The Effect of Methylphenidate on Addictive Behavior and Short-Term Memory Retrieval in Mice

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ABSTRACT

The psychostimulant methylphenidate (MPH) is the most widely prescribed drug for the treatment of attention deficit/hyperactivity disorder (ADHD) in children due to its ability to reduce hyperactivity and improve attentiveness by increasing dopamine levels in the brain. It is thought to be the safest and most effective way to help children with ADHD, but the additional effects of MPH are still relatively unknown. MPH shares a lot of the same characteristics as cocaine and methamphetamine, causing concern that it has the potential to cause addiction. There is also reason to believe that MPH negatively affects short-term memory processes.

In the present study, female mice were housed in either standard housing (SH) or an enriched environment (EE). EE has been proven to be very beneficial to the well-being of the animal, especially in helping protect from pathogenic behaviors. Since MPH is a powerful psychostimulant, it is hypothesized that it will have addictive qualities and mice that are raised in EE will be protected against potential addiction. Methylphenidate’s potential for addiction was tested using a conditioned place preference (CPP) box, but the normal therapeutic dosage of 5 mg/kg was not found to create an addictive behavior in either SH or EE mice groups. Object Recognition was used to test short-term memory (STM) retrieval after a one-time dose of 5 mg/kg of MPH. No significant differences were found for any of the groups, suggesting that

MPH does not interfere with short-term memory. While these results do not show deleterious effects of MPH on addiction or STM, much more research is needed to truly determine the possible implications of chronic methylphenidate use on the developing brain.

INTRODUCTION

In an average classroom in the United States, it is estimated that at least one of the students has attention deficit/hyperactivity disorder (ADHD) (1). ADHD is one of the most common behavioral disorders in childhood, affecting two million school age children, with symptoms often persisting into adulthood (1, 2). The psychostimulant methylphenidate (MPH; trade name Ritalin) is the most widely prescribed drug for the treatment of ADHD due to its ability to reduce hyperactivity and improve attentiveness. While MPH is regarded as relatively safe and efficient when taken at the recommended therapeutic doses, LeBlanc-Duchin and Taukulis (2007) report that the long-term consequences of chronic MPH exposure on immature, developing brains is still unknown (3). Patients with ADHD are thought to have a lower concentration of extracellular dopamine (DA) in the prefrontal cortex (PFC), an area important for personality expression and social intelligence. MPH administration blocks the dopamine transporter, preventing re-uptake and increasing extracellular DA levels; changes in the brain have been reported for dopamine D2 receptors and in dopaminergic transmission in at least the striatum and PFC (4, 5). The behavioral effects of MPH include improved school performance due to increased focus and attention, and overall calmer, less disruptive conduct. When methylphenidate is also paired with behavioral therapy, the treatment is highly effective in helping children and parents dealing with ADHD. However, there is much that remains to be discovered about the long-term effects of this drug.

Methylphenidate is a stimulant whose pharmacodynamics are very similar to frequently abused drugs such as amphetamine and cocaine (6, 7). Just as there is a lack of information on the long-term effects of MPH, there is little known about MPH's potential as a recreational drug. While it is unknown whether MPH has the ability to produce a "buzz" when taken at high doses, MPH is commonly being abused in other ways. In recent years, the prevalence of "study drugs" has increased dramatically with MPH being the most widely misused. The term "study drug" refers to prescription drugs that are used non-medically/illegally, in this case as a way to help concentrate and increase alertness while cramming for exams or pulling all-nighters (8). MPH has potential for various forms of abuse and it is necessary to examine the consequences of short-term abuse of this powerful drug, as well as the effects of chronic therapeutic use.

Past animal studies have shown that housing mice in an enriched environment (EE) can benefit the animals in a multitude of ways, including lessening their susceptibility to drug abuse and addiction (9). The EE is very spacious, with lots of places to play and hide. It also includes running wheels for exercise and other mice for social interaction. Exercise and behavioral stimulation has been shown to sustain and increase human's brain plasticity, which protects against brain insult and improves cognitive performance (12). Previous studies that involved enriched environments exclusively wanted to determine differences between individual and social housing rather than the overall health of the animal (11). Even though these studies proved contradictory in their results about EE, the enriched environment's effect on drug addiction was not assessed. When applied to drug abuse, it was recently reported that enriched environment can trigger long-term modification in neural functions and might prevent the occurrence of pathogenic behaviors (9). Xu et al. (9) studied reward-seeking behavior using morphine administered to mice in standard housing and enriched environment housing. The mice that lived in the EE condition were significantly less inclined to respond to the morphine-induced reward than the standard housing mice, concluding that the enriched environment had the ability to decrease the addictiveness of morphine (9).

Just as methylphenidate's potential for drug abuse is under observation and study, so is its affect on memory formation and storage. Due to the fact that the neurophysiological characteristics of MPH are very similar to those of stimulants known to cause brain damage, it is thought that MPH could negatively influence various important brain processes. One such process is working memory, which allows the mind to create new memories and retrieve old memories from longer-term storage (3). Animals were given an object recognition (OR) task to study memory processes using familiar and novel objects. Greater exploration of the novel object in the second trial suggests that the drug has not interfered with memory formation. This is also known as familiarity discrimination. With this method, MPH can be tested for its affect on memory acquisition (administration prior to Trial 1) and its affect on memory retrieval (Trial 2).

Since MPH is a powerful psychostimulant, it is hypothesized that it will have addictive qualities and mice that are raised in EE will be protected against the potential addiction. Mice raised in EE will also be protected against possible interference of short-term memory retrieval by MPH. By combining the two experiments, we can help advance methylphenidate research more quickly and inform the discussion on the true safety of methylphenidate.

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 approximately 5cm 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.

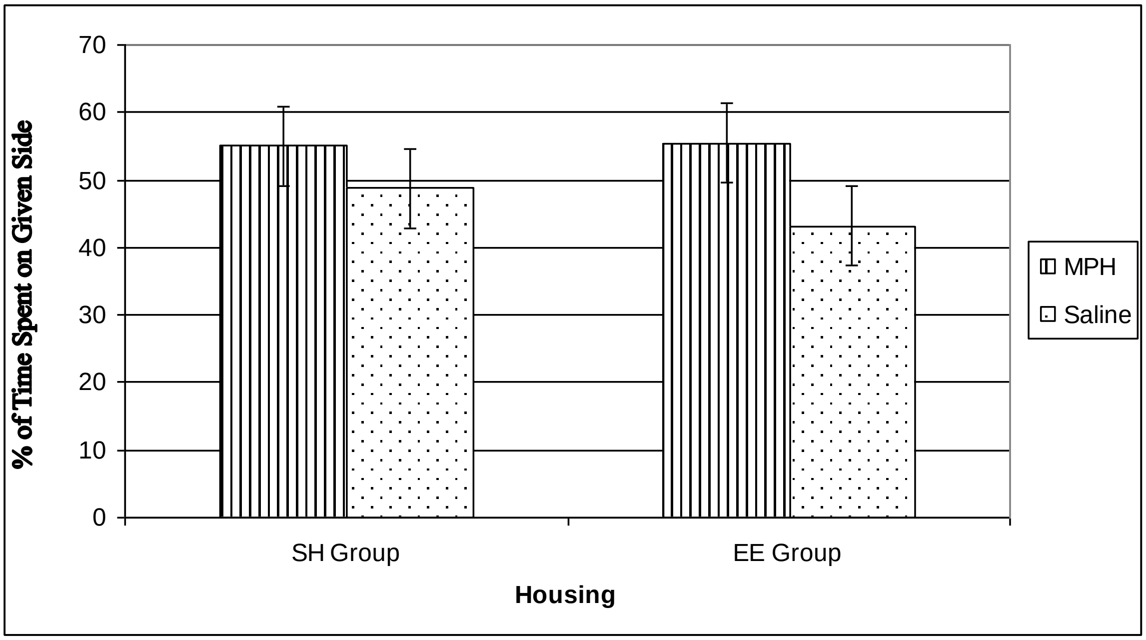

Figure 1. A bar diagram showing the average percentage of time the mice in four treatment conditions spent on the side of the CPP box in which they were administered the drug. A factorial ANOVA yielded no significant differences between housing conditions.

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 (10). 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.

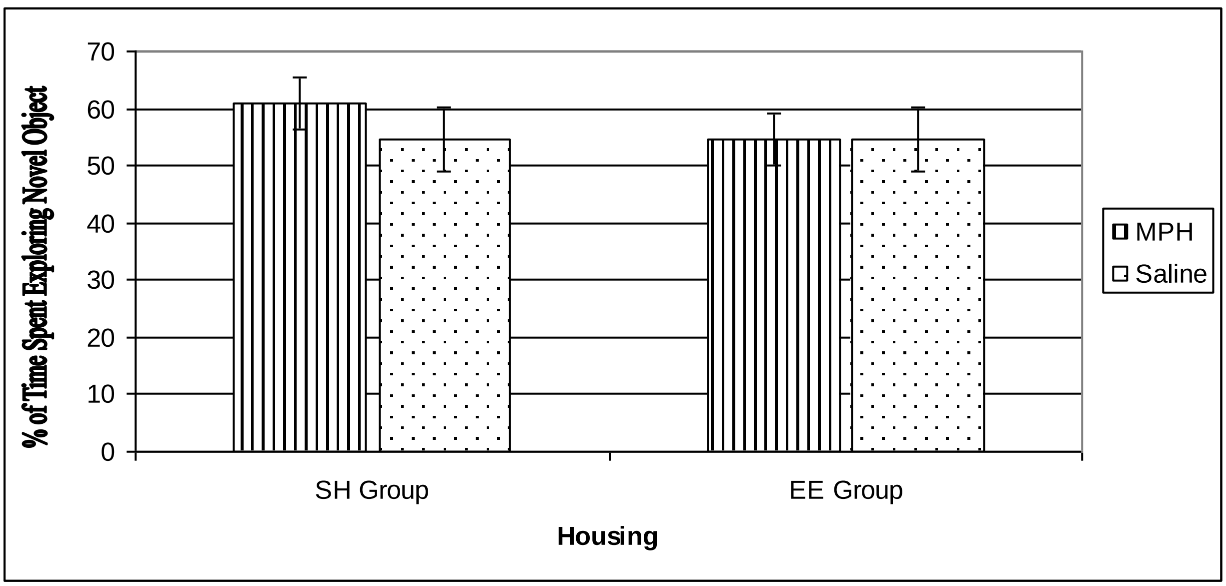

Figure 2. A bar diagram showing the average percentage of time the mice spent exploring the novel object in T2 in four treatment conditions. A factorial ANOVA yielded no significant differences between housing conditions.

DISCUSSION

We hypothesized in our study that enriched environment animals would be more protected against the possible addictive nature of MPH. However, administration of 5 mg/kg i.p., which is the standard therapeutic dosage, for six consecutive days failed to produce a place preference in either SH or EE housed mice. A past study done by Xu et al. (2007) studied the effects of EE on morphine-induced CPP, and found significant results showing that SH mice showed more of an inclination for the morphine-paired side than the EE mice did. This study shows that the CPP test can be successfully performed on mice, but because they used morphine, a drug that is known to be addictive, their study was able to focus more on the effects of EE. As we stated earlier, methylphenidate is not confirmed as an addictive drug, so our experiment was studying dual hypotheses. Since our results were not significant, we can conclude that the short-term therapeutic administration of methylphenidate is not addictive to mice.

There are a number of factors which may account for the differences between these and past results. Many of the materials and methods were altered from previous research done using EE and CPP. Instead of solid black and white compartments, our CPP box was painted with a black and white stripe pattern with horizontal stripes on one side and vertical stripes on the other. We quantified using the stripe pattern rather than solid black and white because mice are more drawn to dark spaces, which could affect results. The stripe pattern has been successful in other experiments, but some of the mice might have found the one side more interesting than the other. This caused them to spend more time on that side and we were forced to exclude them from the study, which lowered our numbers. Low numbers of animals is another implication to the study, but we will discuss that later in the paper.

During the conditioning period we noticed that some of the mice chose to stay in the neutral area of the CPP box. We did the injections in the morning, but as the time approached noon, the mice began to tire and were not as apt to explore their drug-paired side. They would either sit on one side of the box or sit in the middle (neutral) area. Both of these things might have influenced our results. Nevertheless, it is very beneficial that are results were not significant. If we are able to translate a mouse's reaction to MPH to a human's, we can say that the standard dosage being prescribed is safe in terms of addiction. Previous studies have found that administering MPH intravenously can create a "high" due to the quick absorption into the blood system (2). If taken orally though, there is no chance for abuse since it has a very slow influence on plasma levels (2). We administered the drug intraperitoneally for efficiency, as well as to ensure that the mice got the full dose. This is a standard injection method most commonly used in recent research models. Assuming that all injections were properly given, there is no reason to believe that the method of drug administration had an effect on the results.

While mice in this study did not display addictive behavior to methylphenidate, there is much more research to be done before the full effects of MPH can be assessed. Unfortunately, the number of animals that qualified for the CPP experiment was smaller than anticipated, and may have affected the power of the statistics. Replication of the study with higher numbers would give our results much more reliability. Future research should continue to test whether MPH has the potential to be addictive. If it is scientifically proven, then the true effect of EE on MPH addiction could be examined. It would also be helpful to study the design and pattern differences between CPP boxes in past and present studies since we did not find significance with our box, but previous studies were able to successfully find results with the box. Knowing if one pattern or color stimulates a mouse more than another could benefit future addictive drug research. Methylphenidate, while currently an extremely helpful drug, still requires massive amounts of research to determine its long-term effects and possible consequences.

For the second part of our study, we hypothesized that methylphenidate would negatively affect short-term memory retrieval, but that an enriched environment would decrease these effects. However, our results showed no significant differences between the two housing groups suggesting that MPH did not negatively affect memory. During the five minutes that the mice were in the object recognition box, only six of them spent more than 20s exploring the objects during T2. This was unexpected as it has been reported that 20s was an adequate amount of time for memory formation (10). We noticed that many of the mice that received MPH on day 2 were extremely hyperactive, causing them to run circles around the box and barely acknowledging the presence of objects. This was true for both of the housing conditions. To correct for the lack of animals achieving the 20s of exploration, it might be a good idea to extend the five minutes the mice are given in the box. However, we believe that are results are correct in showing that MPH has no effect on short-term memory in either EE or SH.

While there are implications to our study which we will discuss shortly and should be corrected in the future, MPH is not as detrimental to memory processes as previously thought. One of the major differences between our study and past studies that do show MPH affecting memory is the type of animal used. Most of the studies we have read and followed use rats as their test animals, while this study used mice. While rats and mice are quite similar, there is a possibility that they might metabolize MPH differently, which would change how fast or slow the drug affected the animal. They might also respond differently to the drug just as humans have varied reactions to drugs.

Another more logistical divergence from studies that use rats was the size of our OR test box. Our box was designed following the specifications of the Chuhan and Taukulis (2007) study, but scaled it down since rats are much bigger than mice. The initially planned size of the box seemed too small, so we decided to make the box roughly half the size of their box. However, after seeing how much time the mice spent roaming around the box and not exploring the object, we began to wonder if we had given the mice too much room. If that was the case though, it is actually to our benefit. By giving the mice more space, the experiment would also be an open field test, making our method more stringent than the previous study. Our mice were not forced to explore the objects due to a close proximity to them as the rats in other OR experiments might have been.

While we believe our results to be true in saying that a single dose of MPH does not affect short-term memory, there are several things about the experiment that should be changed in order to ensure accuracy. One would be to increase the total number of mice in the experiment. We only had six mice in each condition, which would have made it difficult to retain reliability if there had been any problem mice that needed to be thrown out of the study. With more mice we would also be able to study individual differences between conditions in more detail.

We also believe that a better timing system might improve the study. It was troublesome to actually measure the exact amount of time a mouse would spend exploring in object when it most often happened in milliseconds. We used stopwatches and interrater reliability, but even with those things, the human error could have been quite high. If we were able to devise a more reliable and exact system, the study would be much more qualified scientifically. More advanced technology would probably be the best way to create such a system, so this factor might be harder to resolve in the short-term since that kind of technology is costly.

Future research in this area has endless opportunities. Understanding the effects of methylphenidate on the brain requires much attention, but we are slowly learning what its limits are. In this study, we found that a one-time dose of MPH did not interfere with STM retrieval. It would be beneficial to continue MPH research on memory first, to see if the behavioral reactivity that appears is really from MPH and not from another factor and second, to see if MPH affects any other memory processes. However, more research on the overall short-term and long-term side-effects of methylphenidate is needed due to the high numbers of children who take the drug daily.

Considering how widely it is prescribed, the fact that we know relatively little about MPH's long-term effects is rather frightening. However, based on current evidence its benefits far outweigh its possible risks. Even though previous studies have found problems with MPH, ADHD children treated with MPH are more adjusted behaviorally and socially than those left untreated (13). As long as the drug is taken as prescribed and the child is frequently reassessed for changes and improvements in their ADHD, methylphenidate does not appear to pose an immediate risk. However, MPH is often prescribed outside conditions that have been tested for safety. Prescribing MPH to toddlers, keeping children on the drug longer than intended, and mixing it with other medications are situations that have yet to be studied, so the effects are unknown and therefore risky. There is a pressing need for longitudinal studies that look at children with ADHD who are treated with MPH, as this research is the most valid in determining long-term effects of MPH in humans. Unfortunately, there are relatively few longitudinal studies being conducted at this time and any new studies would not yield results in any less time than a decade. So we are forced to do our best with animal models. While there are limits to animal testing, researchers in this field are constantly obtaining more knowledge about the differences in doses, administration, and reactions of MPH, all of which brings us closer to the goal of discovering the effects of one of the world's most prescribed, yet controversial drugs.

While the results of our study do not show detrimental results, there are many uses and abuses of methylphenidate that are not being studied. This leads to a larger implication for MPH use in general. One increasingly common abuse of MPH, or Ritalin, is found on college campuses across the United States. The abuse of Ritalin now is directly tied to the dramatic increase of prescriptions of it, starting in the early nineties. ADHD was quickly becoming a prevalent diagnosis for children who exhibited signs of hyperactivity and impulsivity. Instead of initially working on the disorder behaviorally through therapy, most doctors immediately prescribed a psychostimulant to treat the condition. Ritalin soon became the most prescribed drug for the treatment of ADD and ADHD, as reflected by statistics regarding the production and use of methylphenidate, which increased six-fold from 1990 to 2000 in the United States (14). The U.S. consumes more than 80 percent of the total world's supply of methylphenidate (14). Due to the increase in prescriptions over the last two decades, Ritalin has become widely available even to those without prescriptions. This is partially caused by the fact that teenagers are usually given the responsibility of administering Ritalin to themselves. After years of chronic use, teenagers often choose to skip doses. By doing this, they have an excess of pills with which they can sell to other teenagers for recreational uses. The street value of Ritalin ranges from about $3 to $15 per pill (15). This is how Ritalin has become so widely abused.

Despite its relative clean track record, Ritalin does have serious risk associated with it. The US Drug Enforcement Administration classifies Ritalin as a Schedule 2 drug, meaning that it has a large potential for abuse. Even though Ritalin is in the same classification of drugs as cocaine and amphetamine, it has not shown the serious effects that these drugs exhibit. Ritalin is affecting the population in a different way. It is quickly replacing coffee and caffeine pills as the method to stay awake and focused for longer periods of time. Evidence shows that Ritalin allows users to maintain abnormal levels of concentration and focus, making it the drug of choice on college campuses in recent years (16). The beneficial effects of Ritalin have turned it into one of the most commonly abused prescription drug. More and more students are turning to Ritalin and other psychostimulants like it in order to cope with the workload of college. While we can tell that Ritalin abuse is prevalent and often rampant in college life, there have unfortunately been very few studies and national surveys documenting this abuse. One survey done in 2000 reported that 16% of students at a small, public liberal arts college had taken Ritalin recreationally at least once (17), a number which has likely grown in the last eight years along with increases in prescriptions.

The alarming amount of abuse of Ritalin is frightening to the research community. Methylphenidate was initially approved for use in young children with ADHD only. These children were only supposed to remain on the drug for a short amount of time with breaks from it on weekends and during the summer. However, Ritalin has become such a popular drug it is being prescribed outside of its original studied parameters. Children under six are now able to receive Ritalin and can stay on it well into adulthood if deemed necessary by their physician. This is a problem because there are absolutely no long-term studies on this kind of use and there are especially no studies on long-term abuse. While teenagers' abuse of Ritalin continues, researchers can be thankful for one thing. Because Ritalin is a prescription drug, it is relatively pure, unlike many other pyschostimulants that are made in home labs. This does not mean that

Ritalin abusers are out of danger's way. When Ritalin is mixed with other drugs and/or alcohol, the chance of overdosing and death increases greatly. There are preliminary studies that have found that mixing Ritalin and alcohol might create another active product even more potent than methylphenidate alone. The vast lack of knowledge and long-term studies of both prescription and recreational uses of Ritalin leaves a huge hole in this growing trend.

While using Ritalin in any way other than how it is prescribed is legitimately wrong, it is often thought extremely necessary to do well in school. The constant pressure from parents, professors, and even themselves to succeed makes students feel like they have no other choice, but to use Ritalin as a study aid. This becomes increasingly prevalent as students rise in academia. Ritalin abuse often begins in high school, especially if it is particularly rigorous and challenging. Abuse continues into college and could increase even more if a student goes to medical or law school. The workload of an overachieving student unfortunately drives their motivation to take Ritalin to get everything done. There is also another aspect to Ritalin abuse. Young people who have been exposed to these kinds of drugs their whole lives often see no problem in taking the drug recreationally, whether it is to study or to party. This generation of young people has grown up with Ritalin. It has long been a topic of discussion between doctors, researchers, and parents. However, there is still a desperate need for research on its long-term effects in all aspects of its use. Without this knowledge, there is no way to truly determine how safe or how dangerous Ritalin can be. For a drug that is so heavily prescribed and used by our nation's youth, we are leaving far too much to chance.

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