Neurofunctional Effects of Methylphenidate and Atomoxetine in Boys with Attention-Deficit/Hyperactivity Disorder During Time Discrimination

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Background: The catecholamine agonists methylphenidate and atomoxetine effectively treat attention-deficit/hyperactivity disorder (ADHD). Furthermore, dopamine agonists have shown to improve time estimation in ADHD, a core cognitive deficit. However, few have compared the effects of methylphenidate and atomoxetine on brain function in ADHD, and none during time estimation. Using single dose challenges, we investigated shared and drug-specific effects in ADHD adolescents on the neural substrates of time discrimination (TD).

Methods: Twenty ADHD adolescent male subjects were compared in a randomized double-blind cross-over design after single doses of methylphenidate, atomoxetine, and placebo in functional magnetic resonance imaging during TD. Normalization effects were assessed by comparing brain activation under each drug condition with that of 20 healthy age-matched control subjects.

Results: Relative to control subjects, patients under placebo showed TD deficits and reduced activation of ventrolateral prefrontal cortex (VLPFC)/insula, inferior frontal cortex, and supplementary motor area. Performance differences were normalized only by methylphenidate, relative to both atomoxetine and placebo. Both medications, however, significantly upregulated right VLPFC/insula activation within patients and normalized its underactivation in ADHD boys under placebo relative to control subjects. The supplementary motor area and inferior frontal cortex activation differences that were observed under placebo were reduced by methylphenidate and atomoxetine, respectively, but neither survived rigorous testing for normalization.

Conclusions: While only methylphenidate had a drug-specific effect of improving TD performance deficits, both drugs significantly upregulated and normalized right VLPFC underactivation in ADHD boys under placebo relative to control subjects, suggesting shared effects of stimulants and nonstimulants on a key prefrontal dysfunction during timing.

Key Words: Attention-deficit/hyperactivity disorder, atomoxetine, functional magnetic resonance imaging, methylphenidate, supplementary motor area, time discrimination

Attention-deficit/hyperactivity disorder (ADHD) is defined by problems with inattention, impulsivity, and hyperactivity (DSM-IV). Children with ADHD are impaired in executive functions (1) but also in temporal processes (2,3). They are particularly impaired in fine temporal discrimination (TD), i.e., the discrimination of intervals that differ in the millisecond range (3–5), which has been shown to be one of the best discriminatory measures for ADHD among a large battery of tasks (5). Using functional magnetic resonance imaging (fMRI), we have shown that TD deficits in ADHD adolescents are underpinned by fronto-striato-cerebellar activation deficits, including right inferior frontal cortex (IFC), dorsolateral prefrontal cortex (DLPFC) supplementary motor area (SMA), anterior cingulate cortex (ACC), the basal ganglia, and cerebellum (2,6,7).

One of the most frequently prescribed medications for ADHD is the stimulant methylphenidate. Methylphenidate blocks dopamine transporters in the striatum and norepinephrine transporters (NET) in NET-rich cortical regions, including prefrontal cortex, where it increases concentrations of both dopamine (DA) and norepinephrine (NE) (8). There is a strong association between DA, the striatum, and fine temporal processes (9): the striatal DA receptor agonist methylphenidate has been shown to improve motor timing and time estimation deficits in children with ADHD in the millisecond (10,11) and second ranges (11,12). fMRI studies in ADHD patients have shown that single doses of methylphenidate consistently upregulate and normalize fronto-striatal activation during cognition (2,13–15). The only fMRI study investigating the influence of methylphenidate during TD showed that a single dose of methylphenidate significantly upregulated and normalized all underactivations observed in ADHD patients relative to control subjects during placebo in DLPFC, ventrolateral prefrontal cortex (VLPFC), ACC, and cerebellum (2).

The only other licensed medication for patients with ADHD is the nonstimulant atomoxetine. While methylphenidate has immediate effects on behavior, atomoxetine takes up to 6 to 8 weeks to show clinical effects (16). Some studies comparing chronic dose effects show that methylphenidate is more effective than atomoxetine (17), while meta-analyses show either comparable efficacy rates in reducing ADHD symptoms or superior effects of the long-acting methylphenidate preparations only (18–20). Atomoxetine is a selective presynaptic blocker of NETs (21), leading to enhanced NE and DA in prefrontal cortex, but also influences other regions including ACC, thalamus, locus coeruleus,

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and cerebellum (22). Compared with methylphenidate, however, atomoxetine has no direct effect upon basal ganglia (22).

In healthy adults, a single dose of atomoxetine increased right VLPFC/superior temporal lobe activation during inhibitory control (23,24). We recently compared single dose effects of atomoxetine with methylphenidate during motor inhibition in ADHD and showed that both medications upregulated and normalized left VLPFC, with drug-specific effects of methylphenidate on normalizing right VLPFC and cerebellum (25). In this study, we wanted to test the effects of a single clinical dose of atomoxetine and of methylphenidate in fMRI during a time discrimination task (6), measuring another key disorder-sensitive function (2–5) shown to be mediated by frontostriatal networks (26–28) and modified by DA agonists in ADHD (10,12).

Given the strong association between DA and frontostriatal networks in TD (26) and evidence for positive effects of methylphenidate on time estimation (2,10,12) and its underlying frontostriatal networks in ADHD (2), we hypothesized that methylphenidate would enhance TD performance and its associated frontostriatal correlates. However, we proposed that atomoxetine would also increase ventrolateral prefrontal activation, as observed in healthy adults during tasks of cognitive control (23,24) and in children with ADHD during motor inhibition (25).

Methods and Materials

Participants

Twenty-eight primarily medication-naive, right-handed adolescent boys between 10 and 17 years old (mean age of final sample: 12 years, 11 months [SD: 1 year, 7 months]) with a clinical diagnosis of inattentive/hyperactive-impulsive combined ADHD, as assessed by an experienced child psychiatrist using the standardized Maudsley diagnostic interview (29), which assesses ADHD according to DSM-IV-Text Revision criteria (30), were recruited from clinics. One patient had a brief medication trial of methylphenidate 9 months before participation. Eight patients were excluded due to neurological abnormalities detected during the scan ($n = 1$), left handedness ($n = 1$), braces ($n = 1$), loss of data ($n = 1$), or intolerance to the scanning situation ($n = 4$). No participant was excluded due to intolerance of medication or increased movement. In line with their diagnoses, all patients scored above clinical cutoff for hyperactive/inattentive symptoms on the parental Strengths and Difficulties Questionnaire (SDQ) (31) and the Conners' Parent Rating Scale-Revised (CPRS-R) (32) and below clinical cutoff on the Social Communication Questionnaire (33) to ensure lack of comorbidity with autism spectrum disorder. They were scanned every Monday between 5:30 PM and 7:30 PM over 3 consecutive weeks using a double-blind, pseudorandomized, crossover drug design, receiving a single dose of either placebo (vitamin C, 50 mg), methylphenidate (Equasym .3 mg/kg: range 5–20 mg), or atomoxetine (Strattera 1 mg/kg: range 16–66 mg), all over-encapsulated. Dosages were determined following National Institute for Health and Clinical Excellence guidelines at the time of the study for typical clinical efficacious dosages with minimal side effects (http://www.nice.org.uk/CG72). As suggested by evidence from pharmacokinetics studies, both medications were administered 1.5 hours before the scan to allow for maximum absorption (34,35).

Twenty-one right-handed, healthy boys between 10 and 17 years old (mean age of final sample: 13 years, 10 months [SD: 2 years, 4 months]) were recruited through advertisements. They scored below clinical cutoff for the SDQ, Social Communication Questionnaire, and CPRS-R. One participant was excluded due to CPRS-R and SDQ scores above clinical threshold. Control subjects were scanned once, unmedicated, for feasibility and ethical reasons. The final subject numbers were therefore 20 ADHD and 20 control subjects.

Participants were excluded if they had comorbid psychiatric disorders (except for conduct disorder and oppositional defiant disorder in the ADHD group: $n = 2$), including learning disability; reading, speech, or language disorder; neurological abnormalities; epilepsy; and drug or substance abuse. Student *t* tests showed no group differences for age ($t = 1.5$; $p =$ ns). All participants had an IQ >70 on the Wechsler Abbreviated Scale of Intelligence (36), but significant group differences were observed (mean control subjects: 113 [10]; mean ADHD: 91 [11]; $t = 6.3$; $p < .0001$), not unexpected given that low IQ scores are typical in ADHD (37,38). IQ was therefore included as a covariate in case-control analyses.

Participants were paid £50 for each visit. Written informed consent and assent were obtained and the study was approved by the local ethics committee.

fMRI Time Discrimination Task

After one practice session outside the scanner, the TD task was visually presented in the magnetic resonance imaging scanner via a prism from a liquid crystal diode projector. The 5-minute block-design task consisted of 5 × 30-sec alternated blocks for two conditions: TD (active condition) and temporal order judgment (TOJ) (control condition), which was always presented first. The TD condition began with the appearance of a centrally located grey circle (5 cm in diameter) with the letter L for 3 sec. This was followed by two equally sized red (left side of screen) and green (right side of screen) circles, appearing consecutively with no intermittent pause and in random order. One circle was randomly presented for 1 sec, and the comparison circle was presented for either 1.3 sec, 1.4 sec, or 1.5 sec, with two trials for each comparison and with 2.1 sec response time for each trial. The subjects were told that in this experimental condition indicated by the letter L, they had to decide which circle stayed on the screen for the longest time by responding with a left-sided button if the red circle (displayed left) lasted longest or a right-sided button if the green circle (displayed right) lasted longest.

The TOJ (control) condition was presented identically. The only difference was that these blocks began with the presentation of the number 2 and required subjects to indicate which circle came second using the same response buttons as described above.

Task Performance Analysis. Repeated measures analyses of variance (ANOVAs) were conducted to test for within-group effects of drug condition on TD and TOJ errors and to test for practice effects. Case-control ANOVAs were conducted to compare healthy control subjects and patients for each of the three medication conditions and for each task condition (TD, TOJ) (6).

Magnetic Resonance Imaging Acquisition and Analysis

Gradient-echo echo-planar imaging data were acquired on a GE Signa 3T Horizon DHx system (General Electric, Milwaukee, Wisconsin) at the Centre for Neuroimaging Sciences, Institute of Psychiatry, Kings' College London, United Kingdom. A semiautomated quality control procedure ensured consistent image quality (39). A quadrature birdcage head coil was used for radio frequency transmission and reception. In each of 48 noncontiguous planes parallel to the anterior-posterior commissure line, 100 T_2*-weighted magnetic resonance images depicting blood oxygen level–dependent (BOLD) contrast covering the whole

Table 1. Mean Percentage of Errors for Temporal Discrimination and Temporal Order Judgment for Control Subjects and Children with ADHD During Placebo, Methylphenidate, and Atomoxetine

	Control Subjects	ADHD (Placebo)	ADHD (Methylphenidate)	ADHD (Atomoxetine)
Errors in Time Discrimination Trials (%)	21.7 (21.9)	40.5 (28.7)[a]	32.5 (25.6)	40.5 (23.8)[a]
Errors in Order Discrimination Trials (%)	5.5 (13.7)	6.0 (8.7)	4.9 (11.1)	7.7 (8.1)

ADHD, attention-deficit/hyperactivity disorder; ANOVA, analysis of variance.
[a]Significant group by condition interaction effect during ANOVA comparison of control subjects and patients, showing enhanced time discrimination errors in patients relative to control subjects.

brain were acquired with echo time = 30 msec, repetition time = 3 sec, flip angle = 90º, in-plane resolution = 3.1 mm, slice thickness = 3.0 mm, and slice skip = .3 mm. This echo-planar imaging dataset provided complete coverage.

We used the nonparametric XBAM software (http://www.brainmap.co.uk) developed at the Institute of Psychiatry, Kings College, London (40–42), which uses median statistics to control outlier effects and permutation rather than normal theory based inference, recommended for fMRI (43). Furthermore, the most common test statistic is computed by standardizing for individual difference in residual noise before embarking on second-level, multisubject testing using robust permutation-based methods. This allows a mixed-effects approach to analysis recommended for fMRI.

After preprocessing (Supplement 1), time series analysis for each individual subject was based on a previously published wavelet-based data resampling method for fMRI data (41,42).

After first-level analysis, the individual statistical maps were then normalized into Talairach standard space (42). A group brain activation map was then produced for the experimental condition (TD–TOJ) and hypothesis testing was carried out at the cluster level.

The detection of activated voxels is extended from voxel to cluster level using a two-pass method (41). We first used a voxel-level threshold of $p < .05$ to give maximum sensitivity and to avoid type II errors. Three-dimensional clusters were then built by joining together adjacent significant voxels. Cluster mass (rather than a cluster extent) threshold was used as second-pass cluster statistic, to minimize discrimination against possible small, strongly responding foci of activation (41). The cluster-level threshold was then computed in such a way as to ensure that the final expected number of type I error clusters was less than one per whole brain.

Between-Group Analyses between Control Subjects and ADHD Under Each Drug Condition

For case-control comparisons, a series of three analyses of covariance (with IQ as covariate) were carried out comparing control subjects with 1) ADHD under placebo; 2) ADHD under methylphenidate; and 3) ADHD under atomoxetine. We used a region of interest (ROI) analysis where we selected brain regions that have consistently been found in previous research to be underactivated in children with ADHD during TD (2,6), including our recent meta-analysis of fMRI studies of timing (7). For this purpose, we applied the Talairach Client (Research Imaging Institute, University of Texas Health Science Center, San Antonio) (44,45) to determine a predefined mask of these regions that included the frontal lobes, including ACC/SMA; the cerebellum; and the striatum (2,6,7). Statistical measures of BOLD response were then extracted for each participant in each of the clusters of between-group differences and post hoc *t* tests (corrected using least significant difference) were conducted to clarify the direction of the differences.

Within-ADHD Repeated Measures Analyses of Drug Conditions

To test for upregulation effects of both drugs on brain areas related to TD within the ADHD group, a repeated measures ANOVA was carried out to test for effects of drug condition (placebo, methylphenidate, atomoxetine) on brain activation within patients using the same predefined regions in the same ROI mask, as described above.

To test for potential order effects, repeated measures ANOVAs were then conducted within patients on the extracted BOLD response measures of the resulting activations.

To test whether the between-group and within-group differences in brain activation were related to performance, statistical measures of BOLD response (sum of squares ratios) were extracted for each participant in each of the clusters of between-group and within-group differences.

Results

Task Performance

Repeated measures ANOVAs within ADHD under each medication condition were significant for TD errors ($F_{2,39} = 4.8$; $p < .02$), due to fewer TD errors in ADHD patients during the methylphenidate condition relative to placebo ($p < .06$) and atomoxetine ($p < .03$), but not for TOJ errors ($F_{2,39} = .1$, $p = .48$) (Table 1).

A series of ANOVAs comparing errors between control and ADHD boys under each medication condition revealed for the ADHD placebo-control comparison, a significant effect of condition ($F_{1,38} = 52$; $p < .0001$) and a group by condition interaction ($F_{1,38} = 6.8$; $p < .013$) and for the ADHD atomoxetine-control comparison, a significant effect of condition ($F_{1,38} = 57$; $p < .0001$) and a group by condition interaction ($F_{1,38} = 6.6$; $p < .014$). However, under methylphenidate, there was a main effect of condition ($F_{1,38} = 40$; $p < .0001$) but no significant group by condition interaction ($F_{1,38} = 2.7$; $p < .107$). The significant effect of condition was explained in each case by superior performance for TOJ compared with TD for all subjects. The significant group by condition interactions were attributable to ADHD patients making more errors than control subjects in TD but not TOJ under placebo ($p < .02$) and under atomoxetine ($p < .02$) but not under methylphenidate ($p < .16$) (Table 1).

Brain Activation

Motion. Multivariate analyses of variance showed no significant effects in the extent of three-dimensional motion as measured by translation (voxels) for x, y, and z axes for the comparison of control subjects and ADHD children under each medication condition.

Within-Group Activation. Within-group brain activation results are described in the text and Figure S1 in Supplement 1.

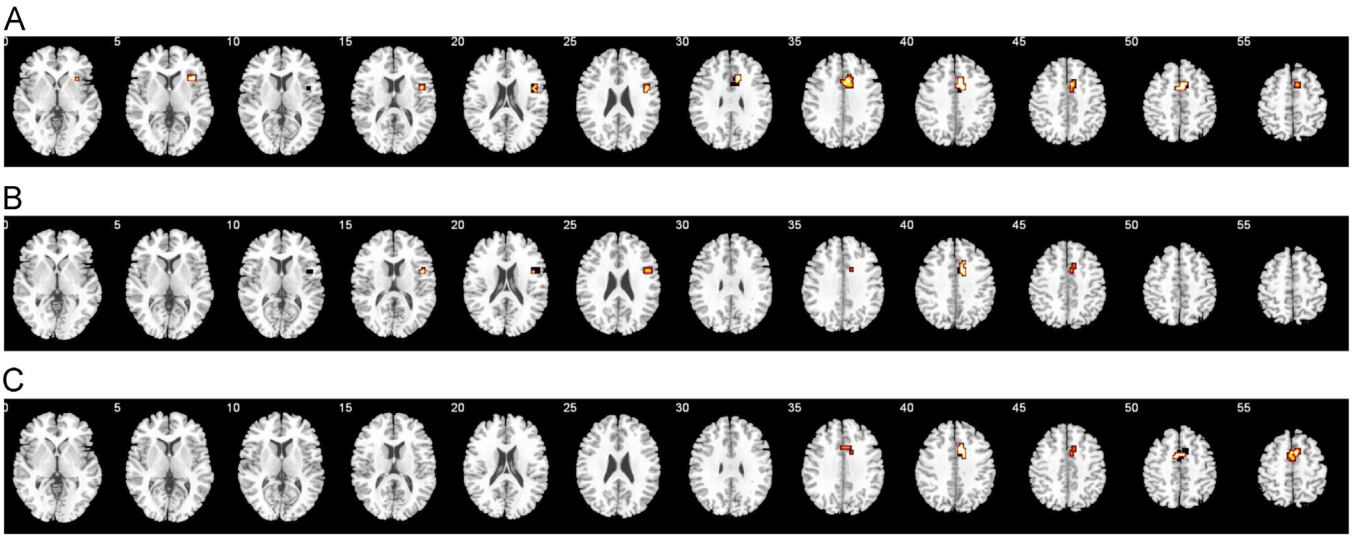

Figure 1. Transversal images of the between-group analysis of variance comparison between healthy control boys and boys with attention-deficit/hyperactivity disorder on (A) placebo, (B) methylphenidate, and (C) atomoxetine during time discrimination. Statistical threshold selected at $p < .05$ for voxel and $p < .01$ for cluster levels. Slices are marked with the z coordinate as distance in millimeters from the anterior-posterior commissure.

ROI Analyses Results for Between-Group Comparisons of Healthy Control Subjects and ADHD Boys Under Placebo, Methylphenidate, or Atomoxetine

Under placebo, ADHD boys compared with control subjects showed reduced activation in right VLPFC (Brodmann area [BA] 47)/insula, right IFC (BA 45), and SMA/ACC (Figure 1, Table 2).

Under methylphenidate, ADHD boys compared with control subjects showed reduced activation in right IFC but no longer in right VLPFC/insula. The activation differences in the ACC cluster were still observed but reduced in size and no longer included the SMA (Figure 1, Table 2). No areas were enhanced in activation for the ADHD boys compared with control subjects.

Under atomoxetine, activation differences between ADHD boys and control subjects were no longer observed in either right VLPFC/insula or right IFC, but SMA/ACC activation was still reduced compared with control subjects (as observed during placebo) (Figure 1, Table 2). No areas of enhanced activation for the ADHD group compared with healthy subjects were observed.

To test for the statistical significance of these apparent normalization effects of each drug on case-control activation differences observed under placebo, we used nonparametric Friedman two-way analysis of variance by ranks on the extracted BOLD responses during each medication condition for each of the three clusters shown to be significantly different in the comparison between control subjects and children with ADHD during placebo. We conducted this test only within patients, given that control subjects were only tested once and hence constant across comparisons.

We found that the BOLD response within the right VLPFC/insula cluster was significantly different for each medication condition ($p < .043$). Post hoc analysis using Wilcoxon signed-rank tests showed that this significant difference was explained by significantly lower BOLD response for patients during placebo compared with methylphenidate ($p < .011$) and atomoxetine ($p < .013$).

Table 2. Between-Group ANOVA Results Showing Differences between Control Subjects and Boys with ADHD Under Either Placebo, Methylphenidate, or Atomoxetine for the Contrast of TD Versus Order Judgment

	Talairach Coordinates			*p* Value		
Number of Voxels	x	y	z	≤	BA	Region
Control Subjects > ADHD Under Placebo						
56	7	11	40	.0008	32/6	R and L SMA/anterior cingulate cortex
15	47	11	20	.0070	45/44	R inferior frontal cortex[a]
11	40	22	0	.0100	47	R VLPFC[a,b]
Control Subjects > ADHD Under Methylphenidate						
13	7	11	40	.025	32	R and L anterior cingulate cortex
12	47	11	20	.025	45/44	R inferior frontal cortex
Control Subjects > ADHD Under Atomoxetine						
20	7	11	40	.02	32/6	R and L SMA/anterior cingulate cortex
23	4	7	46	.02	6	R and L SMA

ADHD, attention-deficit/hyperactivity disorder; ANOVA, analysis of variance; BA, Brodmann area; L, left; R, right; SMA, supplementary motor area; TD, temporal discrimination; VLPFC, ventrolateral prefrontal cortex.

[a] Areas that were no longer observed to be abnormal by atomoxetine. Only the cluster in VLPFC, which was no longer observed under either drug, reached significance after rigorous normalization testing.

[b] Areas that were no longer observed to be abnormal by methylphenidate.

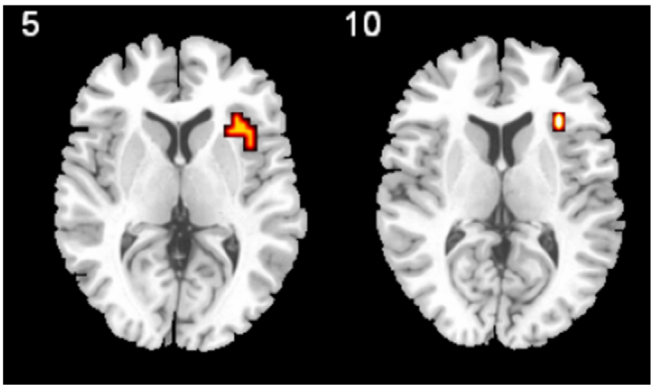

Figure 2. Transversal images of the within-group analysis of variance, showing areas of increased brain activation in boys with attention-deficit/hyperactivity disorder with an acute dose of methylphenidate compared with placebo and atomoxetine during time discrimination contrasted with order judgment. Statistical threshold set at $p < .05$ for voxel-wise and $p < .01$ for cluster-wise analysis. Slices are marked with the z coordinate as distance in millimeters from the anterior-posterior commissure. Mean statistical blood oxygen level–dependent response is shown for each drug condition within ventrolateral prefrontal cortex (Brodmann area 45/47).

ROI Results for Within-Patients Comparison between Placebo, Methylphenidate, and Atomoxetine

Friedman's two-way ANOVA on the preselected ROI regions showed a significant effect of drug condition in right VLPFC/insula activation that reached into the head of putamen, in a similar location to the cluster that was reduced in ADHD under placebo (11 voxels; peak Talairach coordinates [x, y, z]: 40, 19, 4; BA 45/47; $p < .02$) (Figure 2). Within-subjects contrasts revealed that this was attributable to enhanced activation when ADHD boys were under methylphenidate ($p < .004$) and atomoxetine ($p < .02$), relative to placebo. No differences were observed between activations during methylphenidate and atomoxetine.

Correlations between Brain Activation and Performance

There were no significant correlations between TD errors and the statistical measure of brain activation extracted for the cluster of VLPFC activation that showed differences across the three medication conditions in the within-group analysis or for any of the clusters that differed between ADHD and control subjects under either medication condition.

Practice Effects. A repeated measures ANOVA within patients showed that the order of drug administration had no significant effect on TD performance or BOLD response in the VLPFC cluster of activation difference in the within-group analysis (Table S1 in Supplement 1). Although we could not directly measure practice effects in the between-group analyses due to low power, given that there were no differences in the within-group analysis, we assume that they were unlikely to have contributed to performance or brain activation differences between patients and control subjects.

Discussion

This study demonstrates a relatively superior effect of a single dose of methylphenidate compared with placebo and atomoxetine on TD performance in ADHD boys but shared effects on the underlying neurofunctional networks of TD. Compared with control subjects, ADHD boys under placebo made significantly more TD but not TOJ errors and had reduced activation in typical areas of TD in right VLPFC/insula, right IFC, and SMA/ACC. Methylphenidate relative to atomoxetine and placebo significantly decreased TD errors within patients, while only methylphenidate, but not atomoxetine, normalized the TD deficits relative to control subjects. Within-ADHD comparisons showed that both medications significantly upregulated right VLPFC/insula activation compared with placebo. In line with this, case-control comparisons showed that both medications significantly normalized right VLPFC/insula underactivation that was observed in ADHD adolescents under placebo relative to control subjects. The findings show that both methylphenidate and atomoxetine upregulate and normalize a key right ventrolateral fronto-insular area for TD in ADHD.

Attention-deficit/hyperactivity disorder patients had reduced activation relative to control subjects under placebo in key areas that have consistently been found to be involved in time perception in adults (27,28,46–48) and adolescents (48), i.e., in right VLPFC, IFC, insula, SMA, and ACC. The findings replicate, in a larger sample, previous findings of right VLPFC and DLPFC underactivation during the same task in ADHD adolescents (2,6).

The finding of a significant upregulation and normalization effect of methylphenidate on the underactivation in right VLPFC/insula in ADHD boys during placebo replicates previous findings of neurofunctional upregulation and normalization with methylphenidate in this region during the same TD task (2). However, in this study, we show for the first time that the upregulation and normalization effects of methylphenidate on right VLPFC are shared with atomoxetine. Right VLPFC and the insula are key areas of temporal discrimination (3,27), as well as key regions that have been shown to be consistently underactivated in ADHD children during TD (2,6,49) and during other functions such as attention and inhibition (49–53). It has been argued that VLPFC is part of a more generic cognitive control network that subserves several cognitive functions mediated by different VLPFC-striatal neural networks (54), including both executive and timing functions (27).

The normalization effect of right VLPFC underactivation in ADHD relative to control subjects by atomoxetine echoes previous evidence for upregulation with atomoxetine of this region in healthy adults during motor inhibition and performance monitoring (23,24), suggesting similar mechanisms of action of atomoxetine on upregulating right VLPFC activation in healthy subjects and ADHD patients.

The shared normalization effects of both drugs on right VLPFC underactivation in ADHD is intriguing, given the superior performance effects of acute methylphenidate, which parallel the typically faster behavioral effects of this stimulant over atomoxetine (16,55). The findings are important, as they suggest shared mechanisms of action on a key area that has been shown to be consistently underactivated in ADHD patients during timing, as well as during other cognitive functions (7,14,52,53), and that furthermore has been shown to be disorder-specifically underactivated in ADHD relative specifically to other child psychiatric disorders (51,56,57). They extend our recent findings of shared upregulation and normalization effects of both drugs on left VLPFC underactivation in ADHD relative to control boys during a motor inhibition task (25) and may be the underlying mechanism of action for the relatively comparable efficacy rates of both drugs on ADHD behaviors (17,18). These shared effects on ventrolateral frontal regions likely reflect both noradrenergic and dopaminergic mechanisms, given that in frontal regions, both drugs upregulate NE equally or more than DA via reuptake inhibition of NETs that clear DA and NE (8,58–61). These shared neurofunctional modulation effects in VLPFC cortex are hence in line with

the relatively similar catecholaminergic mechanisms of action of both drugs on frontal regions.

It is of note that SMA underactivation was no longer observed under methylphenidate, while right IFC underactivation was no longer observed under atomoxetine. However, neither region survived our rigorous testing for normalization, and hence, unless replicated in future studies, these potential drug-specific modulation effects need to be considered with caution.

The drug-specific enhanced performance effects of methylphenidate are in line with our hypothesis. While the positive effects of dopamine agonists on TD are well documented (26), the relationship between NE and timing functions is less studied, although there is some evidence for improved TD in healthy adults under a single dose of the NE reuptake inhibitor reboxetine (62). These drug-specific performance findings parallel evidence for the immediate behavioral effect of methylphenidate (55) relative to atomoxetine, which takes 6 to 8 weeks to show clinical effects (16). We have previously shown that brain activation is more sensitive than behavior to catecholaminergic drug effects (2,13–15,25), which could explain why brain upregulation effects under atomoxetine appear before behavioral effects. It is possible that methylphenidate operates within a wider DA-innervated network of timing, involving the right VLPFC as well as the DA- innervated striatum and the SMA, which is a crucial region mediating TD (27), whereas the effects of atomoxetine were restricted to right fronto-cortical parts of the network, which may not have been sufficient to elicit performance benefits. However, given that the amelioration effects on SMA with methylphenidate were not significant in our rigorous testing for normalization, this has to remain a speculation.

Future studies should test for comparative effects of both drugs on brain activation and performance under longer chronic administration to adjust for differences in time delays to behavioral effectiveness.

A limitation of the study is the investigation of single rather than chronic doses of each drug. As a single dose challenge, this design reduces long-term confounds such as symptomatic improvement, side effects, or chronic effects on brain activation. However, it may be biased toward methylphenidate, given the immediacy of behavioral effects, while atomoxetine takes maximum clinical efficacy after 6 to 8 weeks of chronic administration (16), possibly explaining normalization of TD performance by methylphenidate only (see above). Future studies should compare long-term effects to accommodate for differences in time to maximum efficacy.

Another limitation is that for ethical reasons, control subjects were only tested once, while patients were tested three times. However, for the within-subjects analysis, potential practice effects were controlled for by the counterbalanced design, and furthermore, no practice effects were observed.

To conclude, to our knowledge, this is one of the first studies to directly compare neurofunctional effects of atomoxetine and methylphenidate in ADHD boys and the first to do so during TD. The study is strengthened by the recruitment of medication-naïve ADHD boys, thus controlling for the confounding long-term stimulant medication effects on brain activation and structure (7,53,63,64). We found that while only methylphenidate normalized TD performance deficits, both drugs showed significant upregulation and normalization effects in a key area of TD in right VLPFC. The findings show that both drugs are equally efficient in upregulating and normalizing right fronto-cortical areas of timing in ADHD.

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