# Chronic Oral Methylphenidate Behavioral, Neurochemical and Developmental Effects

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The majority of animal studies on methylphenidate (MP) use intraperitoneal (IP) injections, subcutaneous (SC) injections, or the oral gavage route of administration. While all these methods allow for delivery of MP, it is the oral route that is clinically relevant. IP injections commonly deliver an immediate and maximum dose of MP due to their quick absorption. This quick-localized effect can give timely results but will only display a small window of the psychostimulant's effects on the animal model. On the opposite side of the spectrum, a SC injection does not accurately represent the pathophysiology of an oral exposure because the metabolic rate of the drug would be much slower. The oral-gavage method, while providing an oral route, possesses some adverse effects such as potential animal injury and can be stressful to the animal compared to voluntary drinking. It is thus important to allow the animal to have free consumption of MP, and drinking it to more accurately mirror human treatment. The use of a two-bottle drinking method allows for this. Rodents typically have a faster metabolism than humans, which means this needs to be considered when administering MP orally while reaching target pharmacokinetic levels in plasma.

methylphenidate ADHD Ritalin psychostimulant dopamine animal model neurochemistry behavior

## 1. Introduction

### 1.1. History of Methylphenidate (MP)

Methylphenidate (MP), more commonly known as Ritalin, was created in 1944 by Panizzon [1]. In 1955, MP was used to treated psychological disorders such as depression, chronic fatigue, and schizophrenia [1]. However, in 1961, MP was approved for use, and demonstrated the most efficacy, on patients diagnosed with hyperactivity [1]. MP is now available in two forms: immediate release and sustained release [2]. Studies have shown that immediate-release MP is more likely to cause stimulant-like side effects, such as tachycardia and sweating, whereas sustained release does not [3]. The use of MP has doubled in the last decade, reaching its peak in 2012; however, it has been steadily declining since [4]. Two-thirds of children, 10% of adolescent boys, and between 1.5% and 31% of college students diagnosed with attention-deficit/hyperactivity disorder (ADHD) are treated with psychostimulants such as MP [5]. In 2003, a study concluded that approximately 4% of undergraduate students use MP illicitly [6]. Illicit use of MP can lead to similar effects on the body as cocaine; both drugs are psychostimulants

and act akin. Illegal use of MP induces a quick and large dopamine (DA) release, creating a euphoric experience for the consumer [7]. On the contrary, therapeutic use of MP provides a constant DA release [7]. Typical dosages of MP range from 10 to 60 mg/kg [2].

## 1.2. MP Prescription and Use in Humans

MP commonly used to treat ADHD has shown progression throughout its existence. Common doses of MP in adults are between 20 and 30 mg/kg and typically exceed 60 mg/kg [4]. Between the years of 2006 and 2016, MP use has increased from 7.9–20 tons, hitting its peak in 2012 at 19.4 tons [4]. Some clinical studies have shown that the abuse of MP increases in participants with drug dependence [8]. Drugs that can be absorbed rapidly (immediate release, IR) have shown association with drug abuse in comparison to those with a slower absorption rate (extended release, ER) [9]. The rate of absorption is highly influenced by the route of administration. Another factor that can lead to the misuse of MP is lack of sleep. In a previous study, it was shown that adult humans who averaged four hours of sleep voluntarily chose to consume 10 mg/kg of MP more often than those who averaged 6–8 h [10]. The dose and illicit use of MP have a proportional relationship. As the dose of MP increases, the probability of substance abuse increases as well [3]. As the human body develops throughout life, a lot of the effects can vary between ages, especially to the DAergic system. Studies have shown that after just four months of treatment, there are increases in cerebral blood flow to the DAergic system in children and these effects tend to be more permanent than if the drug was introduced during adulthood [11]. Studies have also demonstrated that chronic use of MP has long-lasting effects, whereas acute use does not [11]. Narcolepsy has also been successfully treated with MP by using specific dosing of MP, 9 mg/day, and slowly increasing over a year to 18 mg twice per day [12]. Following three months of this treatment, patients became asymptomatic [12]. MP was found to increase heart rate, helping with narcoleptic episodes [12]. In a case study in 1996, a 27-year-old male was treated with 10 mg MP BID (two times a day) and increased to 30 mg BID over the course of four months [13]. In this study, daytime sleepiness was reduced after one month of this treatment; MP was found to stimulate the central nervous system, which helped decrease sleepiness [13].

## 1.3. MP Off-Label Use and Abuse

Illicit use of MP has been an increasing concern among adolescents. The use and abuse among neurotypical people can have a diverse effect compared to those who are neurodivergent. Adolescents have been found to be the population that most frequently uses off-label MP [14]. Long-term recreational abuse of MP can lead to an alarming reduction in weight and even depressive episodes [15]. Due to MP being prescribed, unlike illegal psychostimulants such as cocaine, it is more readily available for consumption among the population. Aside from the direct effects that MP has on a neurotypical person, there is concern that it may lead to the consumption of stronger psychostimulants such as cocaine. If a tolerance to MP is established, the person who is consuming it off-label may seek a stronger drug to acquire the same euphoric effect. Approximately, one-quarter of college-aged illicit prescription stimulant users reported illicit use of MP; these students' reasoning for the off-label use of MP was for enhancement in concentration [6]. Due to the augmentation in cognitive function, there is concern about the abuse of these drugs among students in higher levels of education, such as medical school [16].

## 2. Clinical: Effects of Oral MP on Behavior and Medicine

MP is widely prescribed for patients diagnosed with ADHD, helping to mediate behavior and attention deficits. MP treatment for ADHD leads to a remarkable enhancement in attention [17], improved gait [18], and improved motor function [19] without disrupting sleep [20] or neurophysiology [21]. It has been found that MP helps to alleviate symptoms of ADHD, such as hyperactivity [22]. Additionally, this drug has an efficacy of 70% when relieving symptoms compared to ADHD patients that were treated with a placebo [23]. MP has been found to regulate serotonin and melatonin levels, therefore balancing biological rhythms. This was found to help treat symptoms of ADHD [24]. Children with ADHD have been found to have difficulty in the morning. After a 12 h treatment with MP, these children were found to have an easier time getting ready as they had no functional impairment [25]. MP helps increase working memory; it was found that following two years of treatment, MP successfully improved memory, in comparison to the discontinuation group in which methylphenidate was gradually reduced to placebo, followed by complete placebo [26].

MP is also prescribed for the treatment of narcolepsy [13]. MP can aid in minimizing sleep episodes commonly found in narcolepsy [27]. Following a three-week treatment, MP was found to show improvements in narcolepsy patients [12]. Although modafinil is the drug of choice among adult patients with narcolepsy, it is not well tolerated among children; MP, however, was found to be a successful and well-tolerated treatment regimen among narcoleptic pediatric patients [28]. The success in using MP as a treatment for narcolepsy has been attributed to norepinephrine release [13].

## 3. Clinical: Effects of Oral MP on Brain Function and Neurochemistry

MP works by blocking the reuptake of neurotransmitters dopamine (DA) and norepinephrine (NE) back into the receptors, thus allowing for increased concentrations of these neurochemicals to encourage more effective binding to receptors [29][30]. It is believed that the increased DA and NE signaling may help regulate attention levels by affecting signaling, leading to a decrease in the spontaneous firing of neurons, and an increase in the signal to noise ratio, which may lead to elevated attention levels [29][31][32]. Unlike DA and NE, conflicting results have been reported regarding the influence of MP on the neurotransmitter serotonin; one study conducted by Kucenzki et al. found that, unlike amphetamines, MP does not have an effect on extracellular serotonin levels [33]. However, Daniali et al. reported higher levels of serotonin transporter (SERT) density in the medial frontal cortex (MFC) of adult rats after both short-term and long-term chronic exposure to MP [34].

A study conducted by Volkow et al. [29] analyzed brain glucose metabolism (BGluM) using positron-emission tomography (PET) on 23 healthy adults to find that the whole brain metabolism increases in individuals when working on a more labor-intensive cognitive task, such as a math test, compared to a simple task, such as looking at pictures; however, after oral MP (20 mg) intake, BGluM decreased while performing the cognitive task, implying more efficient use of the brain when focusing on the more mental labor-intensive task. Another PET study [35] on 50 healthy participants, half male and half female, found MP reduced the cost of mental labor and increased the

choice of cognitive task over a leisurely task; they found this effect greatest in participants with the highest levels of striatal DA, indicating a relationship among MP, DA enhancement, and increased attention and cognitive efforts. Similar results have been found when testing ADHD individuals, rather than healthy non-ADHD participants. A study [7] analyzed 20 ADHD individuals who were evaluated before and after 12 months of oral MP treatment to find a reduction in impulsivity and hyperactivity with long-term treatment; a challenge dose of MP was administered and coupled with PET imaging technology to find a significant increase in DA in the ventral striatum of the brain, which was related to the reduction in symptoms.

These findings relating MP to the dopaminergic system led researchers [36] to wonder whether there are gender-based differences in the brain DA system that could affect sensitivity to stimulant medications. They used PET imaging to evaluate MP-based increases in DA in the striatum using different methods of MP administration in 95 healthy adults, 65 male and 30 female, where Cohort A received oral 60 mg MP and Cohort B received intravenous 0.5 mg/kg MP. These researchers found that females reported feeling increased levels of “drug effects” and demonstrated significantly higher DA release in the ventral striatum, but not the dorsal striatum, during both oral and intravenous MP administration compared to males. Researchers suggest that possible gender-specific increases in sensitivity specific to the DA system may be an underlying factor in gender differences seen in ADHD.

Questions regarding the effects of MP on brain structure have also been asked. Researchers [37] analyzed the effects of chronic MP use on brain structure in 131 adult patients with ADHD using MRI technology. Images were taken at baseline, after 3 months, and after 12 months of MP use. The study found that chronic MP use did not lead to any detectable cerebral loss in volume. Evidence from a review paper [38] of structural MRI studies indicate that long-term MP use may actually normalize structural brain changes in the white matter, anterior cingulate cortex, and cerebellum of children with ADHD.

The neurobiological effects through which MP works is still being explored. However, recent studies [39][40][41][42][43] found the use of MP to improve performance during cognitive tasks. A study in healthy men, and following the use of fMRI, found those subjects to have higher activation in the dorsal attention network (DAN) region of their brains, including the parietal and prefrontal cortex (PFC), and more deactivation in the default mode network (DMN) when compared to control groups. The authors suggest that MP, through the elevation of DA and NE signaling, alters activation in the DAN and DMN, ultimately impacting cognitive abilities [41].

Another imaging study [44] attempted to analyze the long-term effects of chronic MP use in children with ADHD, focusing on the neural networks related to executive functioning. Nine boys with ADHD were scanned while drug naive, then a year later after chronic MP treatment, and compared to controls who had never undergone treatment. They found no changes in brain activation patterns when comparing the children who had undergone treatment to those who had not.

A hallmark of ADHD is hyperactivity and researchers [45] conducted a study analyzing regional cerebral blood flow (rCBF) using PET to understand how chronic MP use changes resting brain metabolism and how these results correlate to behavioral changes in response to the drug. Scans were taken for 10 adults with ADHD while

unmedicated and after three weeks of chronic MP use. The study concluded that chronic MP use increases rCBF in the cerebellar vermis and decreases it in the precentral gyrus and caudate nucleus, two areas noted for their role in motor function. Lower brain activity in these regions may correspond to decreased levels of hyperactivity from MP use.

# 4. Preclinical Models of MP Treatment

The use of animal models in clinical studies has been proven to be vital and indispensable in neuroscience and behavioral research. Laboratory rats and mice also provide ideal models for biomedical research and comparative medicine studies due to their similarities to humans in terms of anatomy and physiology [46]. The use of rodents in research also has economic and biological advantages. Rats and mice are small animals and require little space and resources to maintain. They also have shorter gestation times and produce larger numbers of offspring [46]. Due to their relatively larger brain sizes, rats are preferred to mice for brain surgery, imaging, and developmental studies [47]. Rats also have faster developmental stages when compared to humans, where one rat day is equivalent to around 27 human days [48]. This allows researchers to observe desired effects more rapidly than if the studies were conducted in humans. However, drug dose and route of administration are important factors to consider when designing animal studies used for neuropsychopharmacology research. Calculation of drug dosages needs to take the physiological and metabolic systems of rats into account. Almost all physiological and metabolic systems in a rat are faster than a human, including heart rate and respiratory rate [48]. Therefore, the doses administered must be adjusted to account for metabolic differences in each species when conducting research to obtain desired results for drug exposure and to prevent drug overdose.

Route of administration is another factor that is important to consider when creating and conducting animal studies. The method of exposure should be relatively similar between the animal model and what is seen in humans. Prior to the development of the two-bottle method of exposure, previous models for exposure to MP in rats have demonstrated to be unlike the exposure seen in humans. These models of MP exposure include intraperitoneal (IP) injections, subcutaneous (SC) injections, and oral exposure via oral gavage. Depending on the study, the selection of a particular route of administration and assessing the effective dose can affect the pharmacokinetics of the given substance [49][50]. Studies have demonstrated that the choice of route of administration can result in behavioral and neurochemical consequences associated with MP administration in rodents [51].

## 4.1. Intraperitoneal Injection of MP

IP injections are administered in the lower right abdominal quadrant of the animal away from the midline. They are frequently used in experiments to mimic a similar exposure method to oral exposure. IP injections will allow the drug to absorb more efficiently into the mesenteric vessels, in which the drug will likely undergo hepatic metabolism [50]. This route of absorption closely mirrors the route of absorption for oral exposure. However, limitations of this method include potential injury to the animal if the injection is performed incorrectly. By injecting too close to the surface, the drug is administered subcutaneously instead of intraperitoneally which can ultimately change the effects produced by the drug. Errors such as these can decrease the drug's half-life and cause quick peak release

of DA in the brain, which induces behavioral sensitization [14]. Another form of exposure that has been used to administer MP is through the SC route [52]. Dosages of MP administered to rodents are selected based closely on mirrored doses used by humans. By comparison, the process of performing IP injections in rats and mice only differ due to the size of the model [53]. Mice are much smaller than rats, therefore executing an IP injection is much easier. Studies indicate that doses of 2–5 mg/kg reflect the clinical use of MP, while 10–20 mg/kg dose emulates the "recreational" use of MP [54]. Doses of 2.5 mg/kg or greater of MP through IP injections are shown to produce an increase in locomotor activity whereas doses of 1 mg/kg or lower have no effect on locomotor activity [55]. Studies using IP injections have shown to be effective due to its fast absorption rate compared to other methods of administration. This is primarily because IP-administered pharmacological agents are exposed to a large surface area, close to that of the entire skin surface, which leads to rapid and efficient absorption (see **Table 1**) [56].

**Table 1.** Summary of behavioral and neurochemical effects on injected MP.

| MP Exposure | Behavioral Effects | Model Used/References | Neurochemical Effects | Model Used/References |
|---|---|---|---|---|
| Chronic | *Decreased* hyperactive behavior | Naples high-excitability rats [57] | Induces oxidative damage, inflammatory changes, and neurodegeneration to the brain due to *increased* lipid peroxidation or mitochondrial superoxide | Wistar rats [61] [62] |
| | *Decreased* self-administration and reinstatement of drug-seeking behavior | Spotaneously hypertensive rats [58] | | Sprague-Dawley rats [1] |
| | *Decreased* drug sensitization and tolerance | Wistar rats [59] | DNA damage in striatal cells due to dopamine oxidation | Wistar rats [59] |
| | in exploratory and object recognition memory | Wistar rats [60] | Enhanced pyramidal activity in adult rats | |
| | Impaired spatial and working memory results in *decreased* sensitivity to reward stimuli | Sprague-Dawley rats [51] | *Decreased* synaptic transmission and neuronal excitability in juvenile rats | |
| | *Increased* locomotor activity compared to gavage administration | | Loss of astrocytes and neurons with *increased* levels of cytokines and neurotrophins in juvenile rats | |

| MP Exposure | Behavioral Effects | Model Used/References | Neurochemical Effects | Model Used/References |
|---|---|---|---|---|
| | *Increased* depressive and anxiety-like behavior | | | |
| Acute | *Decreased* sensitivity to a given reward, *Decreased* habituation to a familiar environment and *Increased* depressive-like behavior | Sprague-Dawley rats [63] | Neuroprotective effects observed via the reduction in cell damage and *decreased* apoptosis in brain tissue | Sprague-Dawley rats [67] |
| | | Wistar rats [64] | | |
| | *Increased* cross-sensitization suggests *increased* risk of future drug abuse | Sprague-Dawley rats [65] | | |
| | *Increased* cocaine self-administration by rewarding effects and sensitivity of a given drug | Sprague-Dawley rats [66] | | |


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## 4.2. MP Oral Gavage

The oral gavage route of administration has been the most widely used and preferred technique for MP oral dosing in experimental studies. Oral gavage better mimics the oral consumption and metabolism of MP in humans [68]. The oral gavage method involves using a properly fitted tube or a gavage needle that is placed in an animal's mouth and passed into the esophagus (See **Table 2**) [69]. Oral gavage is used for precise, accurate dosing and quick delivery of a drug [50]. The most commonly used dosage of MP through the oral gavage method is 2.5 mg/kg, mainly due to its calming effects shown in "ADHD rats". However, this dose contrarily causes an excitatory response in wild type rats [70]. Doses used in other studies include 0.5–5 mg/kg, with 5 mg/kg on the higher end of the spectrum and commonly used to mirror illicit use of MP [71]. Other studies that used the oral gavage method to administer MP doses of 1, 10, and 90 mg/kg have shown adverse clinical observations including changes in body weight, pathology, and organ weight [72]. Researchers using the oral gavage method to administer drugs need to be extra careful since complications to the animals can occur. When poorly executed, this method can cause serious health concerns to the rat and could potentially cause aspiration and pulmonary injury to the animal [14]. Therefore, it is important to select appropriate tubing size and to handle the animal with extra care to minimize any

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## 4.3. MP Oral Voluntary Drinking

Rat physiology differs significantly from human. Rats typically have a faster metabolism [14], which will automatically decrease the half-life of any drug. Using a two-bottle regimen to administer MP over an 8 h period can potentially compensate for physiological differences between both species. A lower dose administration for the first hour (bottle 1) and a higher dose administered for the other seven hours (bottle 2) allows for a corresponding method of

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exposure, as oral consumption in humans. Each bottle given to the rats is adjusted daily to deliver a consistent concentration of MP [14]. Specific doses used included 4, 20 and 30 mg/kg for the first bottle and 10, 30, and 60 mg/kg for the second bottle [14]. Using this method of exposure of MP allows for a delivery method that is similarly attained in clinical administration while maintaining independence from fluid consumption [78]. Plasma levels obtained from the rats demonstrated that the two-bottle paradigm induced a 30 ng/mL plasma concentration [14]. This plasma concentration closely resembles those used in a clinical setting, 8–40 ng/mL [79].

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