Brief Communication

Methylphenidate amplifies long-term plasticity in the hippocampus via noradrenergic mechanisms

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Methylphenidate treatment is used for Attention Deficit Hyperactivity Disorder and can improve learning and memory. Previously, improvements were considered a by-product of increased attention; however, we hypothesize that methylphenidate directly alters mechanisms underlying learning and memory, and therefore examined its effects on hippocampal long-term potentiation and long-term depression. Methylphenidate enhanced both mechanisms in the absence of presynaptic changes and in a noradrenalin β -receptor-dependent manner. These findings can explain both the improved learning and memory and decreased learning selectivity found with methylphenidate treatment and constitute the first demonstration of direct actions of methylphenidate on mechanisms implicated in cognition.

Attention Deficit Hyperactivity Disorder (ADHD) affects 8%–12% of children (Biederman and Faraone 2005) and is characterized by inattention, hyperactivity, and impulsivity. Consequences of the disorder include poorer academic performance, employment records, social relationships, and a higher risk of drug abuse (Doggett 2004). While behavioral, cognitive, and psychosocial therapies exist for ADHD, they are often ineffective unless combined with pharmacological treatment (Brown et al. 2005), the most common of which is the psychostimulant, methylphenidate (Ritalin, Concerta). Methylphenidate is known to increase extracellular dopamine and noradrenalin (Kuczenski and Segal 1997, 2002), but not serotonin (Kuczenski and Segal 1997) suggesting that therapeutic efficacy relates to alterations in dopamine and noradrenalin. Despite knowledge of these neurochemical effects, the mechanisms contributing to the effectiveness of methylphenidate are unclear (Safer and Allen 1989; National Institutes of Health Consensus Development Conference Statement 2000; Greenhill 2001).

One clue to mechanisms of efficacy can be found at a behavioral level, where methylphenidate improves academic performance (Yang et al. 2004; McGough et al. 2006), working (Wright and White 2003) and visual (Rhodes et al. 2004) memory, nonverbal (O'Toole et al. 1997) and visuospatial (Bedard et al. 2004) learning, and reading skills (Keulers et al. 2007), which may explain why, in addition to its medicinal use, it is used illegally by healthy students to aid study (Teter et al. 2006). Despite evidence of methylphenidate-induced changes in learning and memory, the mechanisms underlying these changes have received little focus and are commonly assumed to be a by-product of improved attention. However, we suggest that these findings are the result of direct actions of methylphenidate on learning and memory mechanisms.

In support of this hypothesis, methylphenidate has been shown to increase hippocampal noradrenalin *in vivo* (Kuczenski and Segal 2002), and such changes are known to impact on plasticity such as long-term potentiation (LTP) and long-term depression (LTD) (Hopkins and Johnston 1984; Izumi et al. 1992; Sah and Bekkers 1996; Thomas et al. 1996; Izumi and Zorumski 1999; Schimanski et al. 2007; Lin et al. 2008), both of which are implicated in learning and memory (Kemp and Manahan-Vaughan

2007). In order to test whether methylphenidate directly impacts learning and memory mechanisms, we investigated the effects of therapeutically relevant doses of methylphenidate on LTP and LTD in the hippocampus *in vitro*.

In order to ensure therapeutic relevance, we used rats aged between P22 and P35. This age is considered to be preadolescent (Brown 2005), and therefore corresponds to the child population commonly prescribed methylphenidate, and has been used in a number of studies on methylphenidate in the past (Kuczenski and Segal 2002, 2005; Carlezon et al. 2003). Horizontal hippocampal slices (400 μ m) were prepared from male Wistar rats ($n = 130$) killed by decapitation under isoflurane anesthesia. The brain was removed and sectioned in ice-cold oxygenated artificial CSF (aCSF) containing (in millimolars): 124 NaCl, 3.7 KCl, 26 NaHCO₃, 2.4 CaCl₂, 1.3 MgSO₄, 1.3 KH₂PO₄, and 10 C₆H₁₂O₆ saturated with 95% O₂/5% CO₂. After sectioning, slices were given 1 h to equilibrate at room temperature before being transferred to a heated submersion recording chamber 30 min before recording commenced, where they were maintained within 0.5°C of their starting temperature for the duration of the experiment (starting temperature 30.5°C–31.5°C) with oxygenated aCSF flowing at a rate of 2.0 mL/minute. Field potential recordings were made from the striatum radiatum in the CA1 region of the hippocampus using glass microelectrodes (3–10 M Ω). Field potentials were evoked by electrical stimulation of the CA1 Schaffer collaterals with concentric bipolar stimulating electrodes (FHC). Stimulus response curves were completed at the start of every experiment, and acceptable field potential amplitudes exceeded 1 mV at the maximum stimulation intensity. The intensity that elicited the half maximal response was used for the remainder of the experiment. Response size was measured as the slope of the response from the point after which the fiber volley finished to the trough. For paired-pulse facilitation (PPF) five interpulse intervals were tested: 25, 50, 100, 200, and 500 ms, and paired-pulse ratios were calculated from averages of 10 stimulation presentations. For LTP and LTD experiments, a stable baseline response to 0.03 Hz stimulation, which is an interstimulus interval of 30 s, was obtained before the conditioning stimuli were applied. To measure LTP and LTD response size, the slope of the field potential was measured 30 min after the tetanus and normalized to baseline. The lowest dose of methylphenidate used corresponds to the blood plasma level of the drug in children receiving treatment for ADHD (10 ng/mL). Methylphenidate is known to reach higher concentrations in the brain than in the

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blood (Hoffman and Lefkowitz 1996), and therefore this dose was taken to be the minimum therapeutically relevant concentration, and two higher doses were also tested (20 and 50 ng/mL). Whenever a drug was applied, an additional 30 min was given for equilibration, and all drugs, once added, were used for the duration of the experiment.

In order to fully characterize the effects of methylphenidate, we examined its effect on baseline responsiveness using a stimulus-response curve and PPF. We found no significant effect of methylphenidate on the stimulus-response curve ($F_{(0.957)} df = 3, 186, P > 0.05$; control $n = 41$, 10 ng/mL $n = 37$, 20 ng/mL $n = 40$, 50 ng/mL $n = 48$), indicating that it does not effect baseline responsiveness in the hippocampus. Wilcoxon tests revealed that in the absence of methylphenidate ($n = 9$), significant PPF was found with interstimulus intervals (ISI) of 25 ms ($P < 0.01$), 50 ms ($P < 0.01$), 100 ms ($P < 0.01$), and 200 ms ($P < 0.05$), but not 500 ms ($P > 0.05$). This pattern of facilitation was maintained with methylphenidate (10 ng/mL $n = 9$, 20 ng/mL $n = 9$, 50 ng/mL $n = 9$) as shown in Figure 1. A two-factor ANOVA (repeated-measures factor = ISI, between-measures factor = dose) was used to compare the paired-pulse ratio at the different doses and revealed a significant main effect of ISI ($F_{(35.33)} df = [4, 128], P < 0.001$) but not dose ($F_{(1.29)} df = [3, 32], P > 0.05$). There was also a significant TIME $\times$ DOSE interaction ($F_{(1.91)} df = [12, 128], P < 0.05$), and Figure 1 shows that the paired-pulse ratio tended to be larger for the drug conditions at shorter ISIs.

In the absence of methylphenidate, LTP was induced by four 1-s trains of pulses at 100 Hz (HFS), with an intertrain interval of 15 s, and resulted in a response of 1.39 ± 0.02 ($n = 6$) times the baseline. There was a dose-dependent increase in LTP with methylphenidate as shown in Figure 2A–C: 10 ng/mL = 1.62 ± 0.12 ($n = 7$); 20 ng/mL = 1.77 ± 0.08 ($n = 6$); 50 ng/mL = 2.13 ± 0.21 ($n = 6$), and Kruskal-Wallis analysis revealed a significant effect of dose ($\chi^2 = 11.37, df = 3, P = 0.01$) on LTP magnitude. Individual Mann-Whitney U-tests showed that in the presence of the lowest dose, 10 ng/mL, LTP was not significantly greater than control ($P > 0.05$), while application of both the 20 and 50 ng/mL resulted in significantly greater potentiation relative to control ($P < 0.005$).

Application of the noradrenergic β -receptor antagonist Timolol (10 μ M) alone had no significant effect on LTP ($t = 0.25, df = 7, P > 0.05; n = 6$). This is in line with previous studies showing that β -receptor antagonists have no effect of LTP in the CA1

region of the hippocampus (Dunwiddie et al. 1982; Swanson-Park et al. 1999), although most studies do find an alteration in other hippocampal regions (Lacaille and Harley 1985; Hopkins and Johnston 1988; Ishihara et al. 1991; Huang and Kandel 1996; Munro et al. 2001). When we applied Timolol in combination with 50 ng/mL methylphenidate ($n = 6$), we found that the β -receptor antagonist could abolish the methylphenidate-induced enhancement ($1.44 \pm 0.16; t = 0.28, df = 5, P > 0.05$ compared with control; $t = 2.57, df = 9, P < 0.05$ compared with methylphenidate alone, see Figure 2D–F), suggesting that the effect of methylphenidate is mediated by action at noradrenalin β -receptors.

When the HFS was repeated 30 min after the initial presentation, no further potentiation could be induced in the presence ($t = 0.75, df = 5, P > 0.05; n = 6$) or absence of methylphenidate ($t = 0.50, df = 5, P > 0.05; n = 6$), suggesting that this stimulus was saturating in both conditions. In order to determine whether methylphenidate altered LTP in the presence of a weaker stimulus, we tested the effects of methylphenidate on responses to 15 pulses at 100 Hz, which would ordinarily be ineffective at inducing LTP. In control conditions this resulted in a mean response of 1.09 ± 0.02 ($n = 5$), that is, within baseline levels. However, in the presence of methylphenidate, a significantly greater enhancement was found ($1.22 \pm 0.05; t = 2.46, df = 6, P < 0.05, n = 5$) suggesting that methylphenidate may reduce the threshold for LTP induction.

Figure 3A shows the normalized magnitude of LTD induced by 900 paired-pulse stimuli (interpulse interval 50 ms) at 1 Hz was 0.95 ± 0.05 ($n = 6$) of the baseline in control conditions, with the majority of records returning to baseline within 30 min. Our failure to induce LTD was initially thought to be due to a general down-regulation of LTD with age often inferred from LTD being less inducible with standard low-frequency stimulation in adults compared with young animals (Bashir and Collingridge 1994; Errington et al. 1995; Heynen et al. 1996; Kemp and Bashir 1999). However, the stimulation paradigm used in the current study uses paired-pulse low-frequency stimulation, which has been shown to be effective in similar age rats to those used here (Kemp et al. 2000), resulting in a depression of $\sim 30\%$. With the exception that the previous work with this age rat used females and the current study used males, we can find no explanation for the discrepancy in the results.

As with LTP, LTD was enhanced with methylphenidate: 10 ng/mL = 0.78 ± 0.07 ($n = 6$); 20 ng/mL = 0.81 ± 0.05 ($n = 7$); 50 ng/mL = 0.75 ± 0.06 ($n = 6$). A One-way ANOVA revealed a significant effect of dose ($F_{(3.21)} df = 3, 21, P < 0.05$) on magnitude, and t -tests corrected for multiple comparisons and effect size (Holm 1979) show a significant increase in LTD for all doses (10 ng/mL: $t = 2.23, df = 10, P < 0.05$; 20 ng/mL: $t = 2.70, df = 11, P < 0.025$; 50 ng/mL: $t = 3.05, df = 10, P < 0.0167$). In line with the LTP, there was no significant effect of Timolol on LTD when applied alone ($n = 6; t = 1.08, df = 10, P > 0.05$), but when Timolol was applied prior to and during application of 50 ng/mL methylphenidate ($n = 6$), it significantly reduced the methylphenidate-induced enhancement ($t = 3.74, df = 7, P < 0.01$) to within control levels ($t = 1.08, df = 10, P > 0.05$).

We have characterized the effects of acute application of methylphenidate on hippocampal LTP and LTD in vitro, demonstrating that methylphenidate

Figure 1. Paired-pulse ratios in the presence and absence of methylphenidate. Significant paired-pulse facilitation is shown in the absence of methylphenidate at all interstimulus intervals except 500 ms. Methylphenidate (10 ng/mL, 20 ng/mL, and 50 mg/mL) does not significantly alter this pattern of responses. Paired-pulse ratio is calculated by dividing the slope of the response to the second pulse by the response to the first pulse.

Figure 2. (A) Methylphenidate (MPH) significantly enhances long-term potentiation in the CA1 region of the hippocampus in response to HFS (indicated by arrowhead). (B) Typical traces from each condition at 30 min post-tetanus (gray) compared with baseline (black). Horizontal scale bar, 10 ms; vertical scale bars, 0.5 mV. (C) Level of potentiation 30 min after the tetanus was applied. There is a dose-dependent increase in potentiation that only reaches significance for the 20 and 50 ng/mL doses. (D) Timolol reverses the effects of methylphenidate application on LTP in the CA1 region. Arrowhead indicates time at which HFS was applied. (E) Typical traces from each condition at 30 min post-tetanus (gray) compared with baseline (black). Control and 50 ng/mL methylphenidate are replicated from B to allow comparison with Timolol and Timolol combined with methylphenidate. (F) Level of potentiation 30 min after the tetanus was applied. Timolol alone has no effect on LTP; however, when applied 30 min prior to 50 ng/mL methylphenidate, it reverses the effect of methylphenidate, returning LTP to within baseline levels. In methylphenidate and methylphenidate + Timolol conditions, the drugs were applied at least 30 min prior to the start of the baseline period and remained present throughout recording.

enhances both mechanisms without significantly affecting PPF. Given that PPF represents a form of short-term plasticity (Creager et al. 1980; Zucker 1989), which is, at least in part, mediated presynaptically as demonstrated by increases in quantal neurotransmitter content or number of quanta released and increased presynaptic calcium (Hess et al. 1987; Foster and McNaughton 1991), the lack of effect on PPF suggests that the drug acts postsynaptically. Enhancements in LTP and LTD were blocked by a selective noradrenergic β -receptor antagonist, indicating that the effects were mediated by noradrenalin acting at β -receptors. These findings are of interest at both a cellular level, where LTP and LTD represent long-term changes in the brain, and at a behavioral level, where they are implicated in learning and memory.

Considering first the cellular level, we suggest that methylphenidate increases noradrenalin levels and in turn alters plasticity. Our finding that the effects of methylphenidate are mediated by noradrenalin is in line with previous work; Kuczenski and Segal (1997, 2002) found that therapeutically relevant doses of methylphenidate increased hippocampal noradrenalin in vivo. In addition to this, noradrenalin is known to be a strong modulator of hippocampal activity; the hippocampus receives adrenergic innervation from the locus coeruleus (Moore and Bloom 1979; Loy et al. 1980) and activation of adrenergic receptors in this region alters excitability of CA1 pyramidal cells, with increases in excitability being attributed to activity at β -receptors, while decreases in excitability are thought to be mediated by α -receptor activity (Mueller et al. 1982; Madison and Nicoll 1988;

Gereau and Conn 1994). It might be expected that if methylphenidate were causing activation of adrenergic receptors, we should see alterations in the baseline responsiveness, which is not the case. However, this may be due to the level of receptor activation; for example, direct application of noradrenalin (10 μ M) has been shown to cause a depression in field potential activity in CA1 (Katsuki et al. 1997), but it is likely that activation of noradrenalin receptors following application of a low dose of methylphenidate is considerably lower than following 10 μ M noradrenalin application, meaning a depression would not necessarily be evident.

As well as changes in baseline excitability in the hippocampus, noradrenalin plays a significant role in hippocampal plasticity. For example, various studies have shown that application of noradrenalin or β -receptor agonists can induce a long-lasting potentiation in the absence of any conditioning stimuli (Dahl and Sarvey 1989; Heginbotham and Dunwiddie 1991; Dahl and Jingmin 1994; Pelletier et al. 1994). In addition, and perhaps more comparable to the current data, are the effects of noradrenalin and its agonist on plasticity induced by electrical stimulation. To date, a number of studies have shown that noradrenalin application increases amplitude of LTP (Hopkins and Johnston 1984; Izumi and Zorumski 1999), enables induction of LTP with lower frequency stimulation (Sah and Bekkers 1996; Thomas et al. 1996), reverses NMDA-mediated inhibition of LTP (Izumi et al. 1992), rescues impaired LTP in transgenic animals (Schimanski et al. 2007), and inhibits associative LTD (Lin et al. 2008). Our results demonstrated that methylphenidate produced a signifi-

Figure 3. (A) Methylphenidate (MPH) significantly enhances long-term depression in the CA1 region of the hippocampus in response to LFS (indicated by arrowhead). (B) Typical traces from each condition at 30 min post-tetanus (gray) compared with baseline (black). Horizontal scale bar, 10 ms; vertical scale bars, 0.5 mV. (C) Level of potentiation 30 min after the tetanus was applied. In the absence of methylphenidate, there is not significant long-term depression. However, LTD becomes evident in the presence of all three doses of methylphenidate. (D) Timolol reverses the effects of methylphenidate application on LTD in the CA1 region. Arrowhead indicates time at which LFS was applied. (E) Typical traces from each condition at 30 min post-tetanus (gray) compared with baseline (black). Control and 50 ng/mL methylphenidate are replicated from *B* to allow comparison with Timolol and Timolol combined with methylphenidate (MPH). (F) Level of depression 30 min after the tetanus was applied. Timolol alone has no effect on LTD; however, when applied 30 min prior to 50 ng/mL methylphenidate, it reverses the effect of methylphenidate, abolishing LTD. In methylphenidate and methylphenidate + Timolol conditions, the drugs were applied at least 30 min prior to the start of the baseline period and remained present throughout recording.

cant enhancement of LTP amplitude, which is in line with this previous data, suggesting that activation of adrenergic receptors can enhance LTP. Our finding that this effect is mediated by β -receptors is also in keeping with previous results (Sah and Bekkers 1996; Thomas et al. 1996; Katsuki et al. 1997; Schimanski et al. 2007), suggesting the importance of this receptor subtype in plasticity. In addition to enhanced LTP, we showed that when methylphenidate is present, a weak, normally ineffective tetanus can induce LTP. This adds support to the work by Thomas et al. (1996) and Sah and Bekkers (1996), the latter of which suggests that this effect is elicited via activation of the noradrenergic system, which results in blockade of afterhyperpolarization.

While we were unable to demonstrate LTD, we were able to demonstrate a dose-dependent increase in LTD with methylphenidate application, which was also mediated by β -receptor activation. The effects of noradrenalin on hippocampal LTD have received less attention than LTP (Dahl and Sarvey 1989; Katsuki et al. 1997; Scheiderer et al. 2003). Dahl and Sarvey (1989) demonstrated that noradrenalin applied to the dentate gyrus could result in long-lasting depression or potentiation in a pathway-specific manner that could be abolished by β -receptor blockade, while Scheiderer et al. (2003) found noradrenalin induced long lasting depression in CA1 in a manner mediated by α -receptors. Perhaps more comparable to the present study, which induced LTD with low-frequency stimulation, Katsuki et al. (1997) found noradrenalin suppressed LTD in the CA1 region. Our data found that methylphenidate, via activity at the β -receptor, was able to

enhance LTD rather than suppress it, which is at odds with the work of Katsuki et al. (1997); but differences in drug concentrations may underlie these discrepancies.

At a behavioral level, where LTP and LTD are implicated in learning and memory, our results are consistent with previous work examining the role of noradrenalin in memory. A recent review by Ramos and Arnsten (2007) stated that noradrenalin increases the signal/noise ratio and enhances long-term memory consolidation in the hippocampus. As well as consolidation, hippocampal noradrenalin is implicated in memory acquisition (Mason and Iverson 1977; Mason and Fibiger 1978) and retrieval (Murchison et al. 2004), indicating that it is fundamental to learning and memory processes in the hippocampus. Interestingly, and very relevant to ADHD treatment, the effects of noradrenalin on retrieval have been found to be independent of attention (Murchison et al. 2004), providing further evidence against the widely held belief that improvements in learning and memory with methylphenidate are due to increased attention.

Our demonstration that a weak stimulus, which ordinarily would not induce LTP, became effective in the presence of methylphenidate, suggests a decrease in stimulus selectivity. ADHD has been characterized as a widening of the attentional window (Shalev and Tsai 2003); therefore, it would be predicted that treatments should increase selectivity rather than decrease it. However, our findings are supported by Horsley and Cassaday (2007), who demonstrated an increase in conditioning to weaker stimuli following acute methylphenidate treatment. They argued

that while this is normally considered detrimental, ADHD sufferers show abnormal conditioning, and decreasing selectivity may represent an improvement. The same may not be true of the healthy methylphenidate users, making it a very real possibility that incorrect plasticity may occur in this group.

While our work uses acutely applied methylphenidate, the mechanisms demonstrated may also underlie some of the effects of chronic use. Indeed, recall that long-term methylphenidate treatment has been shown to improve working (Wright and White 2003) and visual memory (Rhodes et al. 2004), nonverbal (O'Toole et al. 1997) and visuospatial learning (Bedard et al. 2004), and reading skills (Keulers et al. 2007). It has also been found to attenuate memory deficits in Alzheimer's disease (Kittur and Hauser 1999) and improve learning in children left with learning deficits following cancer (Thompson et al. 2001). More firmly correlated to the hippocampal activity investigated in the current study, methylphenidate can increase visual-spatial memory (Bedard et al. 2004) and Morris water-maze performance (Kline et al. 2000). While significant improvements in learning and memory are not always found (Rie et al. 1976; Swanson et al. 1991), a recent review (Pietrzak et al. 2006) suggested that improvements in short- and long-term memory are seen in 58% of studies following methylphenidate treatment, and Swanson et al. (1991) suggest that improvements are not seen in all cases, as doses are calibrated according to behavior and not cognition. Our results are also in line with the recent study showing that atomoxetine, a selective noradrenalin reuptake inhibitor, also used for treating ADHD, can enhance performance on a task aimed at modeling plasticity (Foster et al. 2006).

Although this is the first demonstration that methylphenidate can alter LTP and LTD, there is evidence for methylphenidate affecting other types of plasticity. Recent work has shown that chronic methylphenidate treatment alters dendritic and synaptic development in the anterior cingulate cortex (Zehle et al. 2007), decreases adult neurogenesis (Lagace et al. 2006), and alters a number of factors associated with plasticity including gene expression (Yano et al. 2006) and nitric oxide levels (Itzak and Ali 2006). While the effects on LTP and LTD demonstrated here may seem advantageous, enhanced plasticity and decreased selectivity could be detrimental and result in inappropriate changes in plasticity.

While these experiments were carried out using neonatal rat brains, it is important to understand that the effects seen are highly unlikely to have resulted from changes in neuronal excitability due to alterations in NMDA and GABA receptors during development. It has long been accepted that there are developmental changes in these transmitter systems that extend beyond birth (Mueller et al. 1984; Ben Ari et al. 1989, 1997; Fiszman et al. 1990; McDonald and Johnston 1990), which result in changes in neuronal excitability. GABA is known to have depolarizing effects in the neonatal hippocampus due to high intracellular Cl^- levels (Miles 1999; Herlenius and Lagercrantz 2004). However, evidence from a number of different groups has established that by the end of the first postnatal week, GABA has taken up its role as the dominant inhibitory transmitter in the brain, and there are no changes in GABA_A receptors or the neuron-specific co-transporter of chloride ions (Luhmann and Prince 1991; Gaiarsa et al. 1995; Miles 1999; Rivera et al. 1999; Herlenius and Lagercrantz 2004) during the age range used in the present study. In addition to this, while the NMDA receptors are yet to function as they do in the adult rat at the age used, between P22 and P35 there are no changes in levels of expression of the receptor (Herlenius and Lagercrantz 2004). Finally, we ensured that the age range used in the control condition (P22–P34) and methylphenidate conditions were comparable (P22–P35), such that should any developmental changes occur, this would be true in both

conditions, and as such, there is no reason to believe that the effects seen in the current study were due to receptor and, therefore, excitability changes.

In conclusion, we have demonstrated that acute application of methylphenidate enhances hippocampal LTP and LTD, thus providing direct evidence for methylphenidate altering the mechanisms underlying learning and memory. The fact that improvements in learning and memory are thought to be a result of increased attention makes these findings even more important, as we show they may result from direct action of the drug. We suggest that these noradrenergic-mediated changes are important in the efficacy of ADHD treatment. While the current study investigated acute effects, which may differ from those of chronic treatment for ADHD, we suggest that the mechanisms demonstrated here are relevant to chronic treatment, which can result in improved learning and memory.

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