Distinct Age-Dependent Effects of Methylphenidate on Developing and Adult Prefrontal Neurons

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Background: Methylphenidate (MPH) has long been used to treat attention-deficit/hyperactivity disorder (ADHD); however, its cellular mechanisms of action and potential effects on prefrontal cortical circuitry are not well understood, particularly in the developing brain system. A clinically relevant dose range for rodents has been established in the adult animal; however, how this range will translate to juvenile animals has not been established.

Methods: Juvenile (postnatal day [PD] 15) and adult (PD90) Sprague Dawley rats were treated with MPH or saline. Whole-cell patch clamp recording was used to examine the neuronal excitability and synaptic transmission in pyramidal neurons of prefrontal cortex. Recovery from MPH treatment was also examined at 1, 5, and 10 weeks following drug cessation.

Results: A dose of 1 mg/kg intraperitoneal MPH, either single dose or chronic treatment (well within the accepted therapeutic range for adults), produced significant depressive effects on pyramidal neurons by increasing hyperpolarization-activated currents in juvenile rat prefrontal cortex, while exerting excitatory effects in adult rats. Minimum clinically-relevant doses (.03 to 3 mg/kg) also produced depressive effects in juvenile rats, in a linear dose-dependent manner. Function recovered within 1 week from chronic 1 mg/kg treatment, chronic treatment with 3 and 9 mg/kg resulted in depression of prefrontal neurons lasting 10 weeks and beyond.

Conclusions: These results suggest that the juvenile prefrontal cortex is supersensitive to methylphenidate, and the accepted therapeutic range for adults is an overshoot. Juvenile treatment with MPH may result in long-lasting, potentially permanent, changes to excitatory neuron function in the prefrontal cortex of juvenile rats.

Key Words: ADHD, development, methylphenidate, neuronal excitability, prefrontal cortex, psychostimulant

Attention-deficit/hyperactivity disorder (ADHD) is a commonly diagnosed behavioral disorder. The major symptoms of childhood ADHD include hyperactivity, inattentiveness, impulsivity, and risk taking; if untreated, these behaviors may persist for life (1–4). The etiology of ADHD is unknown, but a delayed maturation of prefrontal cortex (PFC) in ADHD patients has been proposed (5). Functional magnetic resonance imaging studies have revealed decreased blood flow in PFC of affected individuals, and the symptoms resemble those seen in patients with PFC injury (6,7).

The clinical community largely subscribes to a dopamine (DA)/norepinephrine (NE) hypofunction hypothesis of ADHD; therefore, the pharmaceuticals prescribed to treat ADHD focus on raising the concentrations of these neurotransmitters in the brain to correct the attention deficits and hyperactivity (10–14). However, beyond this well-documented biochemical action, the basis for its clinical efficacy is not well characterized, particularly at the level of individual neurons.

The juvenile and adolescent brain is a highly plastic, rapidly developing system and thus may be particularly vulnerable to the actions of chronic drug treatment (13). In this regard, it is particularly noteworthy that most of our knowledge of MPH actions derives from adult animal studies (10,13,15,16) or from investigations using relatively high doses of the drug (13). Few studies have focused on prefrontal functions, although the PFC has been clearly identified as the primary target of the drug actions (10,17,18). Recently, some studies have demonstrated the potential for distinct anatomic and behavioral effects of MPH in juvenile rats not equivalent to those seen in adults; alterations in circadian rhythm, neurogenesis, and working memory tasks have been noted, as well as cellular and molecular alterations in brain regions involved in motivational, attentional, and appetitive behaviors (19–22). However, few physiologic data have been collected that address the specific changes in cellular function and PFC neural circuit operations that may occur following juvenile or adolescent MPH administration.

Because of the paucity of juvenile rodent studies and the limited range of doses that have been explored, it is necessary to examine the effects of clinically relevant doses of MPH in a juvenile rat system. In this study, we examined age- and dose-dependent effects of MPH administration on neuronal excitability and synaptic transmission in the layer 5 pyramidal neurons of rat PFC.

Methods and Materials

Detailed procedures can be found in the Supplement 1. Briefly, rats were divided into four groups: single-dose saline or MPH and chronic saline or MPH. Single-dose animals received a single injection of MPH (1 mg/kg, intraperitoneal [IP]) or equivalent volume of saline and were sacrificed 1 hour later (postnatal day [PD]15–20). Chronic treatment animals received a 1 mg/kg IP injection of MPH or saline once daily beginning at PD15, 5 days per week for 3 weeks, and were, on average, PD40 at conclusion of the treatment. Chronic-treatment animals were sacrificed 1 hour following the final injection. To test dose dependence, animals received either saline or MPHAt .01, .03, .1, .3, or 1.0 mg/kg in a single IP injection. To examine the recovery, three groups of animals were given chronic treatment of 1, 3, or 9 mg/kg IP MPH or saline beginning at PD15 and then allowed 1, 5, or 10 weeks to recover.

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The PFC slices were bathed in a heated (36°–37°C) recording chamber with aerated Ringer’s solution. Layer 5 PFC pyramidal neurons were recorded with electrode pipettes filled a K+-gluconate intracellular solution. For excitability studies, a step current (50-pA increment) protocol was applied with 20 sweeps. Spontaneous excitatory postsynaptic currents (sEPSC) were also recorded. For H current recordings, neurons were held in voltage clamp at −60 mV, and a step-voltage protocol (−60 to −120 mV) was applied (23). All data were analyzed with Clampfit (Molecular Devices, LLC, Sunnyvale, California). Student’s t-test and two-way analysis of variance (ANOVA) were used for significance test.

Results

Effects of MPH Treatment on Membrane Excitability in Juvenile Versus Adult Rat PFC Pyramidal Neurons

We first examined the effects of MPH (1 mg/kg, IP) on the excitability of layer 5 pyramidal neurons in the juvenile rat (PD15–20) PFC. Neurons recorded from single-dose saline control animals (n = 18) exhibited an average of 30.6 ± 3.22 spikes, whereas neurons recorded from MPH-treated animals (n = 11) averaged only 13.7 ± 2.92 spikes (55% decrease, p = .0004; Figure 1A). A chronic treatment of MPH (n = 18) resulted in an average of 4.1 ± 1.01 spikes compared with 24.5 ± 2.75 in chronic saline control (n = 12; 83.3% decrease, p < .0001; Figure 1A). A two-way ANOVA revealed significance across all groups (p = .019, F = 4.50; Figure 1A). These data suggest that MPH treatment has a significant suppressant effect on the excitability of juvenile PFC neurons.

These findings raise questions because previous studies have shown that MPH exerts facilitating effects on motor cortex in healthy adult human subjects (24) and on sensory cortical neurons in adult rodents (25). To investigate this difference, adult rats (PD90, n = 6 rats per group) were treated with MPH (1 mg/kg, IP) for 3 weeks beginning at PD90 or with saline as control. We found that, in contrast to the depressant effects seen in juvenile rats, chronic MPH treatment significantly increased spike numbers in the adult rat layer 5 pyramidal neurons (p < .01, Figure 1B). Resting membrane potential, threshold, peak amplitude, and afterhyperpolarization (AHP) were not affected by chronic MPH treatment in adult rats (p > .05); however, action potential half-width (p = .0104) and input resistance (p = .047) were significantly increased. These results are in agreement with the existing literature and indicate that MPH effects on PFC neuronal excitability are age-dependent.

Effects of MPH Treatment in Juvenile Animals Are Not Attributable to Age

Because chronic treatment lasted for 3 weeks and animals grow from juveniles (PD15–20) to adolescents (PD36–41) by the end of treatment, we postulated that the more prominent excitability decrease seen in chronically treated juvenile rats compared with single-dose-treated animals might be attributable to the older age. To explore this possibility, we first ran a set of control experiments in PD40 and PD90 rats without MPH treatment and compared the results with those in age-matched chronic MPH-treated animals. As shown in Figure 1C, spike number in saline-treated PD40 and PD95 rats decreased and the spike numbers have a negative correlation with animal age (R² = .256, p = .0023; Figure 1C). Nevertheless, the
spike number in normal untreated PD40 (~30 spikes) rats was significantly higher than that in chronically MPH-treated juvenile rats (~4 spikes at ~PD40, \( p < .0001 \); Figure 1A, C). In contrast, in the chronic MPH-treated adult rats, the spike numbers were positively correlated with age, with adult animals showing significantly more spikes after MPH treatment (\( R^2 = .316, p = .001 \)).

We also examined other action potential parameters for age-related changes irrespective of drug treatment. The age difference between single-dose treated and chronically treated rats did not have a significant correlation with AHP (\( R^2 = .009, n = 61 \)) or half-width (\( R^2 = .024, n = 61 \)) but a slightly positive correlation with peak amplitude (\( R^2 = .177, n = 61 \)), despite peak amplitude not being affected by MPH treatment (\( p = .858, F = 1.55 \)). Therefore, the age discrepancy between single-dose and chronically treated juvenile rats in our study did not impact the reliability of our drug treatment nor provide a confounding action for the interpretation of results.

### MPH Effects on the Passive Membrane Properties of Juvenile Rat Pyramidal Neurons Indicates Involvement of Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) Channels

We then further examined numerous passive membrane properties to elucidate a potential cellular mechanism for the decreased excitability seen in juvenile rats. Resting membrane potential is regulated largely by sodium channel activity, whereas input resistance is thought to be a measure of potassium channel sensitivity (26). No significant difference was found in resting membrane potential among treatment groups in juvenile rats (\( p = .085, F = 2.534 \); Figure 2A), and resting membrane potential did not change with age (\( R^2 = .048, p = .201 \)). However, input resistance was significantly decreased in chronically MPH-treated rats (\( p = .006, F = 5.549 \); Figure 2B), despite a significant negative correlation between age and input resistance (\( R^2 = .290, p = .0007 \)). This result suggests that, although input resistance may decrease with age, MPH results in a drug-treatment-specific and significant decrease in input resistance regardless of age. Indeed, as shown in the raw data samples of action potentials in Figure 2C, the negative current (−100 pA, arrows, Rin) applied in baseline and the step currents following baseline (50-pA increments) induced smaller voltage changes in chronic MPH-treated neurons than in cells recorded from saline control animals. Creation of a current–voltage (I-V) curve further revealed a linear relationship between current input and voltage increase, suggestive of potassium channel effects. In addition, a decreased slope was noted for MPH-treated rats, indicative of reduced excitability (\( * p < .05 \) in Figure 2D). These results suggest that the decreased excitability may be mediated by changes in potassium channel gating following drug treatment, either as a direct effect or as a downstream effect of signaling changes due to the presumably heightened levels of DA and NE.

To test this possibility, cyclic adenosine monophosphate (cAMP)-mediated HCN channel current (Ih current) was examined. HCN channels are known to be activated by DA/NE activation of cAMP pathways, and lead to a decrease in neuronal firing. Therefore, we proposed that Ih current amplitude could be increased by MPH. Indeed, acute treatment with 1 mg/kg MPH resulted in a strong increase in Ih current amplitude (Figure 3A and B). Furthermore, the Ih amplitudes were significantly increased at each point in the voltage step protocol from −70 to −120 mV, as well as overall, as revealed by a repeated-measures two-way ANOVA (\( p = .003, F = 5.231, df = 5, 75 \)). Between-subjects measure was also found significant (\( p = .0018, F = 7.0, df = 1.15 \); Figure 3C) as well as effect of injected voltage (\( p < .00001, F = 27.4, df = 5, 75 \)). In addition to overall significance of MPH treatment effects on Ih, each injected voltage beyond −60 mV resulted in significantly greater Ih in MPH-treated animals (\( n = 10 \)) than in saline control animals (\( n = 7, \) Figure 3C). The current produced by the maximum injected voltage (−120 mV) was significantly greater for MPH-treated neurons than for control neurons (491.1 ± 38.82 pA vs. 231.5 ± 70.12 pA; \( p = .0115 \)). MPH effects on excitability in this group of neurons was examined again to verify drug efficacy and reliability of the Ih recordings; 1 mg/kg acute treatment decreased excitability (32.1 ± 3.40 in saline vs. 17.3 ± 1.23 in MPH; \( p = .0036 \)). These findings support that Ih induces hyperpolarization of neuron, which decreases excitability and reduces input resistance (27). Thus the increased HCN channel current induced by MPH treatment in the
Dependent Manner in Layer 5 Pyramidal Neurons

Lower Doses of MPH Also Produce Depression in a Dose-Dependent Manner in Layer 5 Pyramidal Neurons

To test the hypothesis that the juvenile PFC is more sensitive to MPH than the adult PFC, we treated PD15–25 rats with several lower acute doses of MPH at .01, .03, .1, or .3 mg/kg (IP; n = 7 per dosage group) with saline as control (n = 7). A trend toward depression of excitability was noted following all doses except for .01 mg/kg, but significance was not reached until 1 mg/kg. ANOVA analysis revealed significant difference among groups (p = .0025, F = 4.258, Figure 4). However, further examination revealed only trends between saline and .1 mg/kg (30 spikes vs. 19, p = .054; Figure 4), saline and .3 mg/kg (30 spikes vs. 23, p = .086; Figure 4), and .1 mg/kg and .3 mg/kg (19 vs. 23 spikes, p = .377; Figure 4). These data show that the depressive effects of MPH on the juvenile rat brain are evident even at doses at the extreme low end of the range used clinically for ADHD patients and for producing cognitive enhancement in adult rodents, suggesting that there may be a greater sensitivity to the drug effects in the juvenile PFC than in adult PFC.

Synaptic Transmission, as Measured by Spontaneous Excitatory Postsynaptic Current, Is Also Reduced by MPH Treatment in the Juvenile Rat PFC

A decreased excitability is likely to result in, or be reflective of, a concomitant decrease in synaptic transmission. As shown in the sEPSC samples in Figure 5A, a single-dose treatment of 1 mg/kg MPH (n = 11) resulted in significantly decreased frequency, but not amplitude, of sEPSCs compared with saline controls (n = 18; p = .044 and p = .874, respectively; Figure 5B, C). Chronic treatment with MPH (n = 18) also significantly decreased sEPSC frequency and amplitude (p < .0001 and p = .0115, respectively; Figure 5B, C) when compared with chronic saline-treated controls (n = 12). The amplitude of EPSCs in chronic saline-treated neurons was significantly larger than that of the single-dose saline-treated neurons. The increase in chronic saline-treated EPSC amplitude may be attributable to the synaptic strengthening that occurs during development, wherein α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors are trafficked to synaptic membranes, resulting in a larger pool of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors available for synaptic transmission (28). Reduced frequency of sEPSCs represents reduced synaptic input to the layer 5 PFC pyramidal neurons, suggesting decreased synaptic transmission. Furthermore, the reduction in sEPSCs supports reduced excitability in that reduced volume of incoming signals will result in less excitation of the neuron and therefore fewer action potentials. Further analysis indicated that the sEPSC amplitude was positively correlated with age (R² = .325), but the frequency was not (R² = .055). Chronic-treated animals significantly differed from age-matched controls; therefore, MPH treatment decreases sEPSCs in the juvenile rat PFC.

Figure 3. Examination of the cyclic adenosine monophosphate–HCN channel-mediated Ih current reveals an increase in Ih current amplitude concurrent with decreased neuronal excitability following 1 mg/kg methylphenidate (MPH) treatment (n = 10) when compared to saline controls (n = 7). (A) Sample traces showing the voltage steps (lower panel) and currents induced by the injected voltage steps in control and MPH-treated rats (upper two panels). (B) Magnification of the sample traces showing the measurement of Ih currents that are exhibited in panel C. (C) Current–voltage (I–V) curve reveals significant increase of Ih current in MPH-treated neurons from −70 mV to −120 mV injected voltage (treatment by sweep interaction, p = .0003, F = 5.3, df = 5, 75; treatment effect, p = .0182, F = 7, df = 1,15; injected voltage effect, p < .0001, F = 27.4, df = 5, 75). HCN, hyperpolarization-activated cyclic nucleotide-gated.

Figure 4. Examination of lower doses of the purportedly therapeutic range revealed similar depressant effects on juvenile prefrontal cortex pyramidal neurons except for the very low dose of .01 mg/kg (n = 7 per group). Excitability of neurons from rats treated with .03 mg/kg to .3 mg/kg single-dose methylphenidate (MPH) revealed a trend toward a decrease (R² = .668), although not significant at some points compared with control. However, two-way analysis of variance revealed significant difference across groups when 1 mg/kg was included (p = .0025, F = 4.258) (*p < .05; **p < .01).
Recovery from the Effects of Chronic MPH Treatment Is Dose-Dependent

To examine the permanency of the depressive effects of MPH on the developing PFC, juvenile (P15–20) rats were treated for 3 weeks with saline or 1 mg/kg IP MPH and were examined for excitability and synaptic transmission 1, 5, or 10 weeks after treatment ended. Pyramidal neurons from rats tested 1 week after 1 mg/kg treatment were indistinguishable from saline controls in both spike number (p = .997, Figure 6A) and sEPSC frequency (p = .791, Figure 7A). There was also no rebound to drug response after 5 weeks (Figures 6D and 7D), indicating that this dosage of MPH does not cause long-term changes to PFC function. At 1 week postdrug treatment and beyond, no significant differences were seen in resting membrane potential, input resistance, action potential threshold, peak amplitude, half-width, or AHP. However, when animals were treated with 3 mg/kg MPH for 3 weeks, excitability did not recover to control levels even 10 weeks after treatment ended (p = .005, Figure 6E; p = .008, Figure 6G), nor did synaptic transmission (p = .04, Figure 7G). Likewise, neither excitability (p = .028, Figure 6H) nor synaptic transmission (p = .0345, Figure 7H) measures recovered within 10 weeks after 9 mg/kg treatment ended. Interestingly, there was no significant difference in excitability (p > .05) or synaptic transmission (p > .05) between 3- and 9-mg/kg-treated neurons. This apparent lack of a clear dose–response curve could be explained by a “ceiling” effect in which treatment with 3 mg/kg MPH would depress neuronal excitability and synaptic transmission to its maximum, thus preventing additional effects from increasing dosages. These data indicate that, although low clinical doses of MPH may not produce lasting effects, higher clinical doses and abuse doses may cause long-term changes to PFC circuitry by depressing the activity of layer 5 pyramidal neurons.

Discussion

There are several novel findings here. First, we observed distinct age-dependent actions of MPH on prefrontal neurons. In particular, juvenile PFC neurons are supersensitive to even very low doses of MPH. Both single-dose and chronic treatment regimens of MPH at doses that enhance attentional processes and pyramidal neuron activity in adult rats resulted in a significant decrease of neuronal excitability and synaptic transmission in PFC layer 5 pyramidal neurons in juvenile rats. Second, higher doses of MPH induced long-lasting depressant effects on juvenile rat PFC neurons.

MPH is widely used in long-term regimens to treat ADHD in humans. The behavioral and cognitive actions of MPH are observed in both normal human subjects and animal models. Most evidence supports the continued use of MPH as the best available pharmacotherapy for the treatment of children with ADHD, but little is known about possible enduring behavioral and neurodevelopmental consequences of long-term exposure. In particular, the rationale for selection of MPH doses that simulate clinical exposure has not been well defined in juvenile and adolescent animal models. The majority of the studies have been conducted in adult animals or in young animals with relatively high doses. Nevertheless, these studies suggest that MPH elicits reproducible dose–response curves for behaviors expressed in normal adult rats. Low doses of MPH improve working memory and sustained attention in the absence of enhanced arousal or locomotor activation. Collectively, these results indicate that the behavioral and cognitive actions of low-dose MPH are applicable to both normal rats and ADHD patients and are qualitatively distinct from the behavioral effects observed in response to higher doses.

However, it is apparent that developmental age and MPH dose play a critical role in determining the effects of drug administration on prefrontal neurons. Treatment with MPH resulted in significant reduction in both excitability and synaptic transmission in the juvenile rat. This reduction in excitability may seem counterintuitive because the drug has previously been found to increase cortical excitability at low doses and increase locomotion at higher doses in adult rats. The reason for these discrepancies is unknown, but our data indicate that juvenile rat prefrontal neurons are supersensitive to MPH and that the observed effects are age-dependent.
Indeed, in a study of the long-term effects of MPH treatment in adolescent animals, Brandon et al. (34) administered MPH (2 mg/kg, IP) during PD35 to 42 and found an enhanced rewarding effect on subsequent cocaine self-administration. In contrast, the same dose (2 mg/kg, IP) in slightly younger rats (PD20–35) was found to attenuate the rewarding effect (35,51). Our results were opposite to those seen in adult rats, in which prefrontal neurons are excited by low-dose stimulant treatment (10,52) or by systemic administration of 2 mg/kg MPH (53). Taken together these results argue for an age-dependent action of MPH on prefrontal neurons.

ADHD is thought to result from prefrontal hypoactivity, a condition corrected by the administration of stimulants (6,54). However, it is not clear whether the behaviorally effective range of doses identified in adult rodents can be directly translated to juveniles. Therefore, even a dose of MPH thought to be within the clinically relevant range, 1 mg/kg, may in fact cause excessively high levels of DA and NE in the juvenile PFC. If so, the results seen here may in fact be explained through the actions of DA and NE on cAMP-HCN signaling. It has been reported that physiologic levels of NE in the PFC activate HCN2 receptors, which inhibit cAMP signaling (55). Excessive levels of NE, however, activate not only the high-affinity HCN2 receptors but also lower affinity HCN1 and HCN1 receptors (55). This increased cAMP signaling could result in decreased neuronal activity and synaptic transmission via over-activation of the cAMP-HCN channels. Activation of HCN channels results in an influx of cations which hyperpolarize neurons and render them unresponsive to

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1 \text{ Week Recovery} & \quad 1 \text{ mg/kg MPH} \\
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10 \text{ Weeks Recovery} & \quad 1 \text{ mg/kg MPH}
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incoming synaptic stimuli. This results in a decrease in excitability and, therefore, a decrease in signal transmission by the unresponsive neuron. Consistent with this assumption, treatment with 1 mg/kg MPH resulted in increased amplitude of I(h) current concomitant with the decreased excitability in juvenile rat PFC neurons. Thus, the significant decrease in excitability observed in juvenile pyramidal neurons is likely due to MPH creating excessive levels of NE and DA, which in turn increase cAMP-HCN signaling across the PFC, decreasing neuronal responsiveness and transmission (56,57).

However, additional studies are needed to elucidate the mechanisms of the opposing MPH actions in adult rats in which the HCN channel properties appear to be different (58,59). It is known that a developmental decrease in time kinetics of Ih in hippocampus leads to increased adult pyramidal neuron firing rate, and changes in HCN channel isoforms lead to reduced involvement of the channel in Ih (58,59). Furthermore, the expression of DA receptors changes over development, with D2 (inhibitory) higher during juvenile period and D1 (excitatory) levels higher in adulthood (60–62).

The optimal dose–response range for juvenile rats is lower than that for adults. Therefore, clinical dose ranges for use in humans may need to be adjusted for age as well as weight (currently only body weight is taken into account). Furthermore, these effects are

**Figure 7.** Synaptic transmission of neurons was also recorded from recovery groups and recovered from 1 mg/kg chronic methylphenidate (MPH) treatment, but not from 3 and 9 mg/kg. One week after termination of treatment, neurons from (A) 1 mg/kg MPH–treated animals had recovered to saline control levels, but those from (B) 3 mg/kg (p = .007) and (C) 9 mg/kg-treated animals (p = .001) exhibited significantly reduced spontaneous excitatory postsynaptic current (sEPSC) frequency. Five weeks after treatment ceased, neurons from 1 mg/kg-treated animals had recovered (D); however, those from animals treated with 3 mg/kg (E) and 9 mg/kg still exhibited significantly reduced sEPSC frequency; (E) p = .005 and (F) p = .006. Similarly, even after 10 weeks, the reduction of spike numbers in neurons from animals treated with 3 and 9 mg/kg had not recovered; (G) p = .040 and (H) p = .0345; *p < .05.
reversible at the 1 mg/kg dose but not at higher doses, indicating potential lasting effects from long-term treatment or drug abuse and stressing the need for tighter regulation of ADHD diagnoses and more careful observation of patients currently undergoing treatment. This research emphasizes the importance of further examination of MPH effects in the juvenile developing brain and suggests a reexamination of the definition of “therapeutic range” when designing treatment regimens for patients or behavioral paradigms for experimental subjects.

In addition, our data suggest a discrepancy between PFC functional outcomes in adults versus juvenile rats following administration of equivalent doses of MPH and thus reveal a potential for permanent, or at least long-lasting, PFC changes in MPH-treated children. MPH is generally thought to be safe and free of lasting significant side effects when it is administered at the recommended, clinically approved doses (5–15 mg, 3 times daily oral in humans) (63). However, recent studies have shown that the drug may cause changes to brain circuitry and function that persist long after drug clearance (49,64,65). For example, MPH induces deficits in object recognition and spatial working memory for up to 42 days postdrug administration in both adult and periadolescent rats (64,65). These results and other similar studies suggest that MPH may indeed cause lasting, even permanent, changes in neuronal function (41,43,49). Our findings that passive membrane properties of PFC neurons did not recover to control levels following high MPH doses agree with this body of evidence.

This study was limited to examination of layer 5 PFC pyramidal neuron. However, PFC contains gamma-aminobutyric acidergic interneurons that exert inhibitory control over pyramidal neuron activity. When MPH is given systemically, as in this study, it affects all neuronal types. Thus, future examination of MPH effects on interneurons is warranted. Finally, other brain regions are also subject to the effects of systemic MPH administration. Thus, it is possible that some of the effects we observed are indirectly mediated by actions at sites remote from the recording location in the PFC. However, in this context, it is important to acknowledge the relevance of systemic drug administration as examined here and whole-brain exposure to MPH as occurs in the clinical situation.

In conclusion, this study examines the effects of systemic MPH on multiple dimensions of neuronal functions in juvenile brain. Although our results suggest that the currently accepted therapeutic range for MPH treatment may differ between developing and fully developed brain systems, the data also raise the possibilities of differential cell-type involvement in these drug effects and potentially long-lasting impact of MPH on cellular physiology following chronic high dose drug administration.

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Supplementary material cited in this article is available online.


