

Activation in Ventral Prefrontal Cortex is Sensitive to Genetic Vulnerability for Attention-Deficit Hyperactivity Disorder

Sarah Durston, Martijn Mulder, B.J. Casey, Tim Ziermans, and Herman van Engeland

Background: Attention-deficit hyperactivity disorder (ADHD) is a heritable neuropsychiatric disorder, associated with atypical patterns of brain activation in functional imaging studies. Neuroimaging measures may serve as an intermediate phenotype in genetic studies of ADHD, as they are putatively more closely linked to gene expression than a clinical diagnosis.

Methods: We used rapid, mixed-trial, event-related functional magnetic resonance imaging (fMRI) to investigate changes in brain activation during a go no-go task in boys with ADHD, their unaffected siblings, and matched control subjects.

Results: On the hardest inhibitory trials in our task, children and adolescents with ADHD had lower accuracy than control subjects, whereas their unaffected siblings did not. Control subjects activated a network of regions, including ventral prefrontal and inferior parietal cortex. Both children and adolescents with ADHD and their unaffected siblings showed decreased activation in these areas, as well as fewer correlations between performance and activation.

Conclusions: These findings suggest that the magnitude of activation during successful inhibitions is sensitive to genetic vulnerability for ADHD in a number of regions, including ventral prefrontal cortex. If this can be replicated in future studies, this suggests that neuroimaging measures related to inhibitory control may be suitable as intermediate phenotypes in studies investigating gene effects in ADHD.

Key Words: ADHD, fMRI, genetic vulnerability, siblings, ventral PFC, endophenotype

Attention-deficit hyperactivity disorder (ADHD) is a heritable neuropsychiatric disorder, with increased incidence in biological relatives of affected individuals. The siblings of children with ADHD have a three- to fivefold increased risk of having ADHD compared to the siblings of healthy control subjects (Biederman et al 1992; Faraone et al 1993) and the risk is even greater for monozygotic twins with 50-80% concordance compared with up to 33% in dizygotic twins (Bradley and Golden 2001). As full siblings share on average 50% of their genes, even the unaffected siblings of children with ADHD would be expected to share some of the genes involved in the disorder. The genes involved are not yet known, although several candidates have been proposed (see Faraone et al 2005; Heiser et al 2004 for review).

One of the key neuropsychological features of ADHD is that affected individuals are impaired on tasks that tap executive functioning, in particular those that involve cognitive, or inhibitory, control (see Sergeant et al 2002; Willcutt et al 2005 for reviews). Studies using go no-go, stop- and Stroop paradigms have consistently shown poorer performance and slower reaction times for children with ADHD relative to healthy controls (e.g. Grodzinsky and Diamond 1992, Schachar 1995; Seidman et al 1995, 1997; for meta-analysis see Oosterlaan 1998). It has been proposed that deficits in executive functioning associated with

ADHD may even be due to a core deficit in inhibitory control (Barkley 1997). In this view, the ability to inhibit inappropriate behaviors is central to the successful execution of other neuropsychological functions, such as working memory (by inhibiting responses to interfering thoughts or external events), goal-directed behavior (acting towards a future reward and inhibiting actions towards immediate gratification) or emotional self-control (by inhibiting emotional responses in favor of more thoughtful reactions). In ADHD, lack of such behavioral control may then explain the deficits in cognitive functioning and impulsive behaviors associated with the disorder. Imaging studies have shown that cognitive or inhibitory control is associated with a pattern of brain activation involving ventral prefrontal areas, anterior cingulate cortex and parietal cortex, where ventral prefrontal activation has been suggested to be particularly critical to successful task performance (e.g., Bellgrove et al 2004; Casey et al 1997; Durston et al 2002a, 2002b, 2003; Garavan et al 1999; Konishi et al 1999; Liddle et al 2001; Rubia et al 2000).

ADHD has been associated with atypical patterns of brain activation relative to healthy controls (see Durston 2003 for a review). Functional imaging studies consistently show atypical activation in individuals with ADHD, in particular in prefrontal regions (Booth et al 2005; Bush et al 1999; Durston et al 2003; Liotti et al 2005; Rubia et al 1999, 2005; Schulz et al 2004, 2005; Schweitzer et al 2000; Tamm et al 2004; Vaidya et al 1998; Zang et al 2005). While most of these studies show hypofrontality, some have shown localized increases in activation for some prefrontal regions, suggesting that the more frequently reported hypofrontality may be region or task-specific, and that behavioral improvement on medication, and possibly the remission of ADHD symptoms in adolescence may be related to prefrontal increases in activation (e.g., Durston et al 2003; Schultz et al 2004, 2005; Vaidya et al 1998).

Neuroimaging measures may potentially serve as an intermediate phenotype in genetic studies of ADHD (Castellanos and Tannock 2002). Intermediate or endo-phenotypes are putatively more closely related to gene expression than a psychiatric diagnosis, such as ADHD and as such, the effects of risk genes may be greater in these intermediate measures, making them

From the Rudolf Magnus Institute of Neuroscience (SD, MM, TZ, HvE), Department of Child and Adolescent Psychiatry, University Medical Center Utrecht, Utrecht, The Netherlands; and the Sackler Institute for Developmental Psychobiology (SD, BJC), Weill Medical College of Cornell University, New York, New York.

Address reprint requests to Sarah Durston Ph.D., Rudolf Magnus Institute of Neuroscience, Department of Child and Adolescent Psychiatry, Neuroimaging Lab, HP A 01.468, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; E-mail: S.Durston@azu.nl.

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easier to detect. In order to qualify as a potential endophenotype, neuroimaging measures should meet several criteria, such as being heritable, being stable over time, as well as being grounded in neuroscience (Castellanos and Tannock 2002). In ADHD, catecholaminergic modulation of prefrontal functioning may be a good candidate, as it has been implicated in numerous studies of ADHD (see Durston 2003 for review). We previously showed that unaffected siblings of boys with ADHD display subtle reductions in cortical gray matter volume, including in prefrontal cortex, comparable to their affected siblings (Durston et al 2004), suggesting that MRI-based brain measures may be suitable as intermediate phenotypes in ADHD research. To further examine this association, we assessed catecholaminergic gene effects on gray matter volumes, and were able to link prefrontal gray matter to a dopamine gene expressed preferentially in that region (DRD4), as well as show a similar effect in the striatum for a gene expressed preferentially in that region (DAT1) (Durston et al 2005). Recently, it has been suggested that inhibitory control and associated activation in inferior frontal gyrus (IFG) may serve as an endophenotype for ADHD, as this appears to be a stable and heritable trait in this disorder (Aron and Poldrack 2005). Therefore, we set out to address this question by investigating whether changes in inhibitory control and associated brain activation in ADHD could be found in individuals with a genetic vulnerability for the disorder, but free of symptomatology.

In a previous study, we used a go no-go task to show that children with ADHD display atypical patterns of brain activation compared to typically developing children (Durston et al 2003). In that study, we manipulated task difficulty by systematically varying the number of go-trials preceding no-go trials (Durston et al 2002a, 2002b). This manipulation provided an opportunity to examine trials on which the groups had comparable performance. As such, we showed that differences in activation were not solely due to differences in performance of the task. In the present study, we used the same task to investigate inhibitory control and brain activation patterns in a new sample of boys with ADHD, their unaffected siblings and matched control subjects. To maximize our ability to separate ADHD symptoms (in probands) from genetic vulnerability (in probands and unaffected siblings), we required 1) affected siblings to be free of medication at the time of the scan, and 2) unaffected siblings to be free of ADHD-symptomatology, as assessed by structured interview and symptom-rating scales.

Methods and Materials

Subjects

A total of 41 boys, aged 8 to 20 years, participated in the current study. This included 33 individuals who had previously participated in a structural MRI study at our department (Durston et al 2004). Sibling pairs were recruited through the University Medical Center in Utrecht, whereas controls were recruited through schools in the area. All assessments and study participation were conducted in Dutch. After a complete description of the study, written informed consent was obtained from a parent for all subjects. In addition, all subjects were asked to sign for assent. The procedure was approved by the Central Committee on Research involving Human Subjects in the Netherlands. Subjects with major physical or neurological illness, such as migraine, epilepsy, endocrine disorders, head trauma in the past or IQ of below 70 were excluded. All subjects that met the inclusion criteria were asked to participate in a one-hour func-

tional magnetic resonance imaging (fMRI) scanning session and a neuropsychological assessment in order to estimate full-scale IQ (Similarities, Vocabulary, Block Design and Object Assembly subtests of the Wechsler Intelligence Scale for Children - Revised [WISC-R]) (Wechsler 1974). (Dutch norms were not available for more recent versions of the WISC at the time this study was conducted.) For each subject a parent was asked to participate in a semi-structured interview session with a trained rater to objectively determine psychiatric diagnosis (Diagnostic Interview Schedule for Children [DISC-P]) (Shaffer et al 2000). In addition, parents were asked to fill out the Child Behavior Checklist (CBCL) (Achenbach and Edelbrock 1983). ADHD subjects were required to meet DSM-IV criteria (American Psychiatric Association 1994) for ADHD, as assessed by DISC-interview. Subjects with co-morbid disorders other than oppositional defiant disorder (ODD) were excluded. Unaffected siblings and control subjects were excluded if they met DSM-IV criteria for any psychiatric diagnosis, as assessed by DISC interview. In addition, they were excluded if they scored in the clinical range on the CBCL. Control subjects were excluded if they had first-degree relatives who had been diagnosed with ADHD or another disruptive disorder. Functional MRI data from eight subjects were excluded from further analysis, due to excessive motion or, in one case, artifact on the MRI scan caused by dental implants. Data for 11 discordant sibling pairs and 11 matched control subjects were included in the fMRI analyses. Subjects were matched at a group level for age, IQ, and socio-economic status (operationalized as parental education level) for both samples. Ten of 11 subjects with ADHD met DISC-criteria for ADHD, combined subtype. One subject scored subthreshold on the inattention scale of the DISC (4 symptoms) and therefore met criteria for ADHD, hyperactive subtype. In addition, three of the ADHD subjects met DISC-criteria for ODD. There were significant differences between groups on several CBCL scales, including ADHD and attention problems scales. Subjects with ADHD had higher mean values on all significantly different measures (all $F > 4.0$; $p < .03$ for subjects with ADHD; $p > .05$ for unaffected siblings). Six of 11 subjects with ADHD were on stimulant medication at the time they were approached for this study. All discontinued medication for at least 24 hours prior to the scan (see Table 1).

Paradigm

All subjects participated in an fMRI session using a go no-go paradigm as previously described (Durston et al 2002a, 2002b, 2003, 2006). The subjects' task was to press a button in response to visually presented stimuli, but to avoid responding to a rare nontarget. The task consisted of 5 runs, which lasted 3 min and 56 sec each. Each run contained a total of 57 trials, with 75% go trials, resulting in a total of 70 no-go trials, including 20 of each type (with 1, 3 or 5 preceding go trials) per subject. Foil trials (no-go trials after 2 or 4 go trials) were also included, to prevent subjects learning the pattern. The order of presentation of the different types of no-go trials was pseudorandomized. In order to make the task more interesting for children, characters from the Pokemon cartoon series were used as stimuli. Stimulus duration was 500 msec and the interstimulus interval was 3500 msec (total trial length = 4000 msec). Stimuli were projected using a through-projection screen and slide projector. Responses were collected using an MRI compatible air pressure button box.

Analysis of Behavioral Data

All behavioral data were analyzed using the SPSS statistical package (version 11.5, SSPS Inc., Chicago, Illinois). Differences

Table 1. Descriptive Variables per Group

	Controls	Unaffected Siblings	Siblings with ADHD
Age (years)	15.27 (1.92) 12.70–19.00	14.45 (2.58) 10.50–20.40	13.97 (3.14) 8.20–18.20
Total IQ	106 (14) 91–127	107 (15) 80–127	100 (10) 86–114
Verbal IQ	100 (19) 76–124	105 (14) 80–128	99 (16) 74–124
Performance IQ	110 (10) 95–125	108 (20) 85–152	101 (12) 88–123
Father's Education (years)	10.9 (3.9) 6.0–17.0	12.2 (2.4) 10.0–15.0	12.2 (2.4) 10.0–15.0
Mother's Education (years)	111.8 (2.5) 10.0–17.0	111.3 (1.9) 10.0–15.0	111.3 (1.9) 10.0–15.0
Hand preference (no. lefthanded)	0	0	2
ADHD (no. meeting criteria)	0/11	0/11	10/11 combined 1/11 hyperactive
ODD (no. meeting criteria)	0/11	0/11	3/11
ADHD Symptoms (no. on DISC)			
Inattentive	.8 (.8) 0–2	.0 (.0) 0–0	6.5 (2.1) ^b 4–9
Hyperactive/Impulsive	.4 (.7) 0–2	.3 (.9) 0–3	7.4 (1.4) ^b 6–9
CBCL Score			
ADHD	2.0 (1.5)	2.7 (2.3)	7.3 (3) ^b
ODD	1.5 (1.6)	2.0 (2.1)	4.5 (1.8) ^b
Conduct disorder	.5 (.8)	2.0 (2.5)	5.3 (4.1) ^b
Affective problems	1.0 (1.8)	1.0 (1.2)	3.8 (3.8) ^a
Anxiety	.4 (.5)	.9 (1.0)	1.9 (1.6) ^a
Somatic problems	.3 (.5)	.6 (.7)	1.4 (1.4) ^a
Attention problems	2.6 (2.0)	2.6 (2.9)	4.1 (3.5) ^b
Delinquency	.8 (.8)	2.5 (2.5)	5.0 (3.0) ^b
Aggression	1.9 (2.5)	3.0 (4.3)	4.9 (5.5) ^b
Withdrawn	1.2 (1.7)	2.0 (1.8)	2.6 (2.9)
Anxious-depressed	.7 (1.1)	1.7 (1.9)	4.0 (3.2) ^b
Social problems	.5 (.8)	1.2 (2.0)	1.9 (3.0) ^b
Thought problems	.5 (.7)	.7 (1.1)	4.2 (3.5) ^b
Somatic complaints	.4 (.7)	.9 (.8)	1.3 (1.9) ^b
Methylphenidate at Scan, no/group	0/11	0/11	6/11
Performance on Task			
Reaction time (msec)	587 (66)	567 (102)	581 (94)
Accuracy (overall)	.92 (.06)	.88 (.08)	.84 (.12)
after 1 go trial	.96 (.04)	.88 (.12)	.89 (.09)
after 3 go trials	.88 (.12)	.86 (.09)	.86 (.12)
after 5 go trials	.90 (.09)	.87 (.07)	.79 (.17) ^a

Values are mean (standard deviation) and range. ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; CBCL, Child Behavior Checklist; DISC, Diagnostic Interview Schedule for Children.

^a $p < .05$.

^b $p < .01$.

in behavior were investigated in the total sample of 41 subjects, as well as in the smaller sample of 33 subjects for whom imaging data were included. Differences were investigated using a two-tailed General Linear Model (multivariate) analysis of variance ([M]ANOVA), with separate dummy variables coding for ADHD and sibling status (Durston et al 2004). As we hypothesized that there would be a correlation between the number of errors on no-go trials and the number of preceding go-trials, we investigated this using Fisher's R to Z transformation and a one-tailed Z -test. As the age-range included in this study was quite wide, and we have previously shown that the ability to perform this task increases with age (Durston et al 2002b), we re-ran all behavioral and functional MRI analyses including age as a co-variate.

Scan Acquisition

All subjects participated in a practice session prior to scanning, using an MRI simulator, housed at the Department of Child and Adolescent Psychiatry, at the University Medical Center in Utrecht, the Netherlands. The purpose of this session was to acquaint subjects with the scanner environment, the task, and the researchers present during the MRI-session. All subjects successfully participated in both the practice and actual MRI sessions. MRI images were acquired on a 1.5-T Philips Gyroscan (Philips Medical Systems, Best, the Netherlands), housed at the Department of Radiology in the same hospital. Functional MRI scans consisted of a navigated three-dimensional (3D)-PRESTO pulse sequence (time to echo [TE] 11 msec, repetition time [TR] 21.74 msec, flip angle 9.0°, matrix 64 × 64 × 24, field of view [FOV]

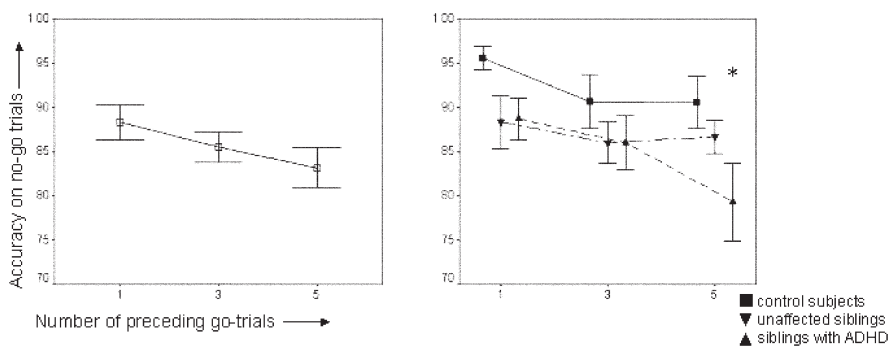


Figure 1. Accuracy on no-go trials for the group as a whole (left panel) and control subjects, children and adolescents with ADHD and their unaffected siblings separately (right panel). In the left panel there is a significant relationship between the number of preceding go-trials and accuracy on no-go trials for the group as a whole. In the right panel accuracy is significantly lower for individuals with ADHD for no-go trials preceded by five go-trials. Differences for other types of no-go trials do not reach significance. ADHD, attention deficit hyperactivity disorder.

256 × 256 × 96 mm, voxel size 4 mm isotropic, and scan duration 2.0 sec per 24-slice volume), covering the whole brain. Anatomical T1-weighted 3D fast field echo (FFE) scans with 130 to 150 1.5 mm contiguous coronal slices of the whole head (TE 4.6 msec, TR 30 msec, flip angle 30°, FOV 256 mm, in plane voxel size 1 mm × 1 mm) were also acquired. A FA30 scan with contrast more similar to the T1 weighted scans was also acquired to aid in the alignment of PRESTO images to the template (TE 12.10 msec, TR 24.24 msec, flip angle 30°, matrix 64 × 64 × 24, FOV 256 × 256 × 96 mm, voxel size 4 mm isotropic). During anatomical scans the projection system was used to play cartoons, to prevent the subjects becoming bored or restless.

Functional MRI Analysis

All data were analyzed using a random effects model in Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neuroscience, London). PRESTO images were realigned and normalized to a standard stereotactic space (Montreal Neurological Institute (MNI) template). Estimated motion parameters were examined on a subject-by-subject basis to ensure that the amount of absolute motion did not exceed 4 mm, or the size of 1 voxel. There were no differences between groups in the average amount of motion.

At the first level, six event types were defined (initial fixation, correct and incorrect go trials and no-go trials and a parametric factor representing the number of go trials preceding a no-go trial (1-5)). These included three effects of interest (go trials, no-go trials the parametric factor) and three effects of no interest (initial fixation, omission errors and commission errors). The event types were time-locked to stimulus by a canonical synthetic haemodynamic response function (HRF) and its first-order temporal derivative.

For the second-level analysis, random effects analyses were performed for each group separately. The first analysis compared no-go to go-trials (one-sample t-test), whereas the effect of the parametric contrast was evaluated in a second analysis, using a one-way ANOVA with no-go and parametric factors entered (see Henson et al 2002). Differences in activation were tested at a threshold of *p* < .001, uncorrected, with a minimum extent of 10 voxels. Correlations between MR-signal change and performance on the task were investigated in an ANCOVA-design, with performance entered as the co-variate. Changes in MR signal related to task performance were then investigated using a masking approach, where the nogo > go contrast was calculated using brain activation associated with performance as an inclusive mask at a more lenient threshold (*p* < .01; extent 5 voxels).

For the between-group analysis, three planned contrasts were performed, comparing subjects with ADHD to controls, siblings to controls and subjects with ADHD to their unaffected siblings.

Whole-brain, between-group analyses were followed up by a region-of-interest (ROI) analysis, implemented in the MarsBaR package (Brett et al 2002) to investigate changes between groups. ROIs included all voxels activated by the control group in the no-go > go condition at the threshold of *p* < .001 with minimum extent of 10 voxels.

As the age-range included in this study was quite wide, all functional MRI analyses were re-run including age as a covariate. MNI stereotactic coordinates were transformed to Talairach and Tournoux space.

Results

Behavioral Results

In the total sample of 41 subjects, there was a significant increase in errors on no-go trials as a function of the number of preceding go trials (*r* = .17; *p* = .034; see Figure 1). This increase did not reach significance for individual subgroups (control subjects, children and adolescents with ADHD, or their unaffected siblings; *p* > .05). There was a trend for differences in accuracy on no-go trials between groups (*F* = 3.76, *p* = .061 for children and adolescents with ADHD and *F* = 1.12, *p* = .30 for their unaffected siblings) that was largely due to a significant difference in the no-go after 5 preceding go-trials condition (*F* =

Table 2. Regions of Activation for Controls

Region	Side	BA	Talairach	Max T-value
Go > No-go				
Precentral G	L	4	-55, -25, 53	7.22
Cerebellum	R		24, -45, -41	8.39
No-go > Go				
Inf frontal G ^a	R	47	40, 20, -8	11.12
	L	47	-36, 19, -7	6.41
Ant cingulate G	R	24/32	4, 40, 16	4.98
	L	24/32	-8, 40, 24	4.57
Inf/mid frontal G	L	10/46	-36, 43, 13	6.82
Mid frontal G	R	9	44, 13, 36	6.11
Premotor cortex	L	6	-44, 6, 51	5.89
Mid/sup frontal G	R	10	28, 55, 8	5.68
Inf parietal lobule ^a	L	40	-60, -49, 29	8.04
Parametric Effect of Preceding Number of Go Trials				
Inf frontal G	R	44	55, 1, 26	4.92
Cingulum	L		-16, -28, -12	5.45
			-36, -31, -2	4.64

Values are *p* < .001; min extent 10 vox; *T* > 4.14. G, gyrus; Ant, anterior; Inf, inferior; Mid, middle; Sup, superior; BA, Brodmann's area; MR, magnetic resonance.

^aRegions where MR signal change on no-go trials correlated with no-go accuracy.

Table 3. Regions of Activation for Unaffected Siblings of Children and Adolescents with ADHD

Region	Side	BA	Talairach	Max <i>T</i> -value
Go > No-go				
Precentral G	L	4	–55, –20, 38	8.56
Cerebellum	R		40, –48, –18	6.65
Sup temporal G	L	22	–55, 0, 4	9.90
No-go > Go				
Inf frontal G ^a	R	44	44, 16, 14	15.05
Ant cingulate G	R	32	4, 40, 16	7.06
	L	24/32	–27, –23, –1	6.15
Inf parietal lobule	R	40	51, –56, 43	9.69
Sup/Mid temporal G	R	21/38	44, 2, –30	4.96
Parametric Effect of Preceding Number of Go Trials				
Inf frontal G	R	47	36, 31, –5	6.62
Cingulum	L		–4, –15, 4	5.71

Values are $p < .001$; min extent 10 vox; $T > 4.14$. G, gyrus; Ant, anterior; Inf, inferior; Mid, middle; Sup, superior; ADHD, attention deficit hyperactivity disorder; BA, Brodmann's area; MR, magnetic resonance.

^aRegions where MR signal change on no-go trials correlated with no-go accuracy.

4.72, $p = .03$ for children and adolescents with ADHD and $F = .57$, $p = .46$ for their unaffected siblings; see Table 1 and Figure 1). There were no significant differences between groups on go-trials or reaction time between groups, even when age was included as a covariate ($p > .05$).

In the sample of 33 subjects for whom fMRI data were included in the analyses, differences between groups in accuracy and reaction time did not reach significance, even after including age as a covariate ($p > .05$). The increase in the number of errors to no-go trials as a function of the number of preceding go-trials no longer exceeded trend level ($r = .14$; $p = .08$).

Functional MRI Results

All groups showed increased activation in left motor cortex and right cerebellum for the go versus no-go comparison (see Table 2, 3 and 4). For the no-go versus go condition, control subjects showed increased activation bilaterally in inferior frontal gyrus (IFG), anterior cingulate gyrus (ACG), regions in the middle and superior frontal gyri and the left inferior parietal lobe, similar to patterns of activation previously described (Durston et al 2002a, 2002b, 2006). Regions in the right inferior frontal gyrus and in the left cingulum area showed an increase in activation with increasing number of preceding go-trials. MR signal change correlated with performance for regions in the right inferior

Table 4. Regions of Activation for Children and Adolescents with ADHD

Region	Side	BA	Talairach	Max <i>T</i> -value
Go > No-go				
Precentral G	L	4	–55, –25, 49	5.91
Cerebellum	R		20, –51, –18	5.40
No-go > Go				
Mid frontal G	R	8	48, 18, 43	6.18
Inf parietal lobule ^a	R	40	44, –40, 46	5.47
Parametric Effect of Preceding Number of Go Trials				
None				

Values are $p < .001$; min extent 10 vox; $T > 4.14$. G, gyrus; Ant, anterior; Inf, inferior; Mid, middle; Sup, superior; ADHD, attention-deficit hyperactivity disorder; BA, Brodmann's area; MR, magnetic resonance.

^aRegions where MR signal change on no-go trials correlated w/ no-go accuracy.

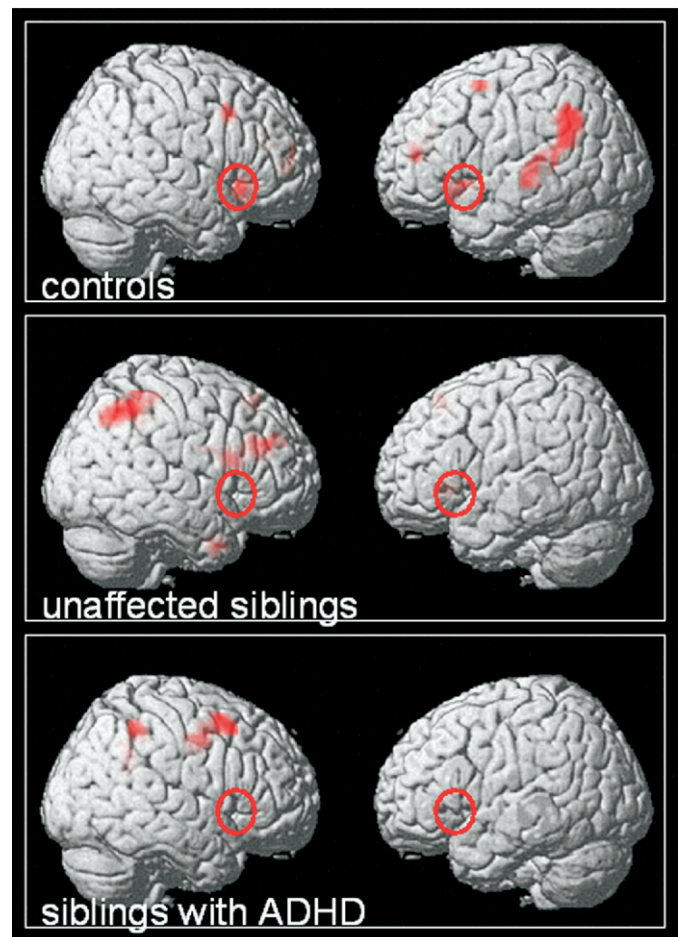


Figure 2. Patterns of activation for no-go > go trials for control subjects, unaffected siblings of children and adolescents with ADHD and children and adolescents with ADHD. Red circles indicate bilateral inferior frontal gyrus, a region critical to performance of this task in control subjects, as activation in this region correlates with performance (40, 20, –8) and is sensitive to manipulations of task difficulty. All images are thresholded at $T > 4.14$; $p < .001$; extent > 10 voxels. ADHD, attention deficit hyperactivity disorder.

frontal gyrus and left parietal lobe (see Table 2; Figures 2 and 3). Unaffected siblings of children and adolescents with ADHD showed increased activation in right inferior frontal gyrus, bilateral anterior cingulate gyrus, the right inferior parietal lobule and the right superior temporal gyrus. Here again, the only regions to show an effect of increasing number of preceding go-trials on activation levels were regions in the right inferior frontal gyrus and in the left cingulum area. Similar to the controls, MR signal change correlated with performance for the region in the right IFG only (see Table 3; Figures 2 and 3). Children and adolescents with ADHD showed increased activation in right middle frontal gyrus and right inferior parietal lobule for no-go versus go trials, but not in inferior frontal gyrus or anterior cingulate gyrus. No regions showed an effect of increasing number of preceding go-trials for this group, but MR signal change correlated with performance for the region in the inferior parietal lobule (see Table 4; Figures 2 and 3).

Whole-brain analyses yielded no regions that were significantly different between groups at the $p < .001$, extent, 10 voxels threshold. A region of interest approach, investigating differ-

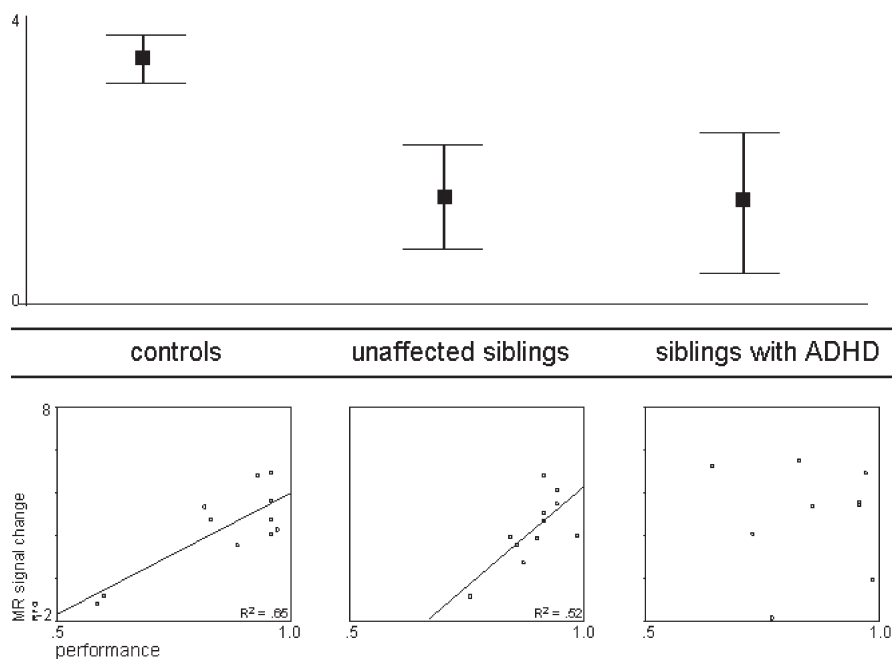


Figure 3. Signal change in right (R) IFG for controls, unaffected siblings, and siblings with ADHD (top panel) and correlations with performance on this task in this region (bottom panel). For the purpose of comparison, signal change was also plotted against performance for siblings with ADHD, although there was no significant correlation. For this group, individual MR signal change values are taken from the ROI defined in the control comparison. ADHD, attention deficit hyperactivity disorder; IFG, inferior frontal gyrus; MR, magnetic resonance; ROI, region of interest.

ences between groups in regions that were significantly activated for the control group for no-go trials compared to go-trials, showed greater activation for controls than children and adolescents with ADHD in inferior frontal gyrus, anterior cingulate gyrus, middle frontal gyrus and inferior parietal lobule. Activation was greater for controls than unaffected siblings in regions in inferior frontal gyrus, middle frontal gyrus and inferior parietal lobule, but not anterior cingulate gyrus. There were no regions where activation was greater for either siblings with or without ADHD than controls. Differences between siblings with and without ADHD were not significant in any region (see Table 5).

Patterns of activation from the analyses treating age as a

Table 5. Significant Differences in Activation Between Children and Adolescents with ADHD, and Their Unaffected Siblings and Control Subjects (for nine clusters from NO-GO > GO condition for controls). G = gyrus, Ant = anterior, Inf = inferior, Mid = middle, Sup = superior.

Region	Side	BA	Talairach	T-value
Controls > ADHD				
Inf frontal G	L	47	-36, 19, -7	2.80
Ant cingulate G	L	24/32	-8, 40, 24	2.25
Premotor cortex	L	6	-44, 6, 51	1.86
Mid/sup frontal G	R	10	28, 55, 8	1.93
Inf parietal lobule	L	40	-60, -49, 29	1.90
Controls > Unaffected Siblings				
Inf frontal G	R	47	40, 20, -8	2.41
Mid frontal G	R	9	44, 13, 36	3.26
Premotor cortex	L	6	-44, 6, 51	2.07
Inf parietal lobule	L	40	-60, -49, 29	4.38
Unaffected Siblings > ADHD				
None				
ADHD > Unaffected Siblings				
None				

Values are for nine clusters from No-go > Go condition for controls. G, gyrus; Ant, anterior; Inf, inferior; Mid, middle; Sup, superior; ADHD, attention-deficit hyperactivity disorder; BA, Brodmann's area.

co-variate were similar to the initial results, and thus not reported separately.

Discussion

In this study, we investigated the effect of genetic vulnerability for ADHD on performance of and brain activation during an inhibitory control task. We showed that unaffected siblings of children and adolescents with ADHD displayed no significant detriment in their performance of this task, whereas children and adolescents with ADHD showed decreased accuracy in the hardest condition of this task. Furthermore, we show differences in their patterns of brain activation, where both children and adolescents with ADHD and their unaffected siblings showed less activation than control subjects in regions in ventral prefrontal and inferior parietal cortex. For control subjects, IFG appeared central to successful inhibitory control in this task, as activation in this region was related to both accuracy on no-go trials and task difficulty. Both children and adolescents with ADHD and their unaffected siblings showed decreased activation in this region, relative to controls. However, for unaffected siblings, activity in this region was associated with performance and task difficulty, similar to controls, whereas for individuals with ADHD, it was not.

Behaviorally, all subjects in this study showed an effect of task difficulty on performance, as accuracy on no-go trials decreased as a function of the number of preceding go-trials. This finding is consistent with previous studies using this manipulation (Durston et al 2002a, 2002b, 2003, 2006). However, this effect did not reach significance for the individual sub-groups, probably due to limited power (see Figure 1). There was a trend-level decrease in accuracy for individuals with ADHD that was significant for the hardest condition of this task. As such, these results are consistent with the numerous reports in the literature of impaired inhibitory control in individuals with ADHD (e.g. Grodzinsky and Diamond 1992; Schachar et al 1995; Seidman 1995, 1997; for meta-analysis see Oosterlaan 1998).

On successfully inhibited no-go trials, control children activated a network of regions, including IFG, ACG and the parietal cortex, very similar to previous normative pediatric imaging

studies (Durston et al 2002b; in press). Furthermore, those studies showed a correlation between performance on this inhibitory control task and activation in IFG and inferior parietal cortex, suggesting that these areas are central to performance of this task, at least in typically developing children. In addition, activation in IFG increased on no-go trials as a function of the number of preceding go-trials, confirming previous reports that this region is critical for inhibitory control, in the context of this (Durston et al 2002a, 2002b, 2006) and other inhibitory tasks (e.g., Bellgrove et al 2004; Casey et al 1997; Durston et al 2003; Garavan et al 1999; Konishi et al 1999; Liddle et al 2001; Rubia et al 2000).

For children and adolescents with ADHD, successful inhibition on no-go trials was not significantly associated with activation of IFG (see Figure 2). Furthermore, no regions were sensitive to the manipulation of no-go difficulty, and the only region where activation was correlated with performance on this task was in parietal cortex. This confirms our previous report of atypical activation in prefrontal and cingulate cortex for individuals with ADHD (Durston et al 2003), as well as other reports of atypical prefrontal activation in ADHD (Booth et al 2005; Bush et al 1999; Liotti et al 2005; Rubia et al 1999, 2005; Schulz et al 2004, 2005; Schweitzer et al 2000; Tamm et al 2004; Vaidya et al 1998; Zang et al 2005). The lack of significant activation of IFG, taken together with the association between parietal activation and performance, suggest that individuals with ADHD may be activating only part of the network associated with this task in controls, or may be relying on additional brain areas to perform this task. Parietal cortex is associated with attentional processes, and activation in this region in the ADHD group may represent compensatory activation, as may the activation in more dorso-lateral prefrontal areas that are implicated in working memory, and other tasks of executive functioning.

Interestingly, unaffected siblings of individuals with ADHD did show a correlation between task performance and IFG activation similar to the control subjects (see Figure 3), as well as a similar association between MR signal change in IFG and no-go difficulty. However, they did not show any association between performance and activation in parietal cortex. In a direct comparison of activation between groups, the control subjects showed significantly more activation in IFG than both children and adolescents with ADHD and their unaffected siblings, whereas there was no difference between the affected and unaffected siblings. Taken together, these results suggest that the unaffected siblings may be employing the prefrontal-parietal network in a manner that is qualitatively similar to control subjects, but less efficiently, as the magnitude of activation in IFG is reduced and correlations with performance do not reach significance in parietal areas. The unaffected siblings included in this study did not have behavioral symptoms of ADHD, as assessed by symptom rating scales and parental interview. As such, these results suggest that the observed changes in IFG and parietal cortex are related to familial risk for ADHD, rather than subthreshold symptomatology. However, as unaffected siblings do show modulation of activation in IFG in response to task difficulty, as well as a relationship between activation and performance in this region, the effect on IFG function may be less severe for at-risk individuals.

Direct comparisons with control subjects were very similar for siblings with and without ADHD, as both groups displayed reductions in activation for no-go trials in prefrontal and parietal regions. Interestingly, the only region that differentiated between the groups was the ACG, where activation was significantly

reduced for subjects with ADHD compared to controls, but not for their unaffected siblings. Tentatively, this could be taken to suggest that activation in this region may be related to compensatory or spared functioning in the unaffected siblings. However, a direct comparison between siblings with and without ADHD did not reach significance in the current study. Therefore this result should be treated with caution until it can be confirmed in a larger sample.

Another noticeable finding was that activation in parietal cortex was left-lateralized for control subjects, but right-lateralized for both affected and unaffected siblings. Potentially, the right-hemisphere findings could be related to compensatory mechanisms with increased activation in attentional areas in both sibling-groups. However, these findings are preliminary and should be treated with caution until they have been confirmed in other studies.

Although our results are consistent with both the inhibitory control and the ADHD literature, there are a number of limitations in our study that need to be acknowledged. First, although the number of subjects included in this study is not atypical of the functional neuroimaging literature, the sample size is relatively small with 11 subjects in each group. As such, we cannot exclude the possibility of type II errors, where we may have missed existing differences. Indeed, differences between groups only reached significance in the post-hoc ROI-analysis, while the between-group whole-brain comparisons yielded no significant results. Second, we have used group averages rather than investigating differences at an individual level. As such, we cannot rule out that differences in activation between groups could be related to increased anatomic variability in the ADHD and unaffected sibling samples, rather than functional differences. An approach that combines anatomical and functional imaging may be better able to tease apart anatomical and functional differences in ADHD. However, our previous study of brain anatomy suggests that volumetric reductions of cortical gray matter are subtle and widespread in both affected and unaffected siblings (Durston et al 2004). It seems unlikely that such global differences in anatomy should result in relatively focal differences in activation, in particular as global scaling procedures, such as implemented in SPM2, remove gross differences between groups. A related concern is the use of a standard adult template, as well as the Talairach atlas in pediatric populations, as adult neuroanatomy does not necessarily generalize well to children. However, there is evidence to suggest that anatomic differences between school-aged children and adults are only modest in their effects on detecting functional differences (Burgund et al 2002). Furthermore, the age range of the sample included in this study spans 12 years and extends into early adulthood, meaning that a child or adolescent template brain would have been equally problematic. Third, although subjects with ADHD were not on medication, or discontinued treatment prior to participating in an MR scan, most were not stimulant-naive. Therefore we cannot rule out the possibility that some of the observed changes in brain activation are due to long-term effects of stimulant medication. However, the unaffected siblings in this study had never taken stimulants, suggesting that changes shared between both groups are unlikely to be fully explained by stimulant treatment. Nevertheless, this point does stress the need for studies of medication-naive subjects, preferably including large samples and multiple methodologies to tease apart these issues. Finally, we have investigated full siblings of individuals with ADHD. As the genetic relatedness is similar (approximately 50%) for all sibling-pairs in this study, we

cannot estimate the heritability of phenotypic measures, such as reduced IFG activation, based on these data. Future studies including both monozygotic and dizygotic twin pairs discordant for ADHD will be better able to address this issue.

In summary, we have shown that individuals with ADHD show detriments in performance and atypical patterns of brain activation during an inhibitory control task. During successful inhibitions, they activate regions in ventral prefrontal cortex less than control subjects. For control subjects, ventral prefrontal and inferior parietal regions appear to be critical in inhibitory control, as activation in these regions is correlated with performance on this task. Unaffected siblings of children and adolescents with ADHD did not show the behavioral deficit evident in their affected counterparts. Furthermore, the relationship between activation in ventral prefrontal cortex and task performance was similar to that for control subjects. However, reductions in activation in a number of regions, including ventral prefrontal areas were similar to those in subjects with ADHD, and there were fewer significant correlations between performance and activation. These findings suggest that the magnitude of activation during successful inhibition is sensitive to genetic vulnerability to ADHD in a number of regions, including ventral prefrontal cortex, even in the absence of differences at a behavioral level. If this can be replicated in future studies, these results suggest that neuroimaging measures related to inhibitory control may be suitable as an intermediate phenotype in studies investigating gene effects in ADHD.

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