The Effects of Methylphenidate on Addictive Behavior and Short-term Memory Retrieval in Mice

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Abstract

Methylphenidate (MPH) is prescribed, perhaps even over-prescribed, for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children and young adolescents. It is generally thought to be free of important side-effects when administered at recommended therapeutic doses. This fact, however, does not calm debate made over the drug, due to the fact that methylphenidate shares a similar pharmacodynamic property to that of cocaine and amphetamine. As well as possible addictive properties of MPH, previous studies have shown preliminary evidence that MPH may have negative effects on memory recognition and behavior. In order to investigate the possibility of cognitive impairment and behavior, mice were given doses of MPH at 5 mg/kg via intraperitoneal injection, while a control group was given the same dose of saline. To test for addiction, animals were assessed using a conditioned place preference test. An object recognition test was then utilized to effectively determine if MPH interferes with short-term memory. In the OR test, exploration time was assessed. In both tests, it was found that results were insignificant and evidence of cognitive impairment was not witnessed, nor was evidence of addiction at recommended therapeutic doses.

Introduction

Medicine has proven to be one of mankind's greatest contributions to science. However, with every drug that is approved for distribution, comes the potential for abuse. Drug abuse is a

prominent issue in America and all over the world, and with more and more drugs being discovered to aid the healing and/or coping with of numerous ailments. One disturbing trend in the United States is the increasing number of adolescents and adults that are abusing prescription drugs, including methylphenidate (Shillington, Reed, Lange, Clapp, & Henry, 2006).

The effects of a particular environment upon susceptibility to drug abuse is not fully understood, but it has been indicated by a recent study that exposure to an enriched environment, which introduces enhanced opportunities for learning, social interaction, and exercise, may decrease drug-induced rewards, and therefore drug seeking behavior in rodent models (Xu et al., 2007). An enriched environment exposes animals to opportunities for mental stimulation and social interaction. For the purposes of our study, the enriched environment consists of toys, running wheels, tubes to crawl through, and free access to food, water, and other mice.

Even though some studies show the positive effects of an enriched environment on drug abuse and protecting against abusive behavior, much remains to be understood about the issue, especially in the context of particular drug effects. Methylphenidate (MPH [Ritalin]) is the most often prescribed psychostimulant utilized in the treatment of children and adolescents with attention deficit/hyperactivity disorder (ADHD). Its pharmacological properties are similar to those of amphetamine and cocaine (Solanto, 200; Volkow et al., 1995). MPH also works along the same neural pathways in the brain as these two drugs and increases synaptic levels of dopamine and norepinephrine in several brain regions. At these higher synaptic concentrations, dopamine and norepinephrine may impair the working memory function of the prefrontal cortex, potentially interfering with both short and long-term memory storage (Arnsten, 2001). Recent research has supported this in the form of memory retrieval studies that test the effect of drug administration on short-term memory (Chuhan & Taukulis, 2005; LeBlanc-Duchin & Taukulis,

2007). This is especially alarming because a psychostimulant that closely resembles not only illegal, but harmful drugs is being widely prescribed to children.

Even though MPH is widely considered to be a drug that is safe for children and pre-adolescents when used at recommended therapeutic doses, the long-term effects of exposure to the drug on immature brains is still unknown. Alarmingly, MPH abuse among adolescents and even adults is rising, according to poison centers across the United States (Klein-Shwartz & McGrath, 2003). The drug is prescribed in America by nurse practitioners, family physicians, pediatricians, psychiatrists, and other physicians. France possesses an official legislation ruling over the distribution of Ritalin, only allowing psychiatrists and pediatricians to prescribe the drug, and for parents to follow a strict set of rules, keeping the medication safely locked away and watching their children take the drug (Frances, Hoizey, Millart, & Trenque, 2004). Because of this protective legislation regarding the method of distribution, France has decreased rates of MPH abuse.

Recent studies performed with laboratory rodent models have shown evidence suggesting that MPH causes potentially devastating changes in brain function, even after the drug administration has ceased (Brandon, Marinelli, Baker, & White, 2001; Carlezon, Mague, & Andersen, 2003; Eckermann et al., 2001; Gaytan, al-Rahim, Swann, & Dafny, 1997). Chronically administered amphetamine and cocaine in laboratory animals, in both adolescence and adulthood, has resulted in functional fluctuations within the nervous system that may be translated into negative behavioral and cognitive changes, and such fluctuations including afflicted performance upon spatial memory (Robinson & Kolb, 2004). Both of these potent drugs are also widely known to cause brain damage and cognitive impairment.

It has been suggested that chronically administered MPH, like amphetamine and cocaine, also produces an apparent impairment of recognition within laboratory rodent models independently of attention interference (Chuhan & Taukulis, 2005). Our study was designed to further investigate the effects of an enriched environment in mice upon drug addiction, as well as whether or not MPH affects memory performance. As an enriched environment has been shown to decrease addiction to morphine (Xu, Hou, Gao, He, & Zhang, 2005), we hypothesize that it will also protect against any addiction to MPH.

MATERIALS AND METHODS

Animals

Forty adult (eight-week old) female C57/B16J mice were obtained from Jackson Laboratories (Bar Harbor, ME). All forty 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.

Conditioned Place Preference

Apparatus

The testing was performed in a small testing room with a divided wooden box consisting of 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 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. 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, EEn=6).

In the conditioning phase, 5 mg/kg MPH or saline was administered via i.p. injection early in the morning to the testing and control groups respectively. The mice were then assigned to either CPP box left or CPP box right and placed in there for twenty minutes following the injection. This occurred 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, as with the CPP pre-test.

Following the eighth 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 7.0). Brains were extracted and post-fixed in PF for 24 hours prior to paraffin embedding.

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 elbow pipes and T-shaped pipes. Both objects were able to fit in a single square of the OR box, and did not greatly resemble one another, 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 a 0.1mL 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 or SAL. 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. Again, they were allowed to explore the box for 5 minutes. Data was recorded with video recording equipment and the time spent exploring each object was analyzed.

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.

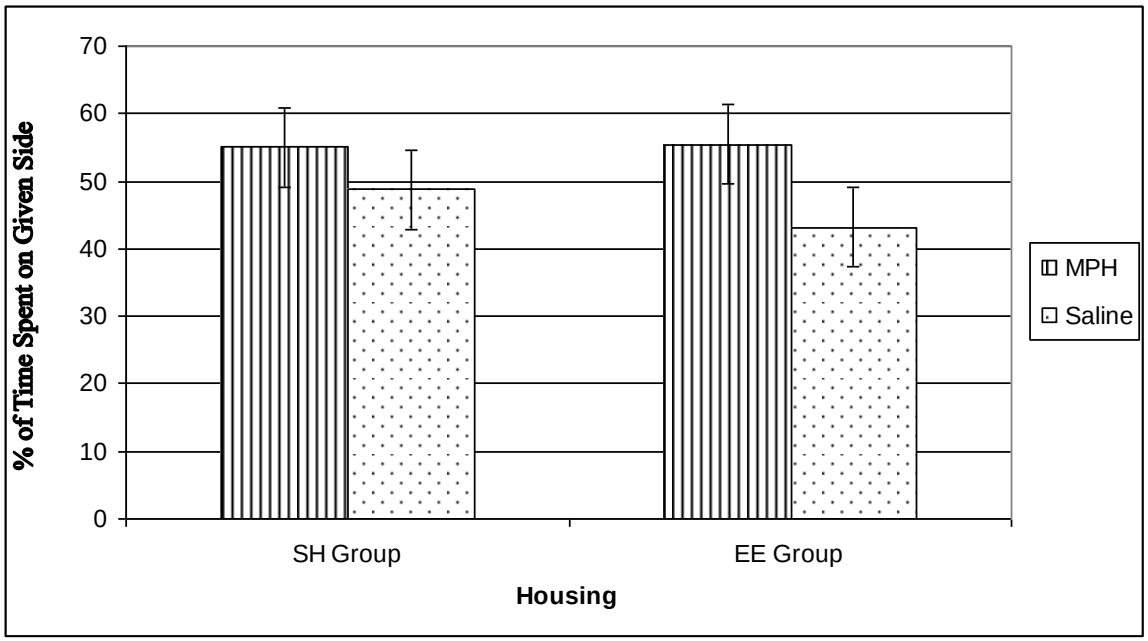

Figure 1. A bar diagram showing the average percentage of time the mice in four treatment conditions spent on the side of the CPP box in which they were administered the drug. A factorial ANOVA yielded no significant differences between housing conditions.

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.

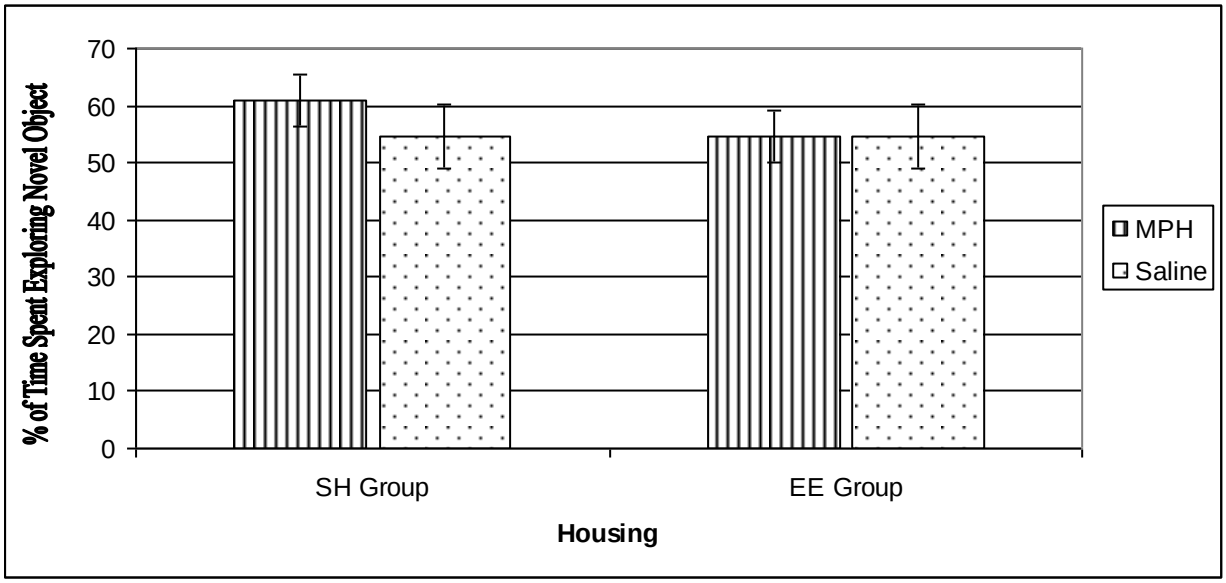

Figure 2. A bar diagram showing the average percentage of time the mice spent exploring the novel object in T2 in four treatment conditions. A factorial ANOVA yielded no significant differences between housing conditions.

Discussion

Both of the tests that we incorporated into this study were performed using small, constant dosages of methylphenidate, in essence modeling appropriate therapeutic use of the drug. The CPP test was utilized to address if MPH treatment may produce significant changes in reward-seeking behavior, while the OR test measured familiarity discrimination, a tendency to explore new, novel objects with greater intensity than objects that are familiar. As the dose of MPH is administered on the day that the new object is introduced, any decrease in exploration time of the new object can be interpreted as MPH interference with short-term memory retrieval.

The results from the CPP test showed no statistical significance. Because of this, it can be deduced that MPH does not appear to cause addiction at this low dose. It does not appear that the

mice learned to associate the effects of MPH to any one particular side of the CPP box. It is important to note that many mice spent much time in the neutral area of the CPP box, between the two sides, which gave us lower numbers than had they chosen to stay on either the horizontal or vertical striped side.

While it does have similar pharmacodynamic properties to that of cocaine, in terms of molecular structure and that they both work upon the dopaminergic pathway, MPH does not appear to create addiction at small doses, taken in the prescribed manner. The drug dose is far too small to create any kind of "high" from therapeutic use. However, the fact remains that it is still abused by those who choose to take much more than the recommended amount, and who take the drug out of prescribed methods (i.e.- snorting or shooting).

The results from the OR test were also not statistically significant, suggesting that MPH did not produce impairment in cognitive function of the mice. It is important to note that while no negative effects appeared to be present for the results of the MPH dosages, we are still uncertain about the anatomical changes within the brain and cells due to constant MPH administration.

The effect of an enriched environment on behavioral response to the drug was also not significant and the same remained true for the standard housing animals, in the case of both the CPP test and the OR test. Even though previous studies showed that evidence of memory impairment after methylphenidate treatment may exist (Taukulis et al., 2007), our study did not confirm this for either the standard housing or the EE animals. The difference that we did notice between the EE animals and the standard housing animals in both tests, is that the EE mice were much more active when placed in the CPP and OR boxes. For both tests, it was a general rule

that the EE mice were somewhat more active for the tests, simply due to the enhanced opportunities for exercise and interaction in the enriched cage.

It is important to note that our results do not agree with the previous results found in the general literature. There are several reasons as to why our results may not agree. Firstly, most similar studies were performed using rats rather than mice, and it is possible that a significant difference exists between mice and rats in response to MPH. Levels of activity may differ due to the difference that can be found within the metabolism of the two species, comparatively. Some concerns were that mice are smaller than rats and because of the difference in size and metabolism, the doses may have a different impact on the rats than it may in mice. However, basing our dosage upon weight addresses the issue of size.

In our OR test, the box was scaled up, meaning the mice in our study had more room in the box proportionally than did the rats involved in other studies (LeBlanc-Duchin, Taukulis, 2007). A possible confound for that study is that animas were forced into engagement with these objects. This could pollute the study, even if the results are desirable, because animals are not exploring the object of their accord, they are simply placed into a box that is too small to appropriately distance the objects from the animals. Our mice had the option to choose the object at which to direct their attention. It is also noteworthy that due to the MPH administration, animals involved in the OR test became hyperactive, which is a typical response even within human children who are given the drug and do not have Attention Deficit Hyperactivity Disorder. It is nearly impossible to effectively model ADHD within rodent models, and due to this fact when MPH is administered to the mice, the effect upon the mice should be similar to that of humans who do not have ADHD. We observed some mice being unable to focus their attention solely on one object, but rather simply running around the OR test box very quickly.

While we did not include behavioral reactivity in our results, it is one possible explanation as to why our OR results were not significant.

The majority of the previous studies that have been performed were carried out utilizing a "wet mash" form of administration, meaning that the MPH was administered orally and mixed with standard rat chow, rather than via i.p. injection. It was assumed for the purposes of our study involving both the CPP and OR tests, that a greater response to the drug could be expected from an i.p. injection, because the drug is absorbed into the bloodstream much faster via injection than oral administration. As such, we expected to see stronger responses than those of previous studies using the oral method (Chuhan & Taukulis, 2005; LeBlanc-Duchin & Taukulis, 2007).

In humans, the oral method of administration is the easiest and relatively least expensive form of giving a drug, and so it remains the most frequently used method of drug administration. However, this method does have its issues. Because of the way a drug moves through the digestive tract, absorption may begin in the stomach, but most of the absorption takes place in the small intestine. Food and/or other drugs in the system may determine how quickly the drug is absorbed. The liver also filters the drug, so the drug may actually be absorbed at a smaller dose than originally given. Injection offers a more concentrated, faster way to administer the drug due to the increase in blood plasma levels. Because the effects of an injection have an onset much faster than that of the oral method, addiction is more feasible with the injection method. In research, injection remains the most effective and reliable way to administer a substance. A previous study involving rats showed that there was a higher concentration of the drug in the body tissues and fewer errors in results in animals given the i.p. injections than did the oral counterparts (Steinbaugh, Taylor, & Pfeiffer, 2007).

With MPH showing no negative results on addiction or short-term memory for both the EE and the standard housing animals, it can be concluded that while MPH has much speculation about its use, it is unable to create addiction at small, controlled doses. For children with the disorder, prescription MPH use can help children in dealing with levels or hyperactivity, focusing and paying attention in school, and make home life much more calm and livable for all household members (Posey et. al, 2007). For children with ADHD, MPH is effective when used at recommended doses (Review- Grund, Lehmann, Bock, Rothenburger, Teuchert-Noodt, 2006). Therefore, it is possible to see why so many parents choose to have their children on the medication.

The lack of effect significance regarding MPH treatment in the OR test suggests that when used at recommended doses, MPH does not appear to impair memory formation, nor does it appear to create addiction. Perhaps further studies could be created that would integrate both methods of MPH distribution, oral and i.p. injection, in order to allow for an apropos comparison of the two methods. This way it might seem to be possible to examine which method would be the preferred method of distribution for the purposes of a certain experiment. Because each mouse is different, this may be a "trial-by-fire" method, but may ultimately relay important information needed for future studies. Because each study has a unique aim, it is important to know which method of distribution is preferable for the intended result. Again, according to previous literature (Steinbaugh, Taylor, & Pfeiffer, 2007), it appears that i.p. injection allows for higher concentrations of the drug to be in the system.

We have pointed out that from our results, it appears unlikely that MPH leads to addiction at small, recommended doses. But again, there are those who choose to take more than the recommended dose outside of prescribed methods. This is where the issue of addiction

begins. It is clear that we need to have newer, more specific guidelines and legislation regarding the use and distribution of methylphenidate. The legislation we have in place within the United States appears to be insufficient, because the fact exists that while MPH is not harmful when used in recommended doses that we know of, those who choose to use it outside of recommended parameters are abusing it. Without a doubt, this is dangerous. A drug that is in the same class as cocaine and amphetamine is being abused, and a message is being spread that this is okay to do. There exists a misconception that what is not illegal will not hurt you. However, this is not true. Abuse of any drug is dangerous and can have terrible consequences.

While is there is certainly no quick fix to this issue, we point once again respectively to the legislation followed by France regarding distribution of the drug. With stricter laws of distribution, lower rates of abuse are reported and occurring. While not all cases may be reported, the fact remains that France has lower rates of MPH abuse than the United States (Frances, Hoizey, Millart, & T. Trenque, 2004). With only pediatricians, nurse practitioners, and psychiatrists being allowed to write prescriptions for MPH, fewer individuals are able to distribute the drug. And in fact, in all of Europe the treatment of ADHD is controversial because the disorder is of unknown etiology. Diagnosis of ADHD in France, for example, requires three different medical opinions for security and certainty. It is the hope, that in this manner, a misdiagnosis is unlikely. It is also required by law for children and adolescents with the disorder to have yearly consultations to examine the progress that methylphenidate has made in their treatment. (Frances, Hoizey, Millart, & T. Trenque, 2004). Because the psychostimulant is so controversial within itself, there is a question that follows: in America, are too many children being given drugs that their parents do not understand, and is ADHD being over-diagnosed?

Does the “there is a pill for everything” attitude prevail in the United States? If so, what can be done to change this attitude?

It is very important to note that there is no clear way to make a drug “ethical” or not. A drug may appear to follow a certain ethical protocol, but again, this does not mean that it will never be abused. What some people find unethical might be ethical and reasonable to others. The problem of a single, consistent opinion is presented here. People will always be different and have different views on subject matters; it is the human condition. There is no real way to fix this issue.

In a positive light, however, it is good that our study failed to find significance within the CPP and OR tests, because it shows that at recommended, therapeutic doses, MPH is fine for children to use and may help with symptoms of ADHD. However, because it is a drug of abuse taken at higher doses, MPH can cause damage. Relatively, the effects of methylphenidate upon brain tissue and the neurons in the brain are unknown, due to the complexity of the organ. We simply do not know how the medication works with the disorder as of yet. So while the behavioral effects and addictive properties appear to be in check for recommended use, we are uncertain about the possible changes that MPH use causes within the brain. It is with further research that more can be discovered about the drug and more can be done to analyze its use.

Little is still known about MPH and the way in which it interacts with the nervous system. Scientists research more and more about other drugs and are certain of long term effects upon the brain, but MPH works in ways that we do not yet comprehend, in conjunction with a disorder that we do not yet fully comprehend. The question remains: How safe is chronic use of methylphenidate in terms of physical effects? The answer is simple but unsatisfying. We just do not yet know. With more research and more time, the effects of MPH on the brain will be further

understood and only then can a complete analysis of the drug be performed. With time, we can learn how the drug works and we may then carry out both appropriate distribution and legislation.

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