as to coincide with the animals' activity level. The mice were then assigned to either CPP box left or CPP box right and were confined to that side for fifteen minutes following the injection. This process was repeated every 24 hours for six days. Twenty-four hours following the sixth injection, mice were placed in the CPP apparatus for fifteen minutes drug-free, and were allowed to freely explore the box. The total time spent on each side of the box was recorded.

One week following the seventh day of testing, mice were fatally anesthetized using 250 mg/kg i.p. injections of tribromoethanol and were perfused with saline followed by 4% paraformaldehyde (PF) in phosphate buffered saline (pH 7.4). Brains were extracted and post-fixed in PF for 24 hours prior to paraffin embedding for future use in immunohistochemistry.

Object Recognition

Apparatus

The object recognition testing box was a black painted square wooden box measuring 35 x 35cm. A grid of 5cm squares was marked in white on the floor of the box. Objects used for the memory test consisted of heavy metal elbow and T-shaped pipes that were the same color and texture. Both objects were able to fit in a single square of the CPP box, and did not greatly resemble one another in shape, so as to elicit differentiating results.

Object Recognition Test

Twenty-four hours prior to testing, the mice were placed in the test box without objects for five minutes in order to become habituated to their surroundings. On the first test day, all mice were given an injection of saline and placed in the same box with two identical objects placed in opposite corners, 5cm from the closest side and 10cm from each other. The mice were allowed to explore the objects for a 5 minute interval. Twenty-four hours later, the mice were randomly assigned the condition of MPH (5mg/kg) (SH n = 6, EE n = 6) or SAL (SH n = 4, EE n = 6). The appropriate injection was given and the mice were placed in a post-injection cage for five minutes. They were then placed in the OR box with both the familiar object from the previous day and a novel object and were again allowed to explore the box for 5 minutes. Data was recorded with video recording equipment and the time spent exploring each object was analyzed. Mice were considered to be exploring the object when they were oriented towards the object with their noses 2cm away from it, but not if they were sitting on or near but not facing the object.

RESULTS

Conditioned Place Preference

In order to determine if enriched environment decreases the potential addictive nature of MPH in mice, we used the traditional model of Conditioned Place Preference. This model has been used extensively and has reliably shown that mice learn to relate the effects of the drug with their assigned side. When drugs are pleasurable, mice will spend a disproportionate time during the trial on the side in which they received the drug. The percentage of time the mouse spent on their assigned side out of the total 15 minutes spent in the box was quantified and analyzed using a 2x2 factorial ANOVA.

The marginal means (±SEM) for each group were: SH/MPH, 55.0(±5.88); SH/Saline, 48.8(±5.88); EE/MPH, 55.4(±5.88); and EE/Saline, 43.2(±5.88) (see Fig.1). There were no significant main effects with the housing and drug variables, $F(1,12) = .196$, $p<.666$ and $F(1,12) = 2.457$, $p<.143$. There was also no significant interaction, $F(1,12) = .258$, $p<.620$. Methylphenidate administered i.p. at a dose of 5 mg/kg did not create a place preference in neither the enriched environment nor the standard housing group.

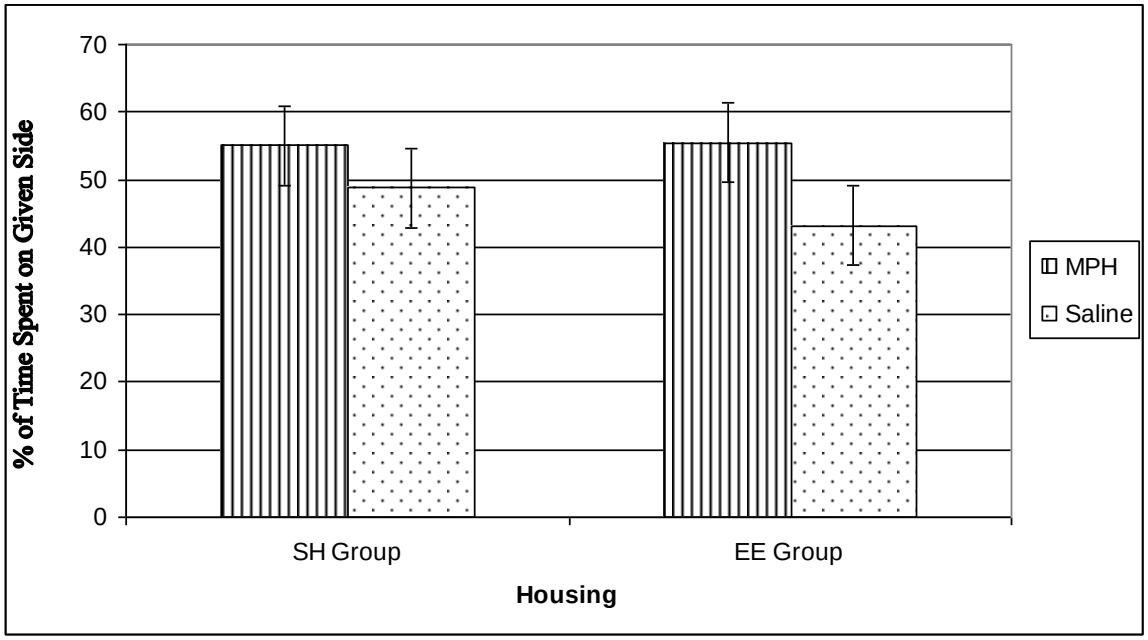

Figure 1. A bar diagram showing the average percentage of time the mice in four treatment conditions spent on the side of the CPP box in which they were administered the drug. A factorial ANOVA yielded no significant differences between housing conditions.

Object Recognition

The effect of methylphenidate on short-term memory was assessed using the object recognition task. In this model, mice spend significantly more time exploring the novel object than the object seen 24 hours prior to the test (Chuhan, Taukulis 2006). Therefore, we hypothesized that mice receiving MPH during T2 would spend less time exploring the novel object, indicating that the drug interfered with short-term memory retrieval. The percentage of time the mouse spent exploring the novel object out of the total object exploration time was quantified and analyzed using a 2x2 factorial ANOVA.

The marginal means (±SEM) for each group were: SH/MPH, 61.0(±4.52); SH/Saline, 54.5(±5.54); EE/MPH, 54.5(±4.52); and EE/Saline, 54.7(±4.52) (see Fig. 2). There were no significant main effects, $F(1,18) = .429$, $p<.521$ and $F(1,18) = .438$, $p<.516$. There was also no significant interaction between housing condition and drug condition, $F(1,18) = .482$, $p<.496$.

These results indicate that methylphenidate had no effect on short-term memory for both standard housing condition and enriched environment conditions.

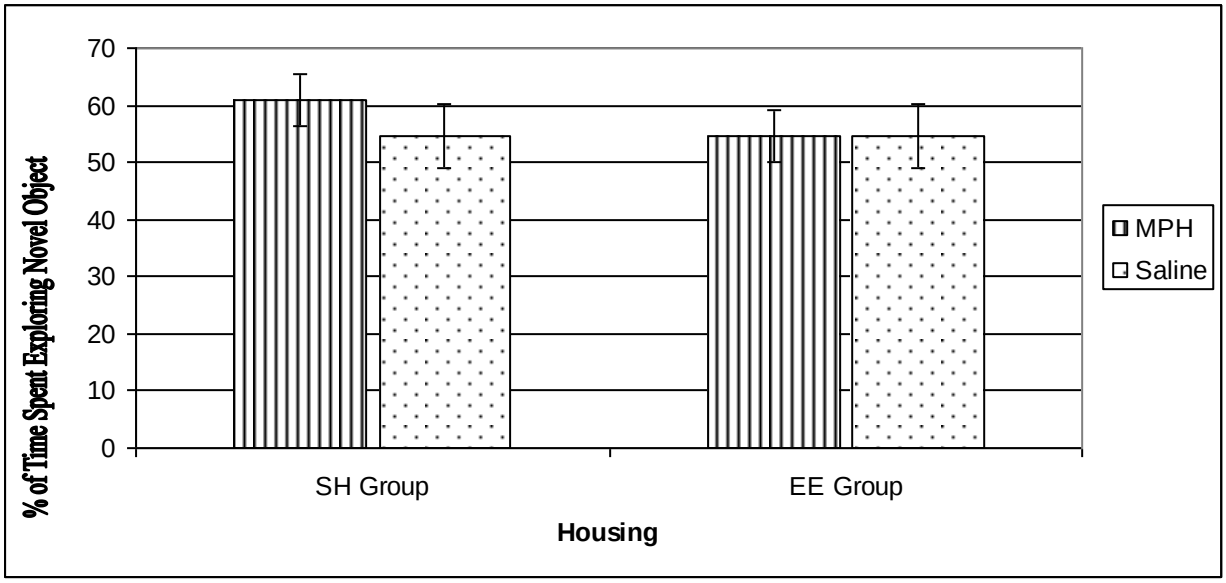

Figure 2. A bar diagram showing the average percentage of time the mice spent exploring the novel object in T2 in four treatment conditions. A factorial ANOVA yielded no significant differences between housing conditions.

DISCUSSION:

Addiction

The CPP test for addiction showed that neither the standard housing (SH) nor enriched environment (EE) MPH mice preferred the side of the box in which they were administered the drug. This implies that when chronically administered at therapeutic dosages MPH does not cause addiction. However, prior research states that MPH can be addictive especially when administered via i.p. injection due to the rapid increase of plasma levels of the drug causing a "high" (Kollins et. al, 2000).

Martin-Iverson et. al (1985) demonstrated that rats administered MPH (5mg/kg) over the course of one week showed a conditioned place preference for the drug. In other words, their experiment demonstrated that chronic therapeutic MPH use may lead to addiction. However, other research suggests that at therapeutic doses MPH actually causes a decreased tendency towards addiction in those individuals affected by ADHD as it does not sensitize the reward pathway like amphetamine and cocaine, but rather desensitizes it as it causes long-lasting effects in the nucleus accumbens (Grund et. al, 2006). As the MPH (5.0mg/kg) in this experiment was introduced via i.p. injection as in the Martin-Iverson experiment, it was hypothesized that the MPH mice would show condition place preference.

There are several possible reasons that our results differ from previously published research. One possibility is that rats and mice metabolize MPH differently. If this is the case, future research should incorporate both rats and mice as a part of the same experimental protocol. Another option is that the CPP box used in this experiment was different from that used in Martin-Iverson et al. While the box used in this experiment used horizontal and vertical stripes other CPP boxes use a black and a white side or stripes and checkerboard. However, varying patterns have also been used in CPP testing and the box in this experiment was developed from a previous model. It was also observed during conditioning that the mice seemed to spend extensive time in the neutral area of the box. It is therefore possible that they were not always experiencing the effect of the drug in the side of the box in which it was administered. In the future, a box could be used with a smaller neutral area. Another possibility is that while most mice were tested in earlier morning in accordance with their activity levels, it was observed that the mice tested later in the morning and in early afternoon had lower activity levels. Therefore they spent less time exploring the box and more time in an idle state.

Lastly, along with the possible differences in the box, opinions on what constitutes a therapeutic dose of MPH varies widely. One paper considered 10mg/kg to be a high dose for mice while another considered it to be a moderate dose within therapeutic range (Grund et al, 2006; Arnsten, 2001). In this instance, it is possible that the 5mg/kg MPH may have been too low a dose to obtain a result. However, we wanted our method to be directly translatable to therapeutic use. Future studies should take into account the large size of the neutral area as well as the time of day the mice were tested. Further testing with more trials would strengthen these results.

Short-term memory impairment

The OR test also yielded no significant results. However, prior research has shown that even one dose of MPH can impair short-term memory (Chuhan et al., 2005). In one experiment MPH (10mg/kg) was administered to rats participating in an OR test. Those administered the drug on the day before they explored the novel objects showed no significant memory for the familiar object when compared with the control. However, those mice administered MPH on the day that they explored the novel objects did show significant memory for the familiar object. This demonstrates that short-term memory storage, but not retrieval was impaired by a single dose of the drug (Chuahan and Taukulis, 2005). This test was used as a preliminary experiment to determine whether it would work in this laboratory with mice. It was hypothesized that the animals receiving MPH(5mg/kg) in this experiment during retrieval would demonstrate impairments in short-term memory formation with the MPH mice not recognizing the novel object. However, neither MPH nor housing condition yielded any significant main effect.

It was expected that while the SAL mice would show memory for the sample object, the MPH mice would not. Though the test was replicated successfully, the SAL animals did not show memory for the sample object. One possible explanation for this is that the OR box in this experiment was also not kept to the same scale used by Chuhan and Taukulis (2005). When the box was initially proportionally scaled down for mice, it appeared to be too small to allow for adequate exploration and clear designation of observation of the objects. As such, it is possible that the OR box used in the previous experiment was so small that it forced interaction with objects. Therefore, the OR box used in this experiment was scaled up in order to allow the mice more room for exploration bearing more resemblance to an open-field test. In fact, the results from using this larger OR testing apparatus may actually be considered stronger as they are closer to the open-field standard.

One possible explanation for the MPH results is that the mice seemed to experience behavioral reactivity with the administration of MPH. Some of the mice, most specifically those that had been housed in the EE spent the majority of testing time running around the OR box, but very little time exploring the objects. As mentioned previously, there is the possibility that mice metabolize MPH more quickly than rats therefore responding differently in this test. However, short-term memory impairment has not been shown during retrieval only during encoding.

There is also the possibility that results could have been obtained with a different strain of mice. Though it is the least likely possibility, C-57 mice do tend to be more hyperactive than other groups and therefore may react more strongly to MPH. For instance, there is a C-57 mouse that is considered to be spontaneously hyperactive which is a behavioral quality that arose from extensive inbreeding (Jackson Laboratories).

Conclusions

Though the results do not support the hypotheses, they should not be discounted. This research does not support the result of negative behavioral effect of addiction with chronic therapeutic MPH use. In addition, it does not support impairment of short-term memory retrieval with a single-dose of MPH. This suggests the possibility that the benefits of MPH may outweigh the risks. The most important benefits of this drug are that it significantly decreases those symptoms related to higher order functioning such as impulsivity.

The pre-frontal cortex is part of the DA concentrated reward system. It is the region of the brain responsible for thinking ahead, planning for the future, visualizing consequences and evaluating actions before they are completed. The decreased activity in the pre-frontal cortex that is seen with ADHD results in symptoms of impulsivity. With the part of the brain responsible for consequences and emotional responses depressed, decisions are made without planning often resulting in self-destructive behavior. Therefore, those individuals affected by ADHD who go untreated may be at a greater risk than those who are treated. However, while the behavioral evidence supports this claim there is much that is unknown about the possible deleterious effects that MPH may have on the brain.

Medical vs. Research Opinion on prescribing MPH for ADHD

There are differing views between the medical and scientific communities about prescribing MPH (Ritalin®). While the scientific community is advising further research into the effects of Ritalin® on the brain, the prevalence for prescriptions of the drug from the medical community increased 6-fold from 1990-1995 (DEA, 1995). The medical model and the model used by the FDA for determining whether or not a drug should be approved for use establishes a

risk/benefit analysis. If the benefits outweigh the known risks, they believe that there is no harm done in prescribing the drug.

Ritalin® is highly affective at treating the symptoms of ADHD and there are few known risks. As many of the symptoms associated with the disorder can be detrimental to an individual, the medical community is justified in continuing to prescribe this medication. However, many questions should be posed regarding its use and misuse. Ritalin® is being prescribed outside of its originally approved parameters and is also a drug with a high abuse potential.

As a result, the scientific community is more cautious about Ritalin® use taking the approach that the maladaptive effects of Ritalin® are simply unknown. One important consideration is that MPH is a drug that is often prescribed during childhood. As this a time of significant neurodevelopment, this causes concerns about the effects of the drug on the developing brain. While this experiment was performed in adult mice, some research suggests that administration of this stimulant during childhood and adolescence may effect neurodevelopment (Grund et al, 2006; Arnsten, 2001). Grund et al demonstrated that i.p MPH can impair development of DA fibers resembling the effect of a single high dose of methamphetamine. However, they also showed that i.p MPH at a therapeutic dose did not show this impairment implying that this may be an issue with abuse, but not with therapeutic use. On the other hand, Le-Blanc- Duchin et al (2007) demonstrated that chronic therapeutic use of MPH caused both short-term and long-term memory impairments in adolescent rats. This also implies that there may be detrimental neuroanatomical effects as memory impairments imply that there is some sort of dysfunction in the ability to form new neuronal connections.

It is important to note that one concern about giving this psychostimulant during development is that there is a programmed round of apoptosis or programmed cell death that

occurs in the brain of children in school age years (Kandel, 2000). This is the body's way of ridding of those neurons that may defective or unused. As Ritalin® is a psychostimulant, it directly effects the neurochemistry of the brain. There are concerns that if the brain undergoes apoptosis while under the influence of this psychostimulant that it may cause possibly detrimental permanent changes in neuroanatomy.

This research shows that there are some known detrimental effects of therapeutic MPH use. However, there are also questions about many other possible detrimental effects. While Ritalin® was originally approved to be given to children ages 5-12 for short-term use, we are now coming upon the generation of individuals who have been using Ritalin® for twenty and thirty years. There are no long-term longitudinal studies that have been done in humans to explore what some of these effects may be. There are also no published animal studies that represent this long-term use.

In addition, as mentioned previously, Ritalin® has a high abuse potential. It is used as a study drug or a party drug. It is taken by those to whom it is not prescribed in manners it was not prescribed be it intranasally or by injection. There is very little research that models the abuse of Ritalin®. The neurochemistry of individuals unaffected by ADHD is believed to be significantly different than affected individuals. There are also very few studies on pairing Ritalin® with other drugs or alcohol. Though abuse is not the intended use for this drug, it is a relatively widespread issue especially in college-age individuals and therefore should be investigated.

While Ritalin® does have a significant effect on symptom management of ADHD, caution should be exercised about large scale chronic use of this drug about which we know so little. The medical community should continue to prescribe Ritalin® for the benefits of affected individuals. Currently the benefits of Ritalin® use outweigh the known risks, but research on this

drug needs to continue. Future research should investigate long-term neuroanatomical effects of the drug as well as develop and investigate various abuse models. In addition long-term investigations in humans involving behavioral, memory and other performance tasks should also be completed.

WORKS CITED

Akay, Ayunar Pekcanlar, et al. 2006. Effects of long-term methylphenidate treatment: A pilot follow-up clinical and SPECT study. Progress in Neuropharmacology & Biological Psychiatry. 30: 1229-1224.

Anderson, Susan L., Carryl P. Navalta. 2004. Altering the course of neurodevelopment: a framework for understanding the enduring effects of psychotropic drugs. Developmental Neuroscience. 22: 423-440.

Angold, Adrian, Erkanli, Alaattin. Egger, Helen L., Costello E. Jane. Stimulant treatment for children: A community perspective. *J. of the American Academy of Child and Adolescent Psychiatry*. 39(8): 975-984.

Arnsten, A.F.T. 2001. Dopaminergic and noradrenergic influences on cognitive functions mediated by prefrontal cortex. *Stimulant Drugs and ADHD: Basic and Clinical Neuroscience.* New York: Oxford University Press: 185-208.

Chuhan, Yadvinder S., Harold K. Taukulis. 2005. Impairment of single trial memory formation by oral methylphenidate in the rat. Neurobiology of Learning and Memory. 85: 125-131.

Cotman, Carl W. Berchtoldt, Nicole C. 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in Neuroscience*. 25:6. 295-301.

Dafny, Nachum, Pamela B. Yang. 2005. The role of age, genotype, sex and route of acute and chronic administration of methylphenidate: A review of its locomotor effects. Brain Research Bulletin. 68: 393-405.

Gatley S.J. Pan D.F., Chen R.Y., Chaturvedi, G., Ding Y.S. 1996. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Science.* 58: 231-239.

Grund, Thorsten, Konrad Lehmann, Nathalie Bock, Aribart Rothenberger, Gertraud Teuchert-Noodt. 2006. Influence of methylphenidate on brain development- an update of recent animal experiments. Behavioral and Brain Function. 2:2.

Kahn, Robert S., Braun, Joe M., Froehlich, Tanya, Auinger, Peggy., Lanphear, Bruce P. 2006. Exposures to Environmental toxicants and Attention Deficit Hyperactivity Disorder in U.S. Children. *Environmental Health Perspective.* 114(12): 1904-1909.

Kandel, Eric R., James H. Schwartz, Thomas M. Jessell, 2000. *Principals of Neuroscience*: Fourth Ed. McGraw-Hill, New York, 1413 pp.

Kollins, Scott H., MacDonald, Emily K., Rush, Craig R. 2000. Assessing the abuse potential of methylphenidate in nonhuman and human subjects: A review. Pharmacology Beiochemistry and Behavior. 68: 611-627.

LeBlanc-Duchin, Denise, Harold K. Taukulis. 2004. Behavioral reactivity to a noradrenergic challenge after chronic oral methylphenidate (Ritalin®) in rats. Pharmacology, Biochemistry and Behavior. 79: 641-649.

LeBlanc-Duchin, Denise, Harold K. Taukulis. 2007. Chronic oral methylphenidate administration to periadolescent rats yields prolonged impairment of memory for objects. Neurobiology of Learning and Memory. 88: 312-320.

Low, K. Graff. Gendaszek, A.E. 2002. Illicit use of psychostimulants among college students: a preliminary study. *Psychology, Health and Medicine.* 7:3. 283-287.

Perry, Jennifer L., Dustin J. Stairs, Michael T. Bardo. 2008. Impulsive choice and environmental enrichment: Effects of d-amphetamine and methlyphenidate, Behavioral Brain Research. doi 10.1016/j.bbr.2008.04.019

Shillington, Audrey M., Mark B. Reed, James E. Lange, John D. Clapp, Susan Henry. 2006. College undergraduate Ritalin abusers in southwestern California: Protective and risk factors. Journal of Drug Issues. 0022-0426/06/04: 999-1014.

Solanto, M.V. 2000. Clinical psychopharmacology of ADHD: Implications for animal models. Neuroscience and Biobehavioral Reviews. 24:1. 27-30.

Teter, C.J., McCabe, S.E., Crawford, J.A., Boyd C.J., Guthrie, S.K. 2005. Prevalence and motives for illicit use of prescription stimulants in an undergraduate student sample. Journal of American College Health. 53: 253-262.

Volkow, Nora D. and Insel, Thomas R. 2003. What are the long-term effects of methyphenidate treatment?. *Biological Psychiatry.* 54-12: 1307-1309.

Xu, Zhiwei, Bing Hou, Yan Gao, Fuchu He, Chenggang Zhang. 2007. Effects of enriched environment on morphine induced reward in mice. Experimental Neurology. 204: 714-719.