

Methylphenidate Exerts Dose-Dependent Effects on Glutamate Receptors and Behaviors

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Background: Methylphenidate (MPH), a psychostimulant drug used to treat attention-deficit/hyperactivity disorder, produces the effects of increasing alertness and improving attention. However, misuse of MPH has been associated with an increased risk of aggression and psychosis. We sought to determine the molecular mechanism underlying the complex actions of MPH.

Methods: Adolescent (4-week-old) rats were given one injection of MPH at different doses. The impact of MPH on glutamatergic signaling in pyramidal neurons of prefrontal cortex was measured. Behavioral changes induced by MPH were also examined in parallel.

Results: Administration of low-dose (.5 mg/kg) MPH selectively potentiated *N*-methyl-D-aspartate receptor (NMDAR)-mediated excitatory postsynaptic currents (EPSCs) via adrenergic receptor activation, whereas high-dose (10 mg/kg) MPH suppressed both NMDAR-mediated and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor-mediated EPSCs. The dual effects of MPH on EPSCs were associated with bidirectional changes in the surface level of glutamate receptor subunits. Behavioral tests also indicated that low-dose MPH facilitated prefrontal cortex-mediated temporal order recognition memory and attention. Animals injected with high-dose MPH exhibited significantly elevated locomotive activity. Inhibiting the function of synaptosomal-associated protein 25, a key SNARE protein involved in NMDAR exocytosis, blocked the increase of NMDAR-mediated EPSCs by low-dose MPH. In animals exposed to repeated stress, administration of low-dose MPH effectively restored NMDAR function and temporal order recognition memory via a mechanism dependent on synaptosomal-associated protein 25.

Conclusions: These results provide a potential mechanism underlying the cognitive-enhancing effects of low-dose MPH as well as the psychosis-inducing effects of high-dose MPH.

Key Words: AMPA receptors, methylphenidate, NMDA receptors, prefrontal cortex, SNAP-25, stress

Methylphenidate (MPH) is a psychostimulant widely used for the treatment of attention-deficit/hyperactivity disorder (ADHD) in adolescents and adults (1). Therapeutic dose of MPH effectively improves cognitive function and reduces hyperactivity in individuals with ADHD (2) as well as normal human subjects and animals (3,4). However, overdose of MPH produces agitation, restlessness, and hallucinations in humans (5) and hyperlocomotion and impaired cognition in animals (6). Intermediate-term administration of MPH in juvenile rodents was found to induce long-lasting behavioral adaptations (7,8). To achieve therapeutic benefit and minimal side effects, it is suggested that dosing of MPH should be titrated to an optimal level.

The biochemical action of MPH is well characterized. The dopamine transporter (DAT) and norepinephrine transporter (NET) are blocked by MPH, resulting in elevated concentration of dopamine and norepinephrine at synapses (3,9,10). However, the mechanisms by which therapeutic dose of MPH acutely improves cognitive functions and overdose of MPH induces psychosis are unclear.

The prefrontal cortex (PFC) is a key brain region mediating cognitive and executive functions, including working memory,

sustained attention, inhibitory response control, and cognitive flexibility (11,12). A delayed maturation in the PFC (13), dysfunction of the frontostriatal circuitry (14), and hypoactivation in the frontal cortex (15,16) have been implicated in individuals with ADHD. Also, the PFC is identified as the primary target of MPH (17). The glutamatergic pyramidal neurons are one of the major cellular constituents in the PFC. Glutamatergic transmission that controls PFC activity is pivotal for cognitive function such as working memory (11,18). Disturbed glutamate receptors are implicated in cognitive dysfunction associated with many mental disorders (19). We speculated that glutamate receptors are potential targets of MPH critically involved in PFC-mediated cognitive functions. In this study, we examined the impact of low-dose versus high-dose MPH on glutamatergic transmission in PFC of adolescent rats and its relevance to behavioral outcomes.

Methods and Materials

Animals and Reagents

Male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, Indiana). On arrival, animals were allowed 4–5 days to acclimate before the experiments. Rats at the early adolescent period (p25–30) (20) were paired-housed on a 12-hour light-dark cycle and provided ad libitum access to food and water. Rats from more than one litter were included in each treatment to avoid litter effects. All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. See Supplementary Methods and Materials in [Supplement 1](#) for details of reagents.

Animal Surgery

The delivery of peptides to the PFC was conducted as we described previously (22). See Supplementary Methods and Materials in [Supplement 1](#) for details.

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Electrophysiologic Recordings

Recordings of evoked synaptic currents in prefrontal cortical slices used standard whole-cell voltage-clamp technique as we described previously (23,24). The paired pulse ratio of *N*-methyl-D-aspartate receptor (NMDAR)-mediated excitatory postsynaptic currents (EPSCs) was calculated as described previously (25). See Supplementary Methods and Materials in Supplement 1 for details.

Biochemical Measurement of Surface and Total Proteins

Surface and total alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) and NMDARs were detected as we described previously (23,24). See Supplementary Methods and Materials in Supplement 1 for details.

Repeated Stress Paradigm

Repeated restraint stress was carried out as we previously described (24,26). Briefly, Sprague-Dawley rats were placed in

air-accessible cylinders for 2 hours daily (10:00 AM–12:00 PM) for 5–7 days (starting at p21–23). The container size was similar to the animal size, which made the animal almost immobile in the container. Experiments were performed 24 hours after the last stressor exposure.

Behavioral Testing

Temporal order recognition memory (TORM), a cognitive behavior controlled by PFC (27); locomotor activity; and attentional set-shifting tasks were performed as previously described (24,26,28). See Supplementary Methods and Materials in Supplement 1 for details.

Statistics

Experiments with two groups were analyzed statistically using unpaired Student *t* tests. Experiments with more than two groups were subjected to one-way or two-way analysis of variance (ANOVA), followed by Bonferroni post hoc tests.

Results

In Vivo Administration of Low-Dose MPH Enhances NMDAR-Mediated Synaptic Currents; High-Dose MPH Reduces Glutamatergic Transmission in Cortical Neurons

To investigate the impact of MPH on glutamate signaling, we examined the NMDAR-mediated and AMPAR-mediated EPSCs in the pyramidal neurons of PFC from adolescent male rats (4 weeks old) subjected to a single administration of low-dose (.5 mg/kg) or high-dose (10 mg/kg) MPH. As shown in Figure 1A and B, two-way ANOVA analysis revealed a significant main effect of MPH treatment on NMDAR-mediated or AMPAR-mediated EPSCs (NMDA [$F_{2,150} = 49.5$, $p < .001$]; AMPA [$F_{2,205} = 18.7$, $p < .001$]). Post hoc analysis indicated that low-dose MPH significantly potentiated NMDAR-mediated EPSCs (38%–57% increase, $n = 10$ –13 cells/4 rats per group, $p < .05$) but not AMPAR-mediated EPSCs (<10% change, $n = 14$ –21 cells/4 rats per group, $p > .05$). In contrast, high-dose MPH markedly reduced both NMDAR-mediated and AMPAR-mediated EPSCs (NMDA, 26%–48% decrease, $n = 10$ cells/4 rats per group, $p < .05$; AMPA acid, 36%–47% decrease, $n = 10$ –21 cells/4 rats per group, $p < .01$). These results suggest that MPH exerts a dose-dependent effect on glutamatergic transmission in the PFC.

To test whether the effects of MPH on NMDAR-mediated EPSCs result from a presynaptic or postsynaptic mechanism, we measured the paired pulse ratio, a readout that is affected by the presynaptic transmitter release (29). As shown in Figure 1C, paired pulse ratio was unchanged by low-dose MPH but was significantly elevated by high-dose MPH (saline, $1.42 \pm .07$, $n = 12$; low-dose MPH, $1.41 \pm .06$, $n = 13$; high-dose MPH, $1.85 \pm .09$, $n = 12$ [$F_{2,36} = 11.24$, $p < .001$, ANOVA]). This finding suggests that low-dose MPH regulates glutamatergic transmission mainly via a postsynaptic mechanism, whereas high-dose MPH might affect presynaptic glutamate release or postsynaptic glutamate receptors. In addition, the decay time constant was not statistically changed in animals treated with MPH at low or high doses (saline, 202.0 ± 15.9 , $n = 11$; low-dose MPH, 252.0 ± 18.8 , $n = 15$; high-dose MPH, 197.4 ± 12.4 , $n = 11$ [$F_{2,47} = .93$, $p > .05$, ANOVA]), suggesting that elevated NMDAR-mediated EPSCs are mediated by both NR2A and NR2B subunits.

In Vivo Administration of Low-Dose MPH Increases Surface Level of NMDAR Subunits; High-Dose MPH Decreases Surface NMDAR and AMPAR Subunits

Because the surface expression of glutamate receptors could determine the strength of glutamatergic transmission, we performed

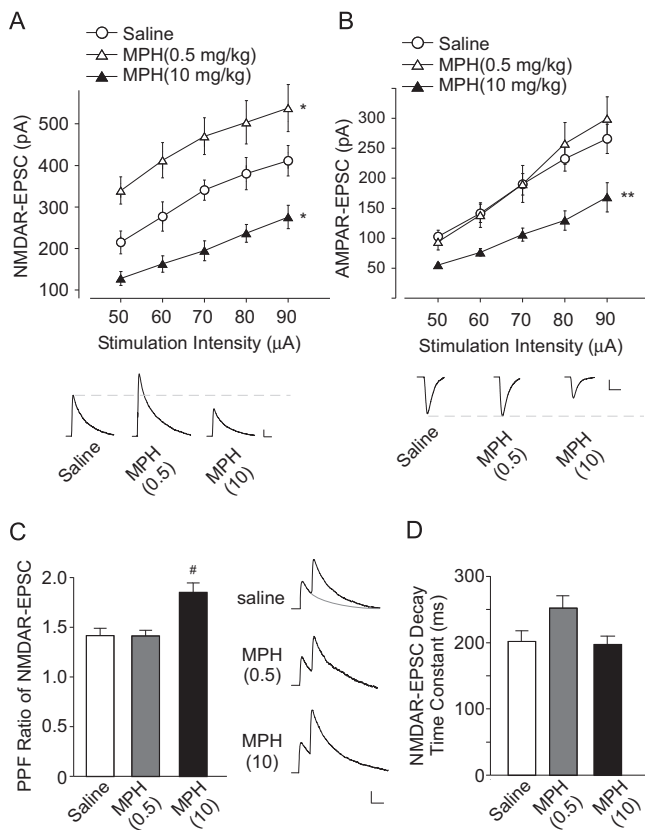


Figure 1. Low-dose methylphenidate (MPH) selectively enhances *N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic currents (NMDAR-EPSC), whereas high-dose MPH reduces both NMDAR-EPSC and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor-mediated excitatory postsynaptic currents (AMPA-EPSC). Input-output curves of NMDAR-EPSC (A) and AMPAR-EPSC (B) evoked by a series of stimulation intensities in prefrontal cortex pyramidal neurons from rats with a single intraperitoneal injection of saline, low-dose MPH (.5 mg/kg), or high-dose MPH (10 mg/kg). * $p < .05$, ** $p < .01$. Inset shows representative EPSC traces. Scale bars = 50 pA, 100 msec (A); 50 pA, 20 msec (B). Bar graphs show the paired-pulse ratio (PPR) of NMDAR-EPSC (interstimulus interval, 100 msec) (C) and decay time constant of NMDAR-EPSC (D) in prefrontal cortex pyramidal neurons taken from animals injected with saline, low-dose MPH, or high-dose MPH. Inset shows representative NMDAR-EPSC traces evoked by paired pulses. # $p < .001$. Scale bar = 50 pA, 100 msec.

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