Published in final edited form as:

Biol Psychiatry. 2012 November 15; 72(10): 880–888. doi:10.1016/j.biopsych.2012.04.018.

Distinct age-dependent effects of methylphenidate on developing and adult prefrontal neurons

Kimberly R. Urban, Barry D. Waterhouse, and Wen-Jun Gao*

Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129

Abstract

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 (P15) and adult (P90) SD 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—1 mg/kg i.p. dose of 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 (0.03 to 0.3 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.

Keywords

methylphenidate; psychostimulant; ADHD; prefrontal cortex; development; neuronal excitability

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a commonly diagnosed behavioral disorder. The major symptoms of childhood ADHD include hyperactivity, inattentiveness,

© 2012 Society of Biological Psychiatry. Published by Elsevier Inc. All rights reserved.

* Correspondence: Wen-Jun Gao, M.D., Ph.D., Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, Phone: (215) 991-8907, Fax: (215) 843-9802, wgao@drexelmed.edu.

Competing interests: The authors declare no biomedical financial interests or potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 MRI 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 levels of DA and NE to restore brain functions associated with attention (6-8). Methylphenidate (Ritalin®, MPH) is the most commonly prescribed medication (9). It has been determined that MPH acts primarily on the dopaminergic and noradrenergic systems through blockade of DA and NE transporters, thereby increasing 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 anatomical 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, little physiological data has been collected that addresses 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.

Materials and Methods

Detailed procedures can be found in the Supplemental Materials. 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, i.p.) or equivalent volume of saline and were sacrificed one hour later (PD15-20). Chronic treatment animals received a 1 mg/kg (i.p.) injection of MPH or saline once daily beginning at PD15, 5 days/week for 3 weeks, and were, on average, PD40 at conclusion of the treatment. Chronic-treatment animals were sacrificed one hour following the final injection. To test dose-dependence, animals received either saline or MPH at 0.01, 0.03, 0.1, 0.3 or 1.0 mg/kg (i.p.) in a single injection. To examine the recovery, three groups of animals were given chronic treatment of 1, 3, or 9 mg/kg (i.p.) MPH or saline beginning at PD15 and then allowed 1, 5, or 10 weeks to recover.

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 EPSCs 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 was analyzed with Clampfit. Student's t-test and two-way ANOVA were used for significance test.

Results

Effects of MPH treatment on membrane excitability in juvenile versus adult rat prefrontal cortical pyramidal neurons

We first examined the effects of MPH (1 mg/kg, i.p.) 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, $p = 0.0004$, Fig. 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, $p < 0.0001$, Fig. 1A). A two-way ANOVA revealed significance across all groups ($p = 0.019$, $f = 4.50$, Fig. 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, i.p.) 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 ($p < 0.01$, Fig. 1B). Resting membrane potential, threshold, peak amplitude, and afterhyperpolarization were not affected by chronic MPH treatment in adult rats ($p > 0.05$); however, AP half-width ($p = 0.0104$) and input resistance ($p = 0.047$) 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 to 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 rats decreased from 30 spikes in adolescent (PD40) to about 15 spikes in adults (PD95). Thus, the spike numbers were progressively reduced with a negative linear correlation with age ($R^2 = 0.256$, $p = 0.0023$, Fig. 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, $p < 0.0001$, Fig. 1A and 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 ($R^2 = 0.316$, $p = 0.001$).

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 ($R^2 = 0.009$, $n = 61$), or half-width ($R^2 = 0.024$, $n = 61$) but a slightly positive correlation with peak amplitude ($R^2 = 0.177$, $n = 61$), despite peak amplitude not being affected by MPH treatment ($p = 0.858$, $f = 0.155$).

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 cAMP-HCN channel

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, while 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 ($p = 0.085$, $f = 2.534$, Fig. 2A) and resting membrane potential did not change with age ($R^2 = 0.048$, $p = 0.201$). However, input resistance was significantly decreased in chronically MPH-treated rats ($p = 0.006$, $f = 5.549$, Fig. 2B), despite a significant negative correlation between age and input resistance ($R^2 = 0.290$, $p = 0.0007$). This result suggests that, while 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 increment) 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 (* note for $p < 0.05$, Fig. 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, cAMP-mediated HCN channel current (I_h 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 I_h current amplitude could be increased by MPH. Indeed, acute treatment with 1 mg/kg MPH resulted in a strong increase in I_h current amplitude (Fig. 3A and B). Furthermore, the I_h 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 2-way ANOVA ($p = 0.003$, $f = 5.231$, $df = 5, 75$). Between-subjects measure was also found significant ($p = 0.0018$, $f = 7.0$, $df = 1, 15$; Fig. 3C) as well as effect of injected voltage ($p < 0.00001$, $f = 27.4$, $df = 5, 75$). In addition to overall significance of MPH treatment effects on I_h, each injected voltage beyond -60 mV resulted in significantly greater I_h in MPH-treated animals ($n = 10$) than in saline control animals ($n = 7$; Fig. 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; $p = 0.0115$). MPH effects on excitability in this group of neurons was examined again to verify drug efficacy and reliability of the I_h recordings; 1 mg/kg acute treatment decreased excitability (32.1 ± 3.40 in saline vs. 17.3 ± 1.23 in MPH; $p = 0.0036$). These findings support that I_h induces hyperpolarization of neuron, which decreases excitability and reduces input resistance (27). Thus the increased HCN channel current induced by MPH treatment in the juvenile rat can explain the decreased excitability and input resistance.

Lower doses of MPH also produce depression in a dose-dependent manner in layer 5 pyramidal neurons

In order 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 0.01, 0.03, 0.1, or 0.3 mg/kg (i.p.; $n = 7$ per dosage group) with saline as control ($n = 7$). A trend towards depression of excitability was noted following all doses except for 0.01 mg/kg, but significance was not reached until 1 mg/kg. ANOVA analysis revealed significant difference among groups ($p = 0.0025$, $f = 4.258$, Fig. 4). However, further examination revealed only trends between saline and 0.1 mg/kg (30 spikes vs. 19, $p = 0.054$, Fig. 4), saline and 0.3 mg/kg (30 spikes vs. 23, $p = 0.086$, Fig. 4), and 0.1 mg/kg and 0.3 mg/kg (19 vs. 23 spikes, $p = 0.377$, Fig. 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 (i.p.; $n = 11$) resulted in significantly decreased frequency, but not amplitude, of sEPSCs when compared to saline controls ($n = 18$; $p = 0.044$ and $p = 0.874$, respectively, Fig. 5B, C). Chronic treatment with MPH ($n = 18$) also significantly decreased sEPSC frequency and amplitude ($p < 0.0001$ and $p = 0.0115$, respectively, Fig. 5B, C) when compared to 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 AMPARs are trafficked to synaptic membranes, resulting in a larger pool of AMPARs 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 ($R^2 = 0.325$), but the frequency was not ($R^2 = 0.055$). Chronic-treated animals significantly differed from age-matched controls; therefore, MPH treatment decreases sEPSCs in the juvenile rat PFC.

Recovery from the effects of chronic MPH treatment is dose-dependent

In order 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 i.p. MPH and were examined for excitability and synaptic transmission 1, 5, or 10 weeks after treatment was ended. Pyramidal neurons from rats tested 1 week after 1 mg/kg treatment were indistinguishable from saline controls in both spike number ($p = 0.997$, Fig. 6A) and sEPSC frequency ($p = 0.791$, Fig. 7A). There was also no rebound to drug response after 5 weeks (Fig. 6D and 7D), indicating that this dosage of MPH does not cause long-term changes to PFC function. At one week post-drug treatment and beyond, no significant differences were seen in RMP, input resistance, AP 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 ($p = 0.005$, Fig. 6E; $p = 0.008$, Fig. 6G), nor did synaptic transmission ($p = 0.04$, Fig. 7G). Likewise, neither excitability ($p = 0.028$, Fig. 6H) nor synaptic transmission ($p = 0.0345$, Fig. 7H) measures recovered within 10 weeks after 9 mg/kg treatment ended. Interestingly, there was no significant difference in

excitability ($p > 0.05$), nor in synaptic transmission ($p > 0.05$) between 3 and 9 mg/kg-treated neurons. This apparent lack of a clear dose-response curve could be explained by a “ceiling” effect, wherein 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, while 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 (29-31). 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 neuroadaptational consequences of long-term exposure (32, 33). In particular, the rationale for selection of MPH doses that simulate clinical exposure has not been well defined in juvenile and adolescent animal models (13). The majority of the studies have been conducted in adult animals (10, 13, 15, 16) or in young animals with relatively high doses (13, 19, 34-49). 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 (16) in the absence of enhanced arousal or locomotor activation (1, 12). 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, as the drug has previously been found to increase cortical excitability at low doses and increase locomotion at higher doses in adult rats. (24, 50) 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 PD35–42 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 physiological levels of NE in the PFC activate α 2A-adrenoceptors, which inhibit cAMP signaling (55). Excessive levels of NE, however, activate not only the high affinity α 2A receptors, but also lower affinity α 1 and β 1 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 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, further 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 $I(h)$ in hippocampus leads to increased adult pyramidal neuron firing rate, and changes in HCN channel isoforms lead to reduced involvement of the channel in $I(h)$ (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 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 vs. 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 post-drug administration in both adult and peri-adolescent 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 GABAergic interneurons which 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. While 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by NIH R01 grant MH232395 to W.-J. Gao.

References

1. Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. *Behavioural Brain Research*. 1998; 94:127–152. [PubMed: 9708845]
2. Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N, et al. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev*. 2007; 17:39–59. [PubMed: 17318414]
3. Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. *Nat Rev Neurosci*. 2002; 3:617–628. [PubMed: 12154363]
4. Barkley RA, Fischer M, Smallish L, Fletcher K. Young adult follow-up of hyperactive children: antisocial activities and drug use. *J Child Psychol Psychiatry*. 2004; 45:195–211. [PubMed: 14982236]
5. Shaw P, Eckstrand K, Sharp W, Blumenthal J, Lerch JP, Greenstein D, et al. Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc Natl Acad Sci U S A*. 2007; 104:19649–19654. [PubMed: 18024590]
6. Arnsten AF. Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *J Clin Psychiatry*. 2006; 67(8):7–12. [PubMed: 16961424]
7. Ohno M. The dopaminergic system in attention deficit/hyperactivity disorder. *Congenit Anom (Kyoto)*. 2003; 43:114–122. [PubMed: 12893970]
8. Russell VA. Dopamine hypofunction possibly results from a defect in glutamate-stimulated release of dopamine in the nucleus accumbens shell of a rat model for attention deficit hyperactivity disorder—the spontaneously hypertensive rat. *Neurosci Biobehav Rev*. 2003; 27:671–682. [PubMed: 14624811]
9. Challman TD, Lipsky JJ. Methylphenidate: its pharmacology and uses. *Mayo Clin Proc*. 2000; 75:711–721. [PubMed: 10907387]
10. Berridge CW, Devilbiss DM, Andrzejewski ME, Arnsten AF, Kelley AE, Schmeichel B, et al. Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function. *Biol Psychiatry*. 2006; 60:1111–1120. [PubMed: 16806100]

11. Kuczenski R, Segal DS. Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. *J Pharmacol Exp Ther.* 2001; 296:876–883. [PubMed: 11181919]
12. Kuczenski R, Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci.* 2002; 22:7264–7271. [PubMed: 12177221]
13. Kuczenski R, Segal DS. Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse. *Biol Psychiatry.* 2005; 57:1391–1396. [PubMed: 15950013]
14. Greenhill L, Kollins S, Abikoff H, McCracken J, Riddle M, Swanson J, et al. Efficacy and safety of immediate-release methylphenidate treatment for preschoolers with ADHD. *J Am Acad Child Adolesc Psychiatry.* 2006; 45:1284–1293. [PubMed: 17023867]
15. Arnsten AF. Stimulants: Therapeutic actions in ADHD. *Neuropsychopharmacology.* 2006; 31:2376–2383. [PubMed: 16855530]
16. Arnsten AF, Dudley AG. Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: Relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. *Behav Brain Funct.* 2005; 1:2. [PubMed: 15916700]
17. Arnsten AF. Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs.* 2009; 23(1):33–41. [PubMed: 19621976]
18. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci.* 2009; 10:410–422. [PubMed: 19455173]
19. Lagace DC, Yee JK, Bolanos CA, Eisch AJ. Juvenile administration of methylphenidate attenuates adult hippocampal neurogenesis. *Biol Psychiatry.* 2006; 60:1121–1130. [PubMed: 16893528]
20. Algahim MF, Yang PB, Bureau KD, Swann AC, Dafny N. Repetitive ritalin treatment modulates the diurnal activity pattern of young SD male rats. *Cent Nerv Syst Agents Med Chem.* 2010; 10:247–257. [PubMed: 20557282]
21. Beckman DA, Schneider M, Yourenoff M, Tse FL. Juvenile toxicity assessment of d,l-methylphenidate in rats. *Birth Defects Res B Dev Reprod Toxicol.* 2008; 83:48–67. [PubMed: 18189274]
22. Gray JD, Punsoni M, Tabori NE, Melton JT, Fanslow V, Ward MJ, et al. Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *J Neurosci.* 2007; 27:7196–7207. [PubMed: 17611273]
23. Kanyshkova T, Pawlowski M, Meuth P, Dube C, Bender RA, Brewster AL, et al. Postnatal expression pattern of HCN channel isoforms in thalamic neurons: relationship to maturation of thalamocortical oscillations. *J Neurosci.* 2009; 29:8847–8857. [PubMed: 19587292]
24. Kirschner J, Moll GH, Fietzek UM, Heinrich H, Mall V, Berweck S, et al. Methylphenidate enhances both intracortical inhibition and facilitation in healthy adults. *Pharmacopsychiatry.* 2003; 36:79–82. [PubMed: 12734766]
25. Drouin C, Wang D, Waterhouse BD. Neurophysiological actions of methylphenidate in the primary somatosensory cortex. *Synapse.* 2007; 61:985–990. [PubMed: 17854047]
26. Hille, B. *Ion Channels of Excitable Membranes.* 3. Sunderland, Mass; Sinauer: 2001.
27. Surges R, Freiman TM, Feuerstein TJ. Input resistance is voltage dependent due to activation of Ih channels in rat CA1 pyramidal cells. *J Neurosci Res.* 2004; 76:475–480. [PubMed: 15114619]
28. Kessels HW, M R. Synaptic AMPA receptor Plasticity and Behavior. *Neuron.* 2009; 61:340–350. [PubMed: 19217372]
29. Franke AG, Bonertz C, Christmann M, Huss M, Fellgiebel A, Hildt E, et al. Non-medical use of prescription stimulants and illicit use of stimulants for cognitive enhancement in pupils and students in Germany. *Pharmacopsychiatry.* 2011; 44:60–66. [PubMed: 21161883]
30. Linssen AM, Vuurman EF, Sambeth A, Riedel WJ. Methylphenidate produces selective enhancement of declarative memory consolidation in healthy volunteers. *Psychopharmacology (Berl).* 2011

31. Smith ME, Farah MJ. Are prescription stimulants “smart pills”? The epidemiology and cognitive neuroscience of prescription stimulant use by normal healthy individuals. *Psychol Bull.* 2011; 137:717–741. [PubMed: 21859174]
32. Grund T, Lehmann K, Bock N, Rothenberger A, Teuchert-Noodt G. Influence of methylphenidate on brain development - an update of recent animal experiments. *Behav Brain Funct.* 2006; 2:2. [PubMed: 16403217]
33. Yano M, Steiner H. Methylphenidate and cocaine: the same effects on gene regulation? *Trends in Pharmacological Sciences.* 2007; 28:588–596. [PubMed: 17963850]
34. Brandon CL, Marinelli M, Baker LK, White FJ. Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology.* 2001; 25:651–661. [PubMed: 11682248]
35. Carlezon WA Jr, Mague SD, Andersen SL. Enduring behavioral effects of early exposure to methylphenidate in rats. *Biol Psychiatry.* 2003; 54:1330–1337. [PubMed: 14675796]
36. Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon WA Jr. Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat Neurosci.* 2002; 5:13–14. [PubMed: 11731802]
37. Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry.* 2003; 54:1317–1329. [PubMed: 14675795]
38. Brandon CL, Steiner H. Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. *European Journal of Neuroscience.* 2003; 18:1584–1592. [PubMed: 14511337]
39. Yang PB, Swann AC, Dafny N. Chronic pretreatment with methylphenidate induces cross-sensitization with amphetamine. *Life Sci.* 2003; 73:2899–2911. [PubMed: 14511774]
40. Sproson EJ, Chantrey J, Hollis C, Marsden CA, Fonel KC. Effect of repeated methylphenidate administration on presynaptic dopamine and behaviour in young adult rats. *J Psychopharmacol.* 2001; 15:67–75. [PubMed: 11448090]
41. Gray JD, Punsoni M, Tabori NE, Melton JT, Fanslow V, Ward MJ, et al. Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *J Neurosci.* 2007; 27:7196–7207. [PubMed: 17611273]
42. Jezierski G, Zehle S, Bock J, Braun K, Gruss M. Early stress and chronic methylphenidate cross-sensitize dopaminergic responses in the adolescent medial prefrontal cortex and nucleus accumbens. *J Neurochem.* 2007; 103:2234–2244. [PubMed: 17924950]
43. Torres-Reveron A, Gray JD, Melton JT, Punsoni M, Tabori NE, Ward MJ, et al. Early postnatal exposure to methylphenidate alters stress reactivity and increases hippocampal ectopic granule cells in adult rats. *Brain Res Bull.* 2009; 78:175–181. [PubMed: 19100815]
44. Adriani W, Leo D, Greco D, Rea M, di Porzio U, Laviola G, et al. Methylphenidate administration to adolescent rats determines plastic changes on reward-related behavior and striatal gene expression. *Neuropsychopharmacology.* 2006; 31:1946–1956. [PubMed: 16319916]
45. Scaini G, Fagundes AO, Rezin GT, Gomes KM, Zugno AI, Quevedo J, et al. Methylphenidate increases creatine kinase activity in the brain of young and adult rats. *Life Sci.* 2008; 83:795–800. [PubMed: 18938183]
46. Andersen SL, Napierata L, Brenhouse HC, Sonntag KC. Juvenile methylphenidate modulates reward-related behaviors and cerebral blood flow by decreasing cortical D3 receptors. *Eur J Neurosci.* 2008; 27:2962–2972. [PubMed: 18588536]
47. Martins MR, Reinke A, Petronilho FC, Gomes KM, Dal-Pizzol F, Quevedo J. Methylphenidate treatment induces oxidative stress in young rat brain. *Brain Res.* 2006; 1078:189–197. [PubMed: 16494852]
48. Vendruscolo LF, Izidio GS, Takahashi RN, Ramos A. Chronic methylphenidate treatment during adolescence increases anxiety-related behaviors and ethanol drinking in adult spontaneously hypertensive rats. *Behav Pharmacol.* 2008; 19:21–27. [PubMed: 18195591]
49. Lee MJ, Yang PB, Wilcox VT, Burau KD, Swann AC, Dafny N. Does repetitive Ritalin injection produce long-term effects on SD female adolescent rats? *Neuropharmacology.* 2009; 57:201–207. [PubMed: 19540860]

50. Andrews GD, Lavin A. Methylphenidate increases cortical excitability via activation of alpha-2 noradrenergic receptors. *Neuropsychopharmacology*. 2006; 31:594–601. [PubMed: 15999146]
51. Andersen SL. Changes in the second messenger cyclic AMP during development may underlie motoric symptoms in attention deficit/hyperactivity disorder (ADHD). *Behav Brain Res*. 2002; 130:197–201. [PubMed: 11864735]
52. Devilbiss DM, Berridge CW. Cognition-enhancing doses of methylphenidate preferentially increase prefrontal cortex neuronal responsiveness. *Biological psychiatry*. 2008; 64:626–635. [PubMed: 18585681]
53. Waterhouse BD, Agster KL, Calrk BD. The effects of methylphenidate on sensory signal processing in the rodent lateral geniculate nucleus. *Society for Neuroscience Abstract:Program No.* 161.165. 2008
54. Casey BJ, Nigg JT, Durston S. New potential leads in the biology and treatment of attention deficit-hyperactivity disorder. *Curr Opin Neurol*. 2007; 20:119–124. [PubMed: 17351480]
55. Arnsten AF. Catecholamine and second messenger influences on prefrontal cortical networks of “representational knowledge”: a rational bridge between genetics and the symptoms of mental illness. *Cereb Cortex*. 2007; 17(1):i6–15. [PubMed: 17434919]
56. Wu J, Hablitz JJ. Cooperative Activation of D1 and D2 Dopamine Receptors Enhances a Hyperpolarization-Activated Inward Current in Layer I Interneurons. *J Neurosci*. 2005; 25:6322–6328. [PubMed: 16000622]
57. Rosenkranz JA, Johnston D. Dopaminergic regulation of neuronal excitability through modulation of Ih in layer V entorhinal cortex. *J Neurosci*. 2006; 26:3229–3244. [PubMed: 16554474]
58. Surges R, Brewster AL, Bender RA, Beck H, Feuerstein TJ, Baram TZ. Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. *Eur J Neurosci*. 2006; 24:94–104. [PubMed: 16882011]
59. Vasilyev DV, B ME. Postnatal development of the hyperpolarization-activated excitatory current Ih in mouse hippocampal pyramidal neurons. *The Journal of Neurosciences*. 2002; 22:8992–9004.
60. Ding L, Perkel DJ. Dopamine modulates excitability of spiny neurons in the avian basal ganglia. *J Neurosci*. 2002; 22:5210–5218. [PubMed: 12077216]
61. Rothmond DA, Weickert CS, Webster MJ. Developmental changes in human dopamine neurotransmission: cortical receptors and terminators. *BMC Neurosci*. 2012; 13:18. [PubMed: 22336227]
62. Surmeier DJ, Ding J, Day M, Wang Z, Shen W. D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci*. 2007; 30:228–235. [PubMed: 17408758]
63. Volkow ND, Insel TR. What are the long-term effects of methylphenidate treatment? *Biological psychiatry*. 2003; 54:1307–1309. [PubMed: 14675792]
64. LeBlanc-Duchin D, Taukulis HK. Chronic oral methylphenidate administration to periadolescent rats yields prolonged impairment of memory for objects. *Neurobiol Learn Mem*. 2007; 88:312–320. [PubMed: 17544718]
65. LeBlanc-Duchin D, Taukulis HK. Chronic oral methylphenidate induces post-treatment impairment in recognition and spatial memory in adult rats. *Neurobiol Learn Mem*. 2009; 91:218–225. [PubMed: 19116174]

Figure 1.

Age-dependent effects of 1 mg/kg MPH on the neuronal excitability in layer 5 pyramidal neurons in rat PFC. A, Excitability of juvenile rat PFC layer 5 pyramidal neurons is suppressed by MPH treatment. Treatment with 1 mg/kg i.p. MPH significantly reduces the excitability (spike numbers) of layer 5 pyramidal neurons in juvenile rat PFC. As shown in these representative sweeps, fewer action potentials were observed in single-dose-treated rats than control animals and even fewer in chronic-treated animals. Right panel, summary histogram shows the significant decrease of spike numbers in single-dose ($n = 11$, $p = 0.019$) compared with single-dose saline control ($n = 18$), and even fewer in chronic MPH-treated rats ($n = 18$, $p = 4.58 \times 10^{-5}$) compared to a chronic saline control ($n = 12$). B, Neurons from adult rats (P90-100) exhibited an increase in excitability following 1 mg/kg MPH treatment ($n = 6$; $p = 0.0006$) when compared to saline control ($n = 6$). C, MPH's depressant effect on spike numbers is independent of animal ages. The spike numbers at saline-treated PD40 and PD95 rats decreased and the spike numbers have a negative correlation with animal age ($R^2 = 0.256$, $p = 0.0023$).

Figure 2.

Generation of an I-V curve plot and examination of passive membrane properties reveals a likely effect of MPH treatment on potassium channel function. A, Resting membrane potential was not affected by single-dose ($n = 11$) or chronic ($n = 18$) MPH treatment ($p > 0.05$). B, Chronically-treated MPH rats ($n = 18$) had significantly lower R_{in} than age-matched, chronically-treated saline controls ($n = 12$; $p = 0.006$). C, Examples of action potential recordings in current clamp mode showing how the I-V curves in D were determined. The arrows indicate the R_{in} change, whereas the dashed line denotes the measurements of voltage differences in response to current injection (at 50 pA increment). D, the neurons were less responsive in chronic treated rats compared with control and other groups ($* p < 0.05$). The I-V curve of following chronic treatment was significantly shifted, suggesting the involvement of K^+ channels, particularly hyperpolarization activated potassium current (I_h).

Figure 3.

Examination of the cAMP-HCN channel-mediated I_h current reveals an increase in I_h current amplitude concurrent with decreased neuronal excitability following 1 mg/kg 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 I_h currents that are exhibited in C. C, I/V curve reveals significant increase of I_h current in MPH-treated neurons from -70 mV to -120 mV injected voltage (treatment by sweep interaction, $p = 0.0003$, $f = 5.3$, $df = 5, 75$; treatment effect, $p = 0.0182$, $f = 7$, $df = 1, 15$; injected voltage effect, $p < 0.0001$, $f = 27.4$, $df = 5, 75$).

Figure 4.

Examination of lower doses of the purportedly therapeutic range revealed similar depressant effects on juvenile PFC pyramidal neurons except for the very low dose of 0.01 mg/kg ($n = 7$ per group). Excitability of neurons from rats treated with 0.03 mg/kg to 0.3 mg/kg single-dose MPH revealed a trend towards a decrease ($R^2 = 0.668$), although not significant at some points when compared to control. However, two-way ANOVA revealed significant difference across groups when 1 mg/kg was included ($p = 0.0025$, $f = 4.258$).

Figure 5.

Effects of MPH treatment on spontaneous excitatory postsynaptic current (sEPSC). A, Examples of spontaneous AMPA receptor-mediated sEPSCs recorded at -70 mV. A single dose of 1 mg/kg MPH ($n = 11$) reduces sEPSC frequency but not amplitude compared to single-dose saline control ($n = 18$), and a chronic MPH regimen ($n = 18$) reduces both frequency and amplitude of sEPSCs compared to chronic-saline control ($n=12$). B, Summary histograms show that sEPSC frequency was significantly reduced by both a single dose ($p = 0.044$) and a chronic regimen ($p = 5.03 \times 10^{-6}$) of 1mg/kg MPH. Frequency of sEPSCs following single vs. chronic dose regimens were not significantly different from each other ($p = 0.331$), but both were significantly reduced compared to saline control frequency ($p = 0.02$, $f = 4.186$, B). C, The amplitude of sEPSCs was only affected by MPH following chronic treatments when compared to a chronic saline control group ($p = 2.25 \times 10^{-5}$, $f = 5.129$). Note the amplitude of EPSCs is significantly larger in chronic saline than single-dose saline ($p = 0.0112$). This is reflective of developmental changes, potentially synaptic strengthening; therefore, neurons can only be compared with age-matched controls. * $p < 0.05$; ** $p < 0.01$.

Figure 6.

Excitability of neurons from animals treated chronically with 1 mg/kg MPH return to saline control level one week after termination of treatment, however excitability of neurons treated with 3 or 9 mg/kg MPH does not recover to saline control levels even 5 or 10 weeks after treatment ended. A. Neurons tested 1 week after termination of chronic treatment. Treatment with 1 mg/kg MPH resulted in recovery back to saline control levels within 1 week ($p = 0.997$, A). However, excitability in neurons from animals treated with 3 mg/kg ($p = 0.010$, B) or 9 mg/kg MPH ($p = 0.021$, C) was still significantly reduced after 1 week. After 5 weeks, excitability of neurons from animals treated with 1 mg/kg was still at saline control levels, with no rebound or overshoot ($p = 0.891$, D). At this time point, neurons from animals treated with 3 or 9 mg/kg MPH still exhibited significantly reduced excitability ($p = 0.005$, E, and $p = 0.0049$, F, respectively). Even after 10 weeks, the reduction of spike

numbers in neurons from animals treated with 3 and 9 mg/kg had not recovered ($p = 0.0082$, G; $p = 0.028$, H). Note: Numbers in the histogram represent the cell numbers in each group, which were also applied to Figure 7.

Figure 7.

Synaptic transmission of neurons was also recorded from recovery groups, and recovered from 1 mg/kg chronic MPH treatment, but not from 3 and 9 mg/kg. One week after termination of treatment, neurons from 1 mg/kg MPH-treated animals had recovered to saline control levels (A), but those from 3 mg/kg ($p = 0.007$, B) and 9 mg/kg- treated animals ($p = 0.001$, C) exhibited significantly reduced sEPSC frequency. Five weeks after treatment ceased, neurons from 1 mg/kg-treated animals had recovered (D); however, those from 3 mg/kg- (E) and 9 mg/kg-treated animals still exhibited significantly reduced sEPSC frequency ($p = 0.005$, E; $p = 0.006$, F). Similarly, even after 10 weeks, the reduction

of spike numbers in neurons from animals treated with 3 and 9 mg/kg had not recovered ($p = 0.040$, G; $p=0.0345$, H).