Dopamine transporter genotype influences the physiological response to medication in ADHD

Donald L. Gilbert,1 Zhewu Wang,3,4 Floyd R. Sallee,4 Keith R. Ridel,1 Stephanie Merhar,2 Jie Zhang,1 Tara D. Lipps,1 Colin White,3,4 Nevert Badreldin3,4 and Eric M. Wassermann5

1Division of Neurology, 2Division of Pediatrics, Cincinnati Children’s Hospital Medical Center, 3Mental Health Care Line, Veterans Administration Medical Center, 4Department of Psychiatry, University of Cincinnati, Cincinnati, OH and 5Brain Stimulation Unit, National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

Correspondence to: Donald L. Gilbert, MD, MS, Cincinnati Children’s Hospital Medical Center, Division of Neurology, ML #2015, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, USA
E-mail: d.gilbert@cchmc.org

Attention deficit hyperactivity disorder (ADHD) is a complex, multifactorial disorder characterized by physical hyperactivity and behavioural disinhibition. Short interval cortical inhibition (SICI), measured in motor cortex with transcranial magnetic stimulation, is reduced in ADHD and correlates with symptom severity. However, ADHD medication-induced changes in SICI vary widely among normal individuals and have not been well studied in children with ADHD. Therefore, we undertook this study to measure and compare effects of two ADHD medications, methylphenidate (MPH), a psychostimulant, and atomoxetine (ATX), a selective norepinephrine reuptake inhibitor, on SICI in children with ADHD. In addition, we wished to determine whether a genetic variation in the dopamine transporter (DAT1), a site of action of MPH, could influence the effects of MPH or ATX on SICI. We performed a randomized, double-blind, single-dose, crossover study comparing 0.5 mg/kg MPH with 1.0 mg/kg ATX in 16 children with ADHD, aged 8-17. Seven were homozygotes and 9 heterozygotes for the DAT1 variable number of tandem repeats 10-repeat allele. Medication and genotype effects on SICI were estimated with repeated measures, mixed model regression. We found that MPH and ATX had similar effects on SICI. However, medication effects differed significantly by DAT1 genotype \[F(2,13) = 13.04, P = 0.0008\]. Both MPH and ATX increased SICI in heterozygotes but not in 10-repeat homozygotes. In conclusion, MPH and ATX have similar effects on SICI in children with ADHD. A genetic variation in DAT1, previously linked to ADHD risk and MPH behavioural responses, influences the neurophysiological effects of both MPH and ATX.

Keywords: ADHD; TMS; Tourette syndrome; children; motor cortex

Abbreviations: ADHD = attention deficit hyperactivity disorder; ATX = atomoxetine; DAT1 = dopamine transporter; ICF = intracortical facilitation; SICI = short interval cortical inhibition; TMS = transcranial magnetic stimulation; VNTR = variable number of tandem repeats; MEP = motor-evoked potential; MPH = methylphenidate


Introduction

In attention deficit hyperactivity disorder (ADHD), attention and impulse control are impaired owing to suboptimal function in frontal-subcortical-cerebellar catecholaminergic circuits (Biederman and Faraone, 2005). At the milder end of the spectrum, ADHD symptoms may blend in indistinguishably with normal human behaviour, complicating research into ADHD as a categorical diagnosis. In moderate and severe cases, ADHD symptoms are associated with significant morbidity, including increased risk of emergency department visits (Leibson et al., 2001), anti-social behaviour, illicit drug use and driving problems (Barkley et al., 2002, 2004). Therefore, a careful search for a quantitative marker of the neurobiological underpinnings of ADHD symptoms and responses to medical interventions is warranted.

One research approach has been to use neurophysiological techniques to study the substrate for behavioural disinhibition. To this end, we and others have used a paired pulse, transcranial magnetic stimulation (TMS) technique to measure short interval cortical inhibition (SICI) (Kujirai et al., 1993) in motor cortex, as a possible neurophysiological marker of the behavioural symptoms in ADHD. In a case–control study in children, SICI was significantly reduced in ADHD children versus non-ADHD children and children with Tourette syndrome (Moll et al., 2001).
In children and adults with Tourette syndrome, reduced SICI has been shown to correlate significantly and consistently with ADHD symptom severity (Gilbert et al., 2004, 2005).

Subsequently, the effect of ADHD medications on SICI has been studied, to determine whether these medications would increase (normalize) SICI. Supporting the SICI/ADHD relationship, in the only TMS study of the psychostimulant methylphenidate (MPH) in children with ADHD, a single 10-mg dose was found to significantly increase (normalize) SICI (Moll et al., 2000). However, in healthy adults, reported effects of MPH on SICI have been quite mixed. The same laboratory that found that MPH increased SICI in ADHD children found no significant effect of MPH on SICI in healthy adults (Moll et al., 2003). Another research group found, similar to the reported effect in ADHD children, that MPH increased SICI in healthy adults (Kirschner et al., 2003). We and one other laboratory have reported the opposite, that MPH decreased SICI in healthy adults (Ilic et al., 2003; Gilbert et al., 2006).

This variability among normal adults and children with ADHD in neurophysiological responses to stimulants has not been explained, but may have a genetic basis. This genetic basis may be related to factors that influence ADHD risk, medication responses, or both. Variations in functionally relevant dopamine receptors, particularly in the variable number of tandem repeats (VNTR) polymorphism in the 3′-untranslated end of the dopamine transporter (DAT1), have been widely studied and appear to affect behavioural responses to MPH (Winsberg and Comings, 1999; Kirley et al., 2003; Cheon et al., 2005; Lott et al., 2005; Stein et al., 2005). Therefore, we felt it would be reasonable to assess this DAT1 polymorphism in this study comparing the neurophysiological responses to two ADHD medications.

The primary objective of this study was to determine whether MPH and atomoxetine (ATX) have distinct neurophysiological effects in motor cortex in children with ADHD. MPH is widely recommended for ADHD treatment (American Academy of Pediatrics, 2001). ATX is a selective norepinephrine reuptake inhibitor shown effective for treatment of ADHD in children (Michelson et al., 2001; Allen et al., 2005) and adults (Michelson et al., 2003). We previously found that MPH and ATX have similar effects on SICI in healthy adults (Gilbert et al., 2006), but the effects of these medications have not been compared in children with ADHD. In addition, because of the unexplained variation in prior studies of the effects of MPH on SICI, we sought to determine whether the number of DAT1 10-repeat VNTR alleles influences the neurophysiological effects of these medications. We included patients with Tourette syndrome in addition to ADHD because of the high rate of co-morbidity between ADHD and tic disorder. We anticipated that the diagnosis of Tourette syndrome would not influence pre-treatment SICI (Moll et al., 2001) or post-treatment SICI since recent studies suggest that most ADHD patients with Tourette syndrome respond favourably to stimulants (Tourette Syndrome Study Group, 2002) and ATX (Allen et al., 2005).

**Patients and methods**

**Subject recruitment, diagnosis, clinical assessment**

Sixteen children and adolescents with clinically diagnosed ADHD, on no ADHD medications at the time of the visits, were recruited through advertisement and were scheduled for two visits, separated by ~1 week. Subjects with Tourette syndrome plus ADHD were recruited from the Cincinnati Children’s Hospital Tourette Syndrome Clinic. Confirmation of the clinical diagnoses was based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2000) criteria, DuPaul ADHD Rating Scale scores >1.5 SD above the age and gender mean and all available clinical information from the examiner and outside raters. Participation was timed such that any medicated patients were able to stop taking stimulant ADHD medications for at least 48 h before the first study visits. Subjects and their parents gave written informed consent for the study, in accord with the Declaration of Helsinki, which was approved by the Cincinnati Children’s Hospital Institutional Review Board.

**Study design and drug administration**

The study was a randomized, double-blind, crossover design comparing the effects of single doses of ATX and MPH. Sixteen children (age = 8–17 years, mean age = 12 ± 3; 2 girls) participated. Nine had Tourette syndrome. Medication doses were clinically relevant, ~0.5 mg/kg for MPH and 1.0 mg/kg for ATX, administered as follows: (i) subjects 20–29 kg, 10 mg MPH, 20 mg ATX; (ii) subjects 30–49 kg, 20 mg MPH, 40 mg ATX; (iii) subjects 50 kg and above, 30 mg MPH, 60 mg ATX. Subjects and investigators were blind to the order of treatment throughout the study. The 1-week interval between study visits was based on published pharmacokinetic data for single doses of ATX (Witcher et al., 2003) and MPH (Markowitz et al., 2003).

**Clinical ratings**

On the day of each study visit, before medication administration, ADHD severity was rated with direct parent interview with the ADHD Rating Scale, with a possible rating scale score of 0–54 (DuPaul et al., 1998). These were performed without knowledge of treatment order, TMS or genotyping results.

**DAT1 genotyping**

Approximately 10-ml EDTA anticoagulated venous blood was obtained from each participant. Genomic DNA was isolated using the Promega Genomic DNA Purification Kit (Promega Corporation, Madison, WI). DAT 3′-untranslated region (3′-UTR) VNTR polymorphisms were genotyped with standard PCR. Technical details are published elsewhere (Persico et al., 1995). Genotyping was performed blind to clinical rating and TMS results.

**Neurophysiology**

Neurophysiological studies were performed in the TMS laboratory at Cincinnati Children’s Hospital Medical Center, using two...
Magstim 200® stimulators (Magstim Co., New York, NY, USA) connected through a Bistim® module to a 90-mm circular coil, and EMG was recorded from the right abductor pollicis brevis (APB) with surface electrodes, amplified, filtered (100/1000 Hz) (Coulbourn Instruments, Allentown, PA) and stored for analysis using Signal® software and a Micro1401 interface (Cambridge Electronic Design, Cambridge, UK), as we have described previously (Gilbert et al., 2004). TMS was performed by an investigator blind to genotype and clinical ratings.

Resting and active motor threshold (RMT and AMT) were measured, using a method described elsewhere (Mills and Nithi, 1997). SICI and intracortical facilitation (ICF) were measured with the APB at rest, according to established paradigms (Kujirai et al., 1993; Ziemann et al., 1997). SICI was measured with a conditioning pulse, 1% of stimulator output below AMT (~70% of RMT), delivered 3 ms before a suprathreshold test pulse. ICF was measured with the conditioning pulse 10 ms before the test pulse. Twenty trials at each interval and 20 unconditioned test pulses were delivered in random order. SICI and ICF were expressed as the ratios of the mean motor-evoked potential (MEP) amplitudes evoked by the pulse pairs divided by the mean amplitude of the MEP from the single test pulses.

After baseline TMS testing, subjects were administered study medication and all measurements were repeated 90 min later, consistent with expected peak serum drug levels (Swanson and Volkow, 2003; Witcher et al., 2003). Each TMS session took ~30 min.

Statistical analysis
All analyses were performed with SAS® version 8.02 (The SAS Institute, Inc., Cary, NC, USA) or SPSS® version 11.5.0 (SPSS, Chicago, IL).

Primary analysis
The primary outcome of interest for this study was ADHD medication, genotype and medication × genotype effects on cortical inhibition (SICI). SICI measures were subjected to a mixed model, repeated-measures analysis of variance using PROC MIXED (Brown and Prescott, 1999). After assuring that baselines were not different and that there were no effects of order, we then modelled SICI = medication + DAT1 genotype + medication × genotype, combining baselines. Age and ADHD severity scores were also entered into the models as potential explanatory covariates. ICF was modelled in the same manner to determine if any effects were specific to cortical inhibition.

To compare ATX and MPH effects on cortical inhibition (SICI), two repeated-measure regressions were performed: (i) the baselines–combined model above, and (ii) a day-of-visit, baseline-adjusted model. Genotypes were entered to estimate genotype-specific effects of ATX versus MPH. Effect sizes of both medications for the two genotype groups were calculated as the differences in the means, $M_{\text{DAT10/10}} - M_{\text{DAT10/10}}$, divided by whichever group’s standard deviation $\sigma$ was larger.

Secondary analyses
The TIC phenotype was entered to estimate TIC × medication and TIC × ATX versus TIC × MPH effects. In addition, using Spearman correlations of changes in SICI and ICF were assessed for ATX and MPH, and for SICI and ADHD severity.

Results
Demographics, adverse events, dropouts
The median weight was 46 kg (26–80 kg). The median medication doses were 20 mg MPH and 40 mg ATX. No events required breaking the blind. Pre-treatment SICI correlated with the ADHD Rating Scale score ($r = 0.68, P = 0.005$), with more severe symptoms correlating with larger ratios (less SICI) (see Fig. 1).

Fifteen subjects attended both visits. One subject’s parents chose not to schedule the second visit owing to safety concerns, despite the absence of side-effects. Thus, data from 62 TMS sessions were analysed. One parent gave highly inconsistent ADHD Rating Scale scores at visit one versus visit two, which were judged unreliable and were excluded from the covariate analysis.

Of 16 subjects, 10 experienced no side-effects, 6 experienced one or more side-effects on the day of one or both visits and one reported several side-effects by phone interview on the days after both visits. Reported side-effects in four subjects after ATX/TMS were numbness/tingling (1), loss of appetite (2), scalp pain (1), stomach pain (1) and headache (3). Reported side-effects in six subjects after MPH/TMS were headache (3), numbness/tingling (1), arm/other pain (2), abdominal pain (1), hearing change (1). All side-effects were rated mild except for one child who reported a moderate headache the day after MPH/TMS.

DAT1 genotypes
Seven patients were homozygous for DAT1 VNTR 10/10 repeats, 8 heterozygous for 9/10 repeats and one heterozygous for 8/10 repeats. Heterozygous subjects were combined...
into one group. Clinical features by DAT1 genotype are shown in Table 1. There were no significant differences in any pre-treatment demographic, clinical or neurophysiological measures between DAT1 9/10 and 10/10 genotypes, although, at baseline, DAT1 10/10 subjects had somewhat worse ADHD scores and less SICI.

### Effects of DAT1 genotype on physiological response to medications

There was no effect of treatment order or difference between visit one versus visit two. There was no main effect of medication \( F(2,13) = 0.51, P = 0.61 \).

The effects of medication treatment on SICI differed with DAT1 genotype (see Fig. 2). In heterozygotes, both MPH and ATX increased SICI. In contrast, in homozygotes both medications decreased SICI (increased MEP amplitude ratios).

This DAT1 genotype by medication interaction was highly robust and significant in the baseline-combined \( F(2,13) = 13.04, P = 0.008 \) analysis. DAT1 genotype was also highly significant in the day-of-visit, baseline-adjusted analyses \( F(1,13) = 9.31, P = 0.0093 \), which was used to compare ATX and MPH effects. The interaction remained significant when the individual with the 8/10 genotype was excluded (data not shown).

Baseline-adjusted estimates of post-MPH and post-ATX SICI are shown in Table 2. In the DAT1 9/10 group, mean MEP amplitude ratios after MPH decreased by 0.075 (SD: 0.19), while in the DAT1 10/10 group, ratios increased by 0.031 (SD: 0.22), for a net difference of 0.11 and effect size 0.47. Similarly, in the DAT1 9/10 group, mean MEP amplitude ratios after ATX decreased by 0.055 (SD: 0.12), while in the DAT1 10/10 group, ratios increased by 0.092 (SD: 0.16), for a net difference of 0.15 and effect size 0.93.

There were no main or interaction effects of medication and DAT1 genotype on ICF. Age and ADHD severity did not interact with the medication effects (data not shown).

There was also no difference detected in the interaction between ATX and DAT1 or MPH and DAT1 effects on SICI. However, after ATX, MEP amplitude ratios at short (SICI-inhibitory) and long (ICF-facilitatory) intervals tended to either both increase or both decrease \( r = 0.52, P = 0.048 \). MPH-induced changes in SICI and ICF were not correlated \( r = 0.032, P = 0.91 \).

### Table 1 Clinical and neurophysiological characteristics by genotype

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>DAT 9/10 (n = 9)</th>
<th>DAT 10/10 (n = 7)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tics</td>
<td>6</td>
<td>3</td>
<td>0.62</td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>Caucasian</td>
<td>8</td>
<td>4</td>
<td>0.26</td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Age mean (SD)</td>
<td>12.9 (2.2)</td>
<td>11.6 (3.5)</td>
<td>0.38</td>
</tr>
<tr>
<td>Inattention score mean (SD)</td>
<td>19 (4.3)</td>
<td>21 (5.3)</td>
<td>0.39</td>
</tr>
<tr>
<td>Hyperactive/impulsive score mean (SD)</td>
<td>14 (8.9)</td>
<td>18 (4.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>Total ADHDRS mean (SD)</td>
<td>33 (10.4)</td>
<td>39 (7.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>SICI mean ratio (SD)</td>
<td>0.50 (0.30)</td>
<td>0.62 (0.17)</td>
<td>0.46</td>
</tr>
<tr>
<td>ICF mean ratio (SD)</td>
<td>1.15 (0.30)</td>
<td>1.16 (0.15)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

ADHDRS = Attention Deficit Hyperactivity Disorder Rating Scale; ICF = intracortical facilitation; SICI = short interval cortical inhibition.
Effects of Tourette syndrome phenotype on physiological response to medications

The effects of medication on SICI \(F(2,13) = 7.20, P = 0.0078\) differed by Tourette syndrome phenotype. In patients with ADHD and Tourette syndrome, ATX and MPH increased (normalized) SICI. In contrast, in individuals with ADHD and no Tourette syndrome, ATX and MPH decreased SICI. Baseline-adjusted estimates, by Tourette syndrome phenotype, of post-MPH and post-ATX SICI are shown in Table 2. There was no statistically significant interaction between Tourette syndrome diagnosis and medication type. Small sample size precluded assessment of three-way interactions between DAT1, Tourette syndrome, and medication.

Table 2 Post-MPH and post-ATX motor cortex inhibition

<table>
<thead>
<tr>
<th>Effect</th>
<th>Cortical inhibition (SICI)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication—ATX versus MPH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATX</td>
<td>0.59</td>
<td>0.51–0.67</td>
<td>0.52</td>
</tr>
<tr>
<td>MPH</td>
<td>0.54</td>
<td>0.46–0.63</td>
<td></td>
</tr>
<tr>
<td>DAT1 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DATI 9,10</td>
<td>0.50</td>
<td>0.43–0.56</td>
<td>0.0086</td>
</tr>
<tr>
<td>DATI 10,10</td>
<td>0.64</td>
<td>0.57–0.71</td>
<td></td>
</tr>
<tr>
<td>Medication × DAT1 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATX and DATI 9/10</td>
<td>0.51</td>
<td>0.40–0.62</td>
<td>0.89</td>
</tr>
<tr>
<td>ATX and DATI 10/10</td>
<td>0.66</td>
<td>0.54–0.79</td>
<td></td>
</tr>
<tr>
<td>MPH and DATI 9/10</td>
<td>0.47</td>
<td>0.36–0.59</td>
<td></td>
</tr>
<tr>
<td>MPH and DATI 10/10</td>
<td>0.61</td>
<td>0.49–0.74</td>
<td></td>
</tr>
<tr>
<td>TIC phenotype</td>
<td></td>
<td></td>
<td>0.0056</td>
</tr>
<tr>
<td>No tics</td>
<td>0.63</td>
<td>0.57–0.70</td>
<td></td>
</tr>
<tr>
<td>Tics</td>
<td>0.50</td>
<td>0.44–0.56</td>
<td></td>
</tr>
<tr>
<td>Medication × TIC phenotype</td>
<td></td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>ATX and no tics</td>
<td>0.62</td>
<td>0.50–0.73</td>
<td></td>
</tr>
<tr>
<td>ATX and tics</td>
<td>0.56</td>
<td>0.45–0.66</td>
<td></td>
</tr>
<tr>
<td>MPH and no tics</td>
<td>0.64</td>
<td>0.52–0.76</td>
<td></td>
</tr>
<tr>
<td>MPH and tics</td>
<td>0.44</td>
<td>0.33–0.54</td>
<td></td>
</tr>
</tbody>
</table>

Post-medication SICI, estimated after adjustment for the day-of-visit, pre-treatment SICI. Effects of ATX and MPH on SICI did not differ, but the effects of ADHD medications differed significantly in patients with DATI 9/10 versus 10/10 genotypes (see text). ATX atomoxetine; DATI dopamine transporter, 9 and 10 indicate numbers of tandem repeats (see text). MPH methylphenidate; SICI short interval cortical inhibition.

Discussion

The primary findings of this study are that the stimulant MPH and the selective norepinephrine reuptake inhibitor ATX have similar effects on cortical inhibition (SICI) in children with ADHD, and that the dopamine transporter influences the neurophysiological effects of both medications. SICI, a measure of cortical inhibitory activity that is reduced in ADHD (Moll et al., 2001) and correlates inversely with ADHD symptom severity (Gilbert et al., 2004, 2005), increased toward normal with both MPH and ATX in DATI 9/10 heterozygotes, similar to MPH-induced changes in SICI previously reported in a German cohort of children with ADHD (Moll et al., 2000). This SICI change differed significantly from the medication effects in DATI 10/10 homozygotes.

This study is novel in several ways. First, with the exception of a single study of a sub-therapeutic dose of MPH (Moll et al., 2000), all prior TMS studies of either psychostimulants and norepinephrine reuptake inhibitors have been performed in healthy adults rather than in children with ADHD. We have compared clinically relevant doses of two ADHD medications in children with ADHD. Secondly, neurophysiological effects of a selective norepinephrine reuptake inhibitor on SICI in ADHD children have not previously been reported. Thirdly, ours is the first study to use functionally relevant dopamine receptor polymorphisms to assess variation in neurophysiological responses to stimulants. Fourthly, this is the first study to report a link between DATI polymorphisms and any response to ATX. That a dopamine receptor could be involved in the responses to this selective norepinephrine reuptake inhibitor is supported by the prior observation that ATX increases both norepinephrine and dopamine in cerebral cortex (Bystam et al., 2002).

The clinical implications of this finding merit further study. Since ADHD children have less SICI (Moll et al., 2001; Gilbert et al., 2004), when MPH and ATX increase SICI, as occurred in the DATI 9/10 heterozygotes, we expect that symptoms would be more likely to improve. In contrast, failure to increase SICI, as occurred in the DATI 10/10 homozygotes, might tend to occur in clinical non-responders. Thus our data are consistent with studies showing a poorer clinical response to MPH in individuals with the DATI 10/10 genotype (Winsberg and Comings, 1999; Roman et al., 2002; Cheon et al., 2005) as well as divergent EEG responses (Loo et al., 2003) to MPH based on this DATI genotype.

ADHD appears to be polygenic, with multiple genes exerting small effects (Waldman et al., 1998). The literature on ADHD risk, treatment response and DATI polymorphisms has not yielded consistent results. For example, in some populations, there may be a better clinical response to MPH in DATI 10/10 homozygotes (Kirley et al., 2003). Epistasis as well as low reliability of clinical assessments may underlie inconsistent results in different cohorts.

The mechanism whereby genetic variation in DATI would influence ADHD and treatment responses remains obscure. The VNTR polymorphism in the 3’-untranslated end of DATI (Vandenbergh et al., 1992) may regulate transcription (Nakamura et al., 1998; Migone et al., 2002; VanNess et al., 2005). DATI 9 and 10 repeat VNTR alleles are most common, and their influence has been studied extensively. Several in vitro studies suggest that this VNTR polymorphism influences the level of gene expression, but whether the 9 repeat (Michelhaugh et al., 2001; Miller and Madras, 2002)
or the 10 repeat (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005) allele increases expression is unresolved. Similarly, in vivo human studies using [123I]beta-CIT single photon emission computed tomography (SPECT) have been inconsistent, showing decreased (Jacobsen et al., 2000), increased (Heinz et al., 2000; Cheon et al., 2005) or no difference (Martinez et al., 2001) in DAT1 binding in 10-repeat homozygous subjects.

The relationship seen in genetic association studies between DAT1 VNTR repeat number and ADHD risk has been somewhat more consistent. Several studies have shown that transmission of the DAT1 10-repeat VNTR allele is linked with increased ADHD risk (Cook et al., 1995; Gill et al., 1997; Waldman et al., 1998; Daly et al., 1999). However, some studies of DAT1 have found weaker (Mill et al., 2005) or no (Muglia et al., 2002; Kim et al., 2005) evidence of this association.

The results of the present study may partly explain variation in MPH-induced changes in SICI in normal adults (Ilic et al., 2003; Kirschner et al., 2003; Moll et al., 2003; Gilbert et al., 2006). Prior studies in adults did not control for genotype. Cognitive factors like baseline memory capacities that can influence responses to stimulants (Kimberg et al., 1997; Mattay et al., 2000), as well as other dopaminergic and norepinephrine receptor genotypes and prior medication use history may also be important.

Our findings that SICI is a marker of both ADHD severity and medication responses, as well as of the influence of DAT1, may be understood in the context of the relationship in cortex between the GABA and dopamine systems in the cortex. SICI has previously been shown to be enhanced by GABA-ergic drugs (Ziemann et al., 1998), neurosteroids (Smith et al., 2002) and dopamine agonists (Ziemann et al., 1996). Dopamine terminals occur on GABA-ergic interneurons closely associated with pyramidal (output) cells (Gaspar et al., 1992; Sesack et al., 1998). Studies of the effects of dopamine in cerebral cortex show inhibitory effects on pyramidal neurons via peridendritic interneurons that are thought to modulate the sensitivity of the pyramidal neurons to excitatory synaptic inputs (Gao and Goldman-Rakic, 2003). These interneurons may include those that result in TMS-induced firing. It is also important to note that motor cortex SICI can be abnormally diminished in other conditions besides ADHD, including schizophrenia and Parkinson’s disease (Ridding et al., 1995; Daskalakis et al., 2002).

It is surprising and potentially important that the DAT1 receptor polymorphism may modulate the effects of ATX. We were unable to detect a difference in the neurophysiological effects of MPH and ATX, similar to our experience in healthy adults (Gilbert et al., 2006). Although ATX has more selective noradrenergic effects in the striatum, both ATX and MPH increase dopamine and norepinephrine acutely in the cortex (Bymaster et al., 2002), and thus their similar effects on SICI may simply reflect common dopaminergic effects. However, since norepinephrine can also act as a dopamine 4 receptor (DRD4) agonist (Lanau et al., 1997; Newman-Tancredi et al., 1997), the common effects of ATX and MPH also reflect noradrenergic activity, and interaction effects of genetic variations in DRD4 receptors and the norepinephrine transporter may be important. Assessing this will require a larger sample, owing to lower prevalence of polymorphisms of interest in these receptor genes.

Our finding of an interaction between treatment and the presence of Tourette syndrome on post-treatment SICI was unexpected. In this sample-of-convenience, it is unclear whether this observation of different effects, predominantly of MPH, on SICI, is real. We suspect it is artefact, and we doubt that Tourette syndrome confounded the DAT1-medication effects, for two main reasons. First, two prior studies in children show that reduced SICI is more tightly linked to ADHD than to Tourette syndrome (Moll et al., 2001; Gilbert et al., 2004). Secondly, studies of psychostimulant medications for ADHD in Tourette syndrome (Sverd et al., 1989; Gadow et al., 1992; Castellanos et al., 1997; Law and Schachar, 1999; Tourette Syndrome Study Group, 2002) generally show similar clinical effect sizes as studies of psychostimulants in children without Tourette syndrome (Multimodal Treatment of ADHD Cooperative Group, 1999). Our study sample size was not adequate to test for a three-way interaction between the effects of DAT1 genotype and Tourette syndrome phenotype on medication responses. However, since it has long been observed that some patients with tics respond less favourably or experience tic symptom exacerbations on stimulants (Lowe et al., 1982; Castellanos et al., 1997), a follow-up study in Tourette syndrome patients with poor stimulant responses would be of interest.

This study was limited by the lack of a DAT 9/9 group and by under-representation of females and inattentive-type ADHD subjects. Our results may not extend to these groups. In addition, we cannot exclude the possibility that other unmeasured cognitive or genetic factors, or prior medication use, were responsible for the highly significant, post-treatment differences we observed in the two DAT1 groups. Recruiting a medication naïve sample would probably result in a non-representative, milder ADHD sample. Our failure to find significant effects of age and baseline ADHD severity may relate to small sample size.

An additional limitation is that we did not collect behavioural-response data in this study. We chose not to assess behavioural responses mainly because MPH has first-dose behavioural effects, but ATX may take 2–4 weeks to achieve efficacy. Selective norepinephrine reuptake inhibitors do have first-dose neurophysiological effects (Herwig et al., 2002; Plewnia et al., 2002; Gilbert et al., 2006), so a single dose study was reasonable for our purposes. The ADHD Rating Scale we used assesses behaviour in the school and home settings for the week before the assessment. This was used to rate baseline ADHD severity in this study, but could not be used as an outcome in a single dose, crossover study. What matters, clinically, are chronic cognitive and behavioural responses in home and school settings.

Cortical inhibitory effects of ADHD medications

Brain (2006), 129, 2038–2046

2043
Owing to the inherent subjectivity and variability of clinical rating scales, as well as issues like medication compliance, combining a meaningful clinical outcome with TMS and genotyping would require a far larger study.

In summary, we used TMS-evoked SICI to compare the effects of a stimulant and norepinephrine reuptake inhibitor on motor cortex in children with ADHD. We found similar effects for both medications on this measure of motor cortex inhibition, shown previously to correlate with ADHD presence and severity. Neuropsychological responses within individuals were similar to both medications. However, between individuals in our cohort, neuropsychological responses varied. Some of this variation, for both medications, appeared to be explained by dopamine transporter DAT1 genotypes previously judged to be important for ADHD risk and stimulant responses in multiple prior genetic, epidemiological and imaging studies. Given the consistent association of TMS-evoked SICI with ADHD severity and its high sensitivity to pharmacological treatment, we believe it should be studied further to clarify genotype-phenotype relationships and clinical responses in ADHD.

Acknowledgements
We gratefully acknowledge the time devoted to this study by the volunteers who participated, and technical assistance by Jared Brandyberry. This research was supported by an independent, investigator-initiated grant from Lilly Research Laboratories, a Division of Eli Lilly and Company (Indianapolis, IN) (D.L.G.); by NINDS K23 NS41920 (D.L.G.); and by the NARSAD Young Investigator Award (Z.W.).

Conflict of interest: This research was supported in part by an independent, investigator-initiated grant from Lilly Research Laboratories, a Division of Eli Lilly and Company (Indianapolis, IN) to Dr Gilbert. Dr Gilbert is the sponsor of the study and had complete control over data analysis and writing of the manuscript. There are no other conflicts of interest to report for the other authors.

References
Herwig U, Brauer K, Connenmann B, Spitzer M, Schonfeldt-Lecuona C. Intracortical excitability is modulated by a norepinephrine reuptake inhibitor as measured with paired-pulse transcranial magnetic stimulation. Psychopharmacology (Berl) 2002; 164: 228–32.
Herwig U, Brauer K, Connenmann B, Spitzer M, Schonfeldt-Lecuona C. Intracortical excitability is modulated by a norepinephrine reuptake inhibitor as measured with paired-pulse transcranial magnetic stimulation. Psychopharmacology (Berl) 2002; 164: 228–32.
Cortical inhibitory effects of ADHD medications


