Scientific Letters

Poster Communication 60

Oral Methylphenidate Induces Sex-Related Differences in Brain Plasticity Proteins in Juvenile Wistar-Kyoto Rats

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Abstract

Background: Methylphenidate (MPH) is widely prescribed as the first-line pharmacological intervention for Attention Deficit/Hyperactivity Disorder (ADHD) among children. However, the increasing cases of misdiagnosis and misuse raise concerns about the neurodevelopmental consequences of exposing children and adolescents to MPH [1,2], given the high brain plasticity during these critical phases [3]. **Objective:** This study aimed to evaluate the impact of repeated exposure to clinically relevant oral doses of MPH on pre- and post-synaptic protein markers involved in synaptic plasticity, and neuronal development, with a focus on sex-related differences. **Methods:** 37 healthy Wistar-Kyoto (WKY) rats (18 males and 19 females), aged 15 days (equivalent to human infancy), were randomly distributed into two groups. The MPH-group received daily oral MPH (5 mg/kg in 5% sucrose) via gavage, while the control group received an equivalent volume of 5% sucrose solution [4]. Treatment continued for 15 consecutive days, with doses adjusted individually based on each animal's weight. On PND 30, the animals were sacrificed and their brains dissected. GAP43 and PSD95 proteins were assessed in brain regions, including the prefrontal cortex (PFC), striatum, hippocampus, and cerebellum, by Western blot. Meanwhile, MAP2 and synaptophysin were evaluated in sections of the PFC, motor cortex, striatum, and hippocampus (CA1, CA3, hippocampal hilum, and dentate gyrus) by immunohistochemistry. **Results:** MPH exposure revealed region- and sex-specific alterations in synaptic plasticity proteins. MPH-treated males showed reduced levels of synaptophysin and GAP43 in the hippocampal CA1 region and cerebellum, respectively, along with increased PSD-95 levels in the striatum. In MPH-treated females, a reduction in PSD-95 levels was observed in the PFC. Additionally, control males showed higher MAP2 levels in the striatum compared to females. **Conclusions:** Early-life exposure to therapeutic doses of MPH can induce changes in neuronal development and synaptic plasticity in both sexes. These findings highlight the importance of considering sex in MPH's brain plasticity research, particularly given the underrepresentation of females in studies conducted on laboratory animals. Also, they emphasize the need to investigate the safety of non-clinical exposure to psychoactive drugs during early development and its potential long-term neurotoxic effects.

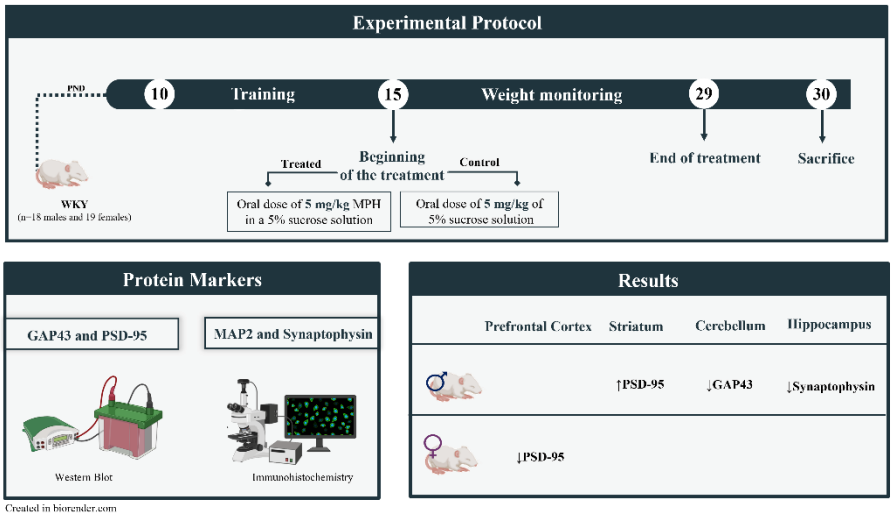

Figure 1. Graphical abstract of the experimental design and main findings.

Keywords: attention deficit hyperactivity disorder (ADHD); methylphenidate (MPH); neuroplasticity; Wistar-Kyoto

Acknowledgments/Funding

This research was funded by national funds from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences (UCIBIO) and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB. Also, supported by national funds through Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project "EXPL/MED FAR/0203/2021".

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