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# Sex Differences in the Kinetic Profiles of d- and l- Methylphenidate in the Brains of Adult Rats

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# Sex differences in the kinetic profiles of d- and l- methylphenidate in the brains of adult rats

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**Abstract. – OBJECTIVE:** Methylphenidate is commonly used in the treatment of Attention Deficit Hyperactivity Disorder and narcolepsy. Methylphenidate is administered as a racemic mixture of the d- and l- threo enantiomers; however, the d-enantiomer is primarily responsible for the pharmacologic activity. Previous studies of the behavioral effects of methylphenidate have highlighted sex differences in the responsiveness to the drug, namely an increased sensitivity of females to its stimulatory effects. These differences may be due to differences in the uptake, distribution, and elimination of methylphenidate from male and female brains. Therefore, we compared the pharmacokinetics of d- and l- threo methylphenidate in the brains of male and female rats.

**MATERIALS AND METHODS:** Adult male and female Sprague-Dawley rats were injected with 5 mg/kg d, l- threo methylphenidate, and whole brains were collected at various time points following injection. We measured methylphenidate concentrations utilizing chiral high pressure liquid chromatography followed by mass spectrometry.

**RESULTS:** Females exhibited consistently higher brain concentrations of both d- and l- methylphenidate and a slower clearance of methylphenidate from brain as compared to males, particularly with the active d-enantiomer.

**CONCLUSIONS:** The increased sensitivity of females to methylphenidate may be partially explained by an increase in total brain exposure to the drug.

*Key Words:*

Methylphenidate, Brain, Chiral separation, Sex differences, Liquid chromatography-mass spectrometry.

## Introduction

Methylphenidate (MPH) is commonly used in children and adolescents for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and narcolepsy. According to current estimates, 5.9-7.1% of children and 5.0% of adults are affect-

ed by ADHD, and MPH is the first line treatment, accounting for a majority of the prescriptions written for the disorder<sup>1-3</sup>. Additionally, increases in off-label use and diversion of MPH have been reported<sup>4-6</sup>. MPH works in the brain by blockade of both the dopamine transporter (DAT) and the norepinephrine transporter (NET), which leads to an increase in both dopamine and norepinephrine in the synaptic cleft. Given the fact that MPH increases extracellular dopamine, it has the potential for abuse<sup>7</sup>. In fact, it is more potent at blocking the DAT than cocaine<sup>8</sup>. However, its limited abuse in the context of clinical use appears to be due to pharmacokinetics, namely its slow, steady uptake in the brain when taken orally<sup>9</sup>.

Methylphenidate has two chiral centers; however, only the *threo* enantiomers are used in therapy, as they are more pharmacologically potent<sup>10</sup>. Although the drug is administered as a racemic mixture of the d- and l- *threo* enantiomers, the d-MPH enantiomer is thought to be primarily, if not entirely, responsible for the pharmacologic activity<sup>11-14</sup>. Interestingly, MPH is also subject to stereoselective metabolism. MPH is subject to hydrolysis of the methyl ester linkage via the enzyme carboxylesterase 1 (CES1) to the pharmacologically inactive d- or l- ritalinic acid<sup>11,15</sup>. It is well-recognized the CES1 is considerably more efficient in the de-esterification of l-MPH relative to d-MPH, resulting in a significantly higher bioavailability of the d- enantiomer<sup>14,15</sup>.

Most of the previous studies analyzing the effects of MPH on the brain and behavior have utilized males as subjects. This trend is likely due to the recognized higher prevalence of ADHD in males versus females and the biological complexity of females due to their reproductive cycling<sup>16</sup>. However, both human and animal studies have indicated some important gender differences in ADHD manifestations, as well as responsiveness to psychostimulants<sup>17-20</sup>. For exam-

ple, Patrick and colleagues reported that females described a significantly greater stimulant effect of MPH than males when asked in a self-report analysis<sup>21</sup>. Interestingly, a number of animal studies also indicate the presence of sex differences in response to MPH. In one study, adult female rats demonstrated increased conditioned hyperactivity to both moderate and high doses of MPH as compared to adult male rats<sup>22</sup>. Additionally, in two recent studies, females demonstrated behavioral sensitization (or increased locomotor responses to subsequent exposure) to MPH whereas males did not<sup>23,24</sup>.

In sum, several studies seem to indicate an increased responsiveness of females to MPH. One of these studies was conducted in collaboration with our laboratory, and it revealed that female rats demonstrated more robust sensitization to MPH and increased locomotor activation compared to males<sup>23</sup>. These sex differences could be due to pharmacodynamic and/or pharmacokinetic factors. Since drug effect is directly related to brain concentrations, here, we investigate MPH pharmacokinetics in male and female brains. Specifically, we compare the uptake, distribution, and elimination of d- and l- *threo* MPH in the brains of male and female rats.

## Materials and Methods

### Animals

Fifty day old rats were chosen for this study in order to follow up on previous behavioral work conducted in collaboration with our laboratory<sup>23</sup>. In that particular study, rats received injections of MPH or saline every other day from P33 through P50; locomotor activity and behavioral sensitization was analyzed throughout this time period, and in females, the most robust effect occurred after day 50 with a dose of 5 mg/kg MPH. As such, we aimed to investigate the pharmacokinetics of MPH in animals at this developmental age (P50) using an identical dose. Furthermore, the 5 mg/kg dose has been utilized in other studies to mimic an “abusive dose” of MPH, and intraperitoneal (IP) administration is believed to mimic the absorption seen in snorting<sup>23,25,26</sup>. As such, 50 day old Sprague-Dawley rats (150-200 g) were injected intraperitoneally with 5 mg/kg d, l- *threo* methylphenidate HCl (Sigma Aldrich, St. Louis, MO, USA) prepared in sterile physiological saline. Animals were sacrificed via decapitation at the following time points post injection: 1 min,

5 min, 10 min, 30 min, 60 min, and 120 min, and an n of 6 to 7 for each sex at each time point was used to ensure appropriate statistical power. Whole brains were flash frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  for later use. All animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) accredited facility with food and water provided *ad libitum*. All procedures were carried out according to NIH guidelines and were approved by the East Tennessee State University Committee on Animal Care.

### Tissue Collection and Sample Preparation

Solid phase extraction was utilized to extract MPH from brain tissue according to our previously validated method<sup>27</sup>; this method allows for extraction recovery of 72-75% for both d- and l- *threo* methylphenidate and both low (10 ng/mL) and high (100 ng/mL) calibration concentrations.

### Liquid Chromatography-Mass Spectrometry

Methylphenidate concentrations were measured using liquid chromatography-mass spectrometry (LC-MS). Since MPH is administered as a racemic mixture of the d- and l- *threo* forms, these enantiomers were separated and measured individually as described previously<sup>27</sup>. This method allowed for a lower limit of detection (LLOD) of 0.5 ng/mL and a lower limit of quantification (LLOQ) of 7.5 ng/mL, with desirable intra- and inter-day precision and accuracy (% RSD and % error were  $< 15\%$  for every calibration point).

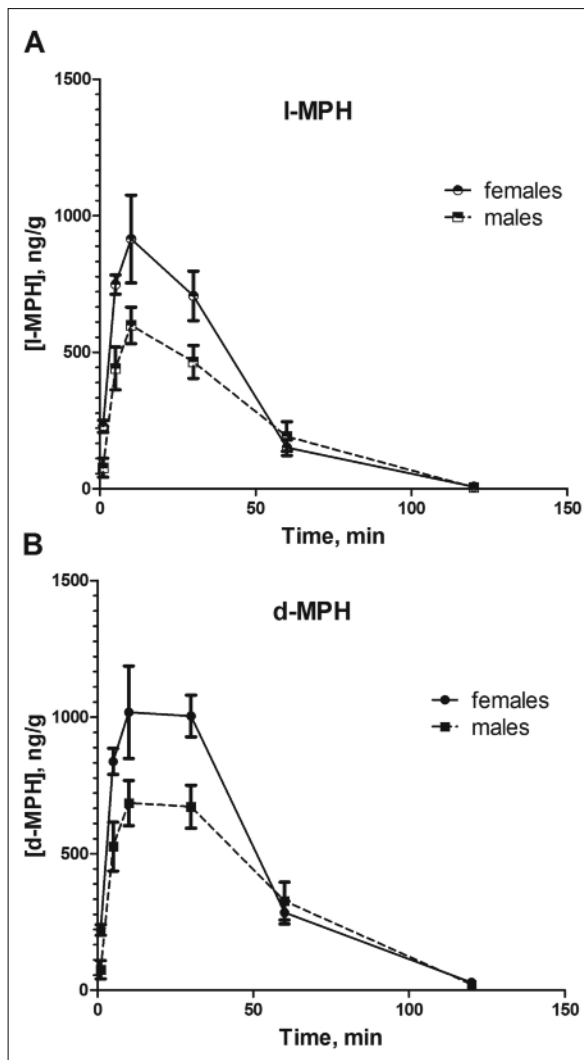
### Statistical Analysis

All data were subjected to a noncompartmental analysis utilizing the Phoenix 64/WinNonLin software. Parameters of interest included the area under the curve (AUC), the maximal concentration ( $C_{\text{max}}$ ), the time of maximal concentration ( $T_{\text{max}}$ ), the clearance from the brain (Cl), and the elimination half-life ( $T_{1/2}$ ) from the brain. If standard errors were reported, the results of these analyses were compared using an ANOVA followed by Newman-Keuls Multiple Comparison Tests. Data were considered to be statistically significantly different when  $p < 0.05$ .

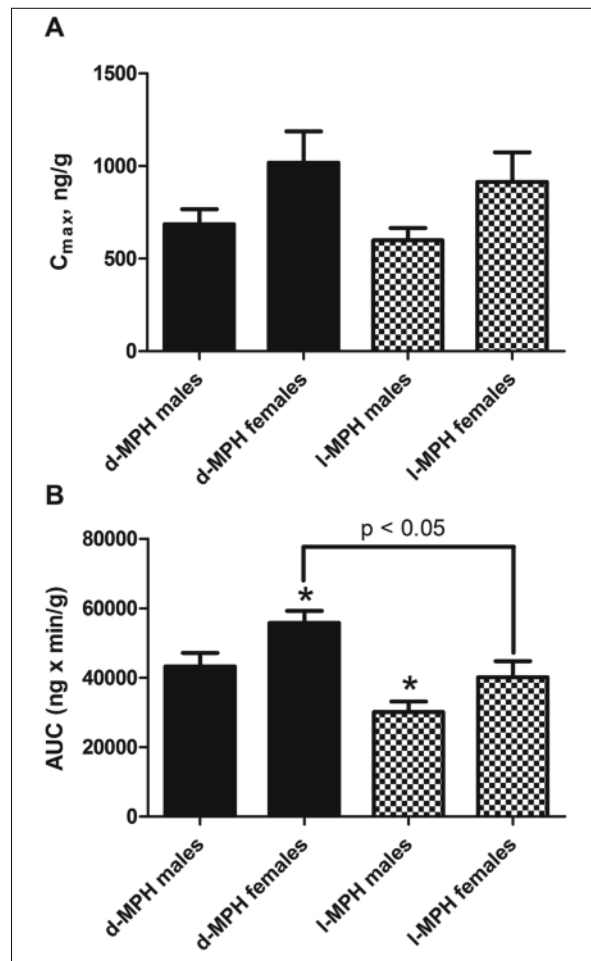
## Results

The d- and l- *threo* enantiomers of MPH were measured using LC-MS. Interestingly, females

appeared to exhibit consistently higher brain concentrations of both d- and l- MPH (Figure 1). Additionally, the d-enantiomer appeared to be maintained in the brain at higher concentrations as compared to the l-enantiomer in both females and males (Figure 1). Subsequently, the pharmacokinetic data were analyzed via noncompartmental analysis with Phoenix 64/WinNonLin software. Although the maximal concentrations ( $C_{max}$ ) of d- and l- MPH were not significantly different in males as compared to females (Figure 2A), the total brain exposure to the drug, as indicated by the area under the curve (AUC), was significantly higher in females versus males (Figure 2B).



**Figure 1.** Pharmacokinetics of **A**, l- and **B**, d- methylphenidate (MPH) following intraperitoneal injection of 5 mg/kg MPH in females (solid line) and males (dotted line). Data are expressed as the mean  $\pm$  SEM, n = 6-7.



**Figure 2.** **A**, Maximal concentrations of d- and l-MPH ( $C_{max}$ ) following injection of 5 mg/kg MPH. No statistically significant differences were achieved. **B**, Total brain d- and l- MPH exposure as calculated by the area under the curve (AUC) in males versus females. \* $p < 0.05$  versus the AUC of d-MPH in males (n = 6); One way ANOVA followed by Newman-Kuels multiple comparisons test.

Based on the pharmacokinetic model created by the noncompartmental analysis, several other parameters were calculated including the time of maximal concentration ( $T_{max}$ ), the half-life of MPH in the brain ( $T_{1/2}$ ), and the clearance of MPH from the brain (Cl). (Table I). Since the data are theoretical, standard errors could not be calculated. Interestingly, the  $T_{1/2}$  for either enantiomer did not appear to be substantially different between the sexes. Additionally, the  $T_{max}$  for both d- and l- in males and females was 10 min. However, there did appear to be some sex differences that should be noted; females exhibited a slower Cl of MPH as compared to males, particularly with the d-enantiomer (88.45 g/min versus

**Table I.** Theoretical pharmacokinetic parameters as calculated through pharmacokinetic modeling. Values for half-life ( $T_{1/2}$ ), time of maximal concentration ( $T_{max}$ ), and clearance (Cl) from the brain are presented.

	I-MPH Males	I-MPH females	d-MPH males	d-MPH females
$T_{1/2}$ (min)	13.55	14.33	17.52	17.62
$T_{max}$ (min)	10	10	10	10
Cl (g/min)	165.68	124.24	144.22	88.46

Values for half-life ( $T_{1/2}$ ), time of maximal concentration ( $T_{max}$ ), and clearance (Cl) from the brain are presented.

144.22 g/min). Finally, when comparing the enantiomers, the  $T_{1/2}$  appeared to be slightly longer and the Cl slightly less for d-MPH as compared to l-MPH.

## Discussion

In this study, we examined the brain pharmacokinetics of d- and l- *threo* MPH in male and female rats following intraperitoneal injection of 5 mg/kg MPH. Interestingly, we discovered the females had consistently higher brain concentrations of both d- and l- MPH than males, resulting in a significantly higher overall brain exposure to MPH (as represented by the AUC). This was accompanied by a notable decrease in the rate of clearance of MPH from the brain for both enantiomers, but most profoundly with the pharmacologically active d-enantiomer. The reason for the sex differences in the pharmacokinetics of MPH is currently unclear. To date, there is little known regarding the extent to which transporters contribute to movement of MPH across the blood-brain barrier; however, one study indicates that a carrier-mediated process is at least partially responsible<sup>28</sup>. This carrier is saturable and is also responsible for the uptake of amphetamine and  $\beta$ -phenethylamine. In addition, one study indicated that d-MPH is a weak substrate of P-glycoprotein<sup>29</sup>. Given the fact that transporters are at least partially responsible for access of methylphenidate to the brain, sex differences in the levels of these transport proteins may contribute to the differences observed in this study. Another possible explanation for the increased levels of MPH in females as compared to males would be sex differences in the metabolism of methylphenidate. As

noted earlier, methylphenidate is subjected to hydrolysis via the CES1 enzyme; if males have higher CES1 activity than females, this would result in lower bioavailability of MPH and thus lower brain concentrations. Regardless, these results may at least partially explain the previous findings that females are more sensitive to the psychostimulant effects of MPH. Specifically, in a study completed in conjunction with our laboratory, females demonstrated robust locomotor sensitization in response to 5 mg/kg MPH, whereas males did not.

In this study, we also quantified the levels of d- and l- *threo* MPH separately. Of note, the AUC for d-MPH was also significantly greater than the AUC for l-MPH in both males and females, thereby indicating greater exposure to the more active enantiomer. This is likely due to the enhanced bioavailability of this enantiomer due to preferential hydrolysis of l- *threo* MPH by CES1, which in rodents is active in both the liver and the plasma<sup>14,15</sup>. Brain concentrations found in this study ( $725 \pm 87.0$  at 10 min and  $552.7 \pm 60.2$  at 30 min, pooled d- and l-MPH, male and female data) utilizing LC-MS were similar to previous reports employing other methodologies; Levant and colleagues used an ELISA-based assay to find that adult rats at postnatal days 42 and 70 had brain concentrations of  $985 \pm 44$  ng/g and  $1006 \pm 32$  ng/g, respectively, 20 min following subcutaneous administration of 5 mg/kg MPH<sup>30</sup>. In that particular study, the enantiomers were not quantified separately, and multiple time points were not examined. A few other studies have examined brain concentrations of MPH in rats, yet the doses and/or methods of administration used in these studies were vastly different, making direct comparisons difficult<sup>31-35</sup>.

## Conclusions

In summary, we have found significant sex differences in the pharmacokinetics of MPH; namely, females have a higher overall brain exposure to MPH as compared to males, especially with the d-enantiomer. Additionally, the elimination of MPH from the brain as represented by the clearance appears to be substantially slower in females. Future studies are needed to determine the reason for the sex-related differences, but they could be related to variance in metabolism between the sexes or differences in the rate of transport of methylphenidate across the blood brain barrier. Nonetheless, these data may explain many of the previously documented sex differences in the responses to this psychostimulant indicating an increased sensitivity of females to the drug.

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## Conflict of Interest

The Authors declare that they have no conflict of interests.

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