fMRI activation during response inhibition and error processing: the role of the DAT1 gene in typically developing adolescents and those diagnosed with ADHD.

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Running title: DAT1 effects on response inhibition in ADHD

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Abstract

The DAT1 gene codes for the dopamine transporter, which clears dopamine from the synaptic cleft, and a variant of this gene has previously been associated with compromised response inhibition in both healthy and clinical populations. This variant has also been associated with ADHD, a disorder that is characterised by disturbed dopamine function as well as problems with response inhibition. In the present study we used fMRI to investigate the role of dopaminergic genetic variation on executive functioning by comparing how activation associated with successful and unsuccessful inhibitions differs based on DAT1-genotype and ADHD-diagnosis in adolescents performing a go/nogo task. The results identify regional specificity concerning which functional differences can be attributed to the possession of the high risk DAT1 genotype, the clinical condition or an interaction between the two. During response inhibition, individuals with two copies of the 10-repeat allele showed increased activation in frontal, medial, and parietal regions, which may indicate that inhibition is more effortful for this group. Conversely, this group displayed a reduced error response in the parahippocampal gyrus, suggestive of reduced learning from errors. There were also a number of frontal, parietal, medial and occipital regions, where the relationship between genotype and fMRI-activation differed between the ADHD group and typically developing adolescents. Finally, the ADHD group displayed decreased activation in parietal and (pre)frontal regions during response inhibition, and in frontal and medial brain regions on error trials.

Introduction

Controlling and inhibiting actions are important cognitive abilities that allow us to function successfully in a changing and complex environment. Individual differences in executive functioning such as response inhibition and error monitoring have been related to genetic variation (Goldberg & Weinberger, 2004), for example, by studying unaffected family members of clinical groups with deficits in response inhibition (Slaats-Willemse et al., 2003) and by comparing groups who differ in genotype. Earlier studies suggest an important role for the dopamine system in response inhibition: typically developing children homozygous for the 10-repeat allele of DAT1 perform less well on a test of response inhibition (Cornish et al., 2005), though this effect can be modulated by genotypic variation on other dopaminergic genes such as DRD4 (Congdon, Lesch & Canli, 2007). In this study we investigate the role of dopaminergic functioning in response inhibition and error-monitoring, by comparing the effects of variation in DAT1-genotype in typically developing adolescents and in adolescents diagnosed with ADHD.

Attention Deficit/Hyperactivity Disorder (ADHD) is a childhood disorder characterised by symptoms of inattention, hyperactivity and impulsivity (DSM-IV). The disorder is relatively common, with between 3-5% of school-age children affected (Buitelaar, 2002). ADHD symptoms persist into adulthood in about 65% of cases (Faraone, Biederman, & Mick, 2006) and individuals with ADHD often show impairments of executive function, including the ability to control and inhibit behaviour (Nigg, 2005).

ADHD is thought to have a strong genetic component, with additive effects of multiple genes explaining around 80% of an individual's susceptibility to ADHD

(Albayrack, Friedel, Schimmelmann, Hinney & Hebebrand, 2008). More than a decade of molecular genetics research into ADHD has confirmed associations of small effect size for a number of candidate genes, including those for the dopamine transporter (DAT1) (Mick & Faraone, 2008). Since individual genetic influences on clinical phenotypes, such as ADHD, are likely to be small and potentially heterogeneous, recent studies have sought to link DNA variation in candidate genes to objective constructs such as a specific psychological process or markers of brain function that are hypothesised to lie intermediate between gene and disorder.

Within the cognitive genetics literature of ADHD, multiple lines of evidence suggest that executive function measures, such as response inhibition, might index susceptibility to ADHD. First, recent twin studies have demonstrated a remarkably strong genetic contribution to executive functions, including response inhibition (Friedman et al., 2008). Second, familial risk profiles for response inhibition deficits in ADHD have also been established. Response inhibition deficits are more pronounced in ADHD probands of affected as compared to non-affected parents (Crosbie, Pérusse, Barr & Schachar, 2008). Non-affected siblings of children with ADHD have response inhibition deficits that fall intermediate between typically developing children and children with ADHD (Slaats-Willemse, Swaab-Barneveld, de Sonneville, van der Meulen & Buitelaar, 2003). Neuroimaging studies of response inhibition have shown similar hypo-activation of prefrontal areas in ADHD probands and their unaffected siblings, compared with typically developing children (Durston, Mulder, Casey, Ziermans, & van Engeland, 2006). Finally, a number of molecular genetic studies in both children with ADHD and non-clinical participants have reported associations between allelic variation in DRD4

and DAT1 and measures of response inhibition (Cornish et al., 2005; Johnson et al., 2008; though also see Kebir, Tabbane, Sengupta, & Joober, 2009; Rommelse et al., 2008). More recently, Durston and colleagues (2008) found that DNA variation in DAT1 influenced brain activation during response inhibition in the striatum and cerebellum.

In the present study, we used functional magnetic resonance imaging (fMRI) to investigate the effects of the DAT1 40 base pair (bp) variable number of tandem repeat (VNTR) polymorphism on the neural networks underlying response inhibition, in adolescents diagnosed with ADHD as well as typically developing adolescents. The dopamine transporter gene (DAT1) codes for the production of the dopamine transporter (DAT) which clears dopamine from the synaptic cleft back into the neuron and is the primary site of action for psychostimulants. Allelic variation within DAT1 has been shown to affect the level of DAT expression, with increased expression associated with the 10R-allele (Mill, Asherson, Browes, D'Souza, & Craig, 2002; though also see Van Dyck et al., 2005). Thus, it is plausible that tonic levels of available dopamine are lower in individuals homozygous for the 10R-allele, compared to other genotypes, and this may affect cognitive control processes that rely on dopamine, such as response inhibition and error-related processing. Individuals diagnosed with ADHD often show impairments in error-monitoring (van Meel, Heslenfeld, Oosterlaan, & Sergeant, 2007) and/or response inhibition (Vaidya et al., 1998).

Comparing the relative contribution of genotype and diagnosis allows us to distinguish which brain differences in activation are affected by variation in DAT1 genotype, and which are likely caused by other (genetic or environmental) factors that contribute to the disorder. Consequently, a main effect of genotype in the absence of

effects of diagnosis suggests that DAT1 genotype explains most of the variation in activation in this region, with little additional contributions from other (genetic or environmental) factors associated with ADHD. Similarly, regional brain functioning for which ADHD-related impairment is mediated by genotype may be expected to show genotype by diagnosis interactions while ADHD-related impairments not mediated by genotype might be expected to show just main effects of diagnosis. In total, this approach enables us to determine the contribution that the DAT1 gene makes to specific cognitive and specific regional functional impairments that might be observed in those with ADHD.

MATERIAL AND METHODS

Participants

A total of 58 right-handed adolescents were included in this study: 20 diagnosed with ADHD (mean age: 14.1, SD: 2.1; 3 female) and 38 typically developing adolescents (mean age: 13.26, SD: 1.98; 7 female); the proportion of genders did not differ between the two groups (p=.476, Fisher's exact test), nor did their ages (t(56)=1.5, p=.14) (also see Table 1, left panel). The participants with ADHD were referred by consultant psychiatrists in Ireland. All participants with ADHD met DSM-IV criteria for ADHD, as determined through semi-structured interviews by psychiatrists using the parent form of the Child and Adolescent Psychiatric Assessment (CAPA) (Angold et al., 1995) or the Parental Account of Children's Symptoms (PACS) (Taylor, 1986). Control participants were recruited through local schools. Participants who were taking stimulant medication

for ADHD were asked to discontinue their medication for at least 24 hours[1] prior to the scan session.

Exclusion criteria included known neurological conditions or pervasive developmental disorders, serious head injuries and below average intelligence (below 70 on the WISC (Wechsler's Intelligence Scale for Children)), and MRI-exclusion criteria (metal anywhere in the body (excluding the mouth), pregnancy, claustrophobia). In addition, the parents of all participants completed the Conners' ADHD Rating Scale-Revised: Long Version (Connors, 1996). Control adolescents had Conners' Global Index H-scores ≤60, and adolescents with ADHD had scores ≥65. Participants diagnosed with ADHD had lower scores on the WISC, as well as on both spelling and reading subtests of the WRAT (Wide Range Achievement Test), and scored higher on the Conner's scale (all $F \geq 11.6$, $p \leq .001$). There were no significant effects on these variables of genotype (all $F \leq .67$, $p \geq .42$), nor were there significant interactions between genotype and diagnosis (all $F \leq 1.2$, $p \geq .28$). We also present analyses on a subsample of the participants, who were matched for their scores on the WISC and WRAT (all $F < 2.8$, $p \geq .105$), and which included only boys (see Table 1, right panel).

After the study was described, participant and parental consent was provided. The study was approved by the local ethics committee, and was carried out in accordance with the Declaration of Helsinki (1964; 2000).

The data from some of the controls were previously reported in a developmental study of inhibitory control (Braet et al., 2009).

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[1] We chose a relatively brief stimulant wash-out period, as this is less disruptive for participants. Acute effects of stimulant withdrawal have been reported for both behavioural (e.g. Carlson & Kelly, 2003) and neuroimaging data (e.g. Langleben et al., 2002). Our results are broadly consistent with those of a recent study by Bédard et al., 2009, where a two-week stimulant withdrawal period was used.

Table_1_(a+b)

Genotyping

DNA was extracted from blood, or from saliva using Oragene DNA self-collections kits (DNAgenotek, Canada). Polymerase chain reaction (PCR) amplification and genotyping of the variable number of tandem repeats (VNTR) of the 3' untranslated region (UTR) of the DAT1 gene were conducted as described by Cook and colleagues (1995). All genotypes were in Hardy Weinberg Equilibrium. Children were grouped according to possession of the 10 repeat VNTR: the "high risk" (HR) group possessed two copies and the "low risk" (LR) group possessed one or no copies of the 10 repeat allele [ADHD: 9 HR, 11 LR and Control: 18 HR and 20 LR]. There was no significant association between allele possession for the 3' UTR VNTR DAT1 and clinical diagnosis [$\chi2(1)=0.03$, p=0.864]. The categorisation of the genotypes as either HR or LR was done similar to prior studies (see e.g. Bellgrove, Hawi, Kirley, Gill, & Robertson, 2005), based on statistical grounds.

Sustained attention to response task (SART)

Participants performed the random SART, a go/no-go test that measures both inhibitory function (no-go trials) and sustained attention (go trials) (see O'Connell et al., 2009). In this paradigm, numbers from 1-9 are presented in a random (i.e. non-sequential)

order, and participants are asked to make a buttonpress response to each number, except '3'. Each trial consisted of the (go or no-go) target (313 ms), after which a mask was presented (563 ms, which consisted of 125ms mask, 63ms response cue, and a further 375ms mask), followed by a fixation cross (563 ms); inter-stimulus interval (ISI) was 1439 ms. (see Figure 1). The task was presented using E-prime (Psychology Software Tools, Pittsburgh, USA), in a single block of 450 trials (consisting of 400 go trials and 50 no-go-trials), which included two 30s breaks after 150 and 300 trials.

We chose this design to be optimised to detect activation related to both successful and unsuccessful nogo-trials (which are assumed to reflect response inhibition and error-monitoring, respectively). To ensure a sufficient number of nogo-trials, while keeping their proportion low (compared to the total number of trials, to encourage a prepotent tendency to respond), the duration of individual trials was kept relatively short. This design allows for reliable estimation of response maps for response inhibition and error-monitoring, but is not ideal to investigate activation relating to tonic processes such as sustained attention or motor processes given the fast pace of go-trials (faster than the scan acquisition time) which means that hemodynamic response curves cannot be fitted to go-events. Consequently, the baseline for assessing successful and unsuccessful nogo-related activation is comprised of these tonic attentional and motoric processes. An alternative approach would be to include a number of fixation-only trials which could then serve as a low-level baseline against which tonic attentional and motor activity could be assessed. Several factors motivated the choice of our design. First, our main interest was response inhibition/error detection rather than sustained attention, and for this we only required activation maps for nogo-trials. Similarly, it was not pertinent to our

research question to assess go-related activation: Previous studies have shown that go- and nogo-trials lead to distinct maps with minimal overlap (Garavan, Ross & Stein, 1999), and their differential time courses as revealed by ERP studies (e.g. O'Connell et al., 2009) further confirm that both trial types elicit independent cognitive processes. Second, the present design ensures that every trial is used in the analysis (either as baseline, or as an effect of interest), which reduces the time that participants are in the scanner which, in turn, makes it easier for participants (particularly the ADHD group) to keep movement to a minimum. Third, response inhibition is commonly investigated by comparing activation during nogo-trials to a baseline of go-trials (Braet et al., 2009; Durston et al., 2008; Ramautar, Slagter, Kok & Ridderinkhof, 2006) with one advantage being that one employs an active baseline condition with similar visual stimuli to the nogo trials.

Figure_1

MRI data acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system. Each scanning sequence began with a reference scan to resolve sensitivity variations. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV 230 mm, thickness 0.9 mm, voxel size $0.9 \times 0.9 \times 0.9$) were then acquired (total duration 325 s), to allow subsequent activation localization and spatial normalization.

Functional data were collected using a T2*-weighted echo-planar imaging (EPI) sequence that acquired 32 non-contiguous (10% gap) 3.5 mm axial slices covering the

entire brain (TE = 35 ms, TR = 2000 ms, FOV 224 mm, 64 × 64 mm matrix size in Fourier space). The functional scans had a total duration of 730 s, and were collected in a single sequence.

fMRI analysis

The data were analysed under the general linear model using AFNI (http://afni.nimh.gov) (Cox, 1996). Images were corrected for motion (using a least-squares alignment allowing translations and rotations). These motion parameters were also included as variables-of-no-interest in the first-level fMRI analyses; there were no reliable differences in any of the 6 motion parameters between participants diagnosed with ADHD and controls (all t≤1.5, p≤.139) (no participants were excluded due to excessive motion), and activation outside the brain was removed. MR signal drift was estimated by fitting a 5th-order polynomial function. Separate impulse response functions (IRFs) were estimated for successful inhibitions and commission errors using deconvolution techniques (the time course of the two 30s breaks was also included as a variable-of-no-interest). Gamma-variate functions were fit, voxelwise, to these IRFs using a non-linear regression programme. A percentage signal-change score (%SC) was calculated by dividing the area under the curve of these functions by the area under the baseline which, in this case, reflects tonic ongoing processes involved in Go trial responses[2]. Individual %SC maps were then spatially blurred using a 3mm rms isotropic

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[2] Go-trials were not analysed separately, as the timing of our design was optimized to identify activation maps for (successful and unsuccessful) nogo trials, rather than for go-trials, given the high frequency (typically more than one in a single TR) of go-trials. Nogo-trials (which comprise approximately 10% of the total number of trials) were spaced further apart, leading to more reliable IRFs. Prior studies (e.g.

Gaussian kernel, and transformed into MNI space using the MNI (Montréal Neurological Institute) 152-brain template.

For both commission errors and successful inhibitions, activation maps for each group of interest were then determined using one-sample t-tests against 0 (i.e. against the null hypothesis of no change in activation compared with the baseline). Significant voxels passed a voxelwise statistical threshold (ADHD HR: t=5.04; ADHD LR: t=4.78; control HR: t=3.97; control LR: t=3.88, p≤.001; these t-values varied as the sample sizes differed between groups), and were required to be part of a cluster of significant voxels with a minimum volume of 135 μl. This minimum cluster-size was determined using Monte-Carlo simulations (1000 iterations), resulting in a 0.05 (corrected) probability of a cluster surviving due to chance. Separate maps were generated for the four groups, and these were subsequently combined into separate maps (containing every voxel that was part of a significant cluster in any of the four groups) for successful inhibition and commission errors. The clusters of activation in these resulting maps (one map for successful inhibitions and one for commission errors) were then used for functionally-defined region of interest (ROI) analyses wherein we extracted mean activation values for each region for every participant (these same ROIs were used for the analyses on the matched sample). These data were analysed as two-way ANOVAs with diagnosis and genotype as grouping factors.

This method was chosen (rather than constructing a single map for all participants), as it ensures that every region which was activated by at least one of the 4 groups is included in the ROI-analyses, rather than just regions which were commonly

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Garavan, Ross & Stein, 1999) have investigated activation maps of both go- and nogo-trials, and found relatively little overlap between the two maps.

activated (and which would otherwise likely be determined primarily by the (larger) control group). This method tends to be more sensitive to identify group-differences (and particularly, interaction-effects between multiple grouping-factors), as activation at the ROI-level is more reliable than at the level of individual voxels (due to the reduction of noise by averaging over a larger cortical area). While there are differences in the number of events (e.g. ommission errors) between the adolescents diagnosed with ADHD and the controls, we have previously shown that performance-matching (in terms of the number of omission and commission errors as well as response variability) between two groups has only limited effects on the results of the ROI-analysis (Braet et al., 2009). This makes it unlikely that the effects reported here are directly related to differences between the groups in the number of errors they make, or even differences in performance on go-trials (which were used as the baseline).

Analysis of behavioural data

Errors of commission (responses made on the no-go digit 3, which indicate failure of inhibitory processes) and omission (non-responses on the go-trials, believed to reflect temporary lapses in attention) and the mean and standard deviation (SD) of the response times (RTs) on the go-trials were calculated for each participant. Differences between the groups (as well as interactions between genotype and diagnosis) were assessed using univariate analyses under the general linear model, with diagnosis (ADHD or control) and DAT1-genotype (HR or LR) as fixed factors, and with the alpha level set at .05.

RESULTS

Behavioural performance

Adolescents diagnosed with ADHD made significantly more omission errors $(F(1,54)=18.48, p<.001)$, and were significantly more variable in RT (higher SD) on go-trials $(F(1,54)=6.46, p=.014)$ than typically developing adolescents, as well as displaying a trend for a higher number of commission errors $(F(1,54)=3.36, p=.072)$. Participants diagnosed with ADHD and typically developing adolescents had similar RTs for both go trials (485s and 512s, respectively, $p>.05$) and commission errors (425s and 426s, respectively, $p>.05$). There were no interactions between diagnosis and genotype on any of the behavioural measures, nor were there any main effects of genotype.

The matched sample showed the same pattern of results: significantly more omission errors $(F(1,32)=16, p<.001)$ and higher variability on go-trial RTs $(F(1,32)=4.8, p=.035)$ for adolescents diagnosed with ADHD compared to typically developing adolescents, but there were no other significant main effects or interactions (all $F\leq2.6$, $p\geq.117$).

Participants successfully responded to the majority of go trials (93% for adolescents diagnosed with ADHD, and 98% for the control group), suggesting that both groups had a prepotent tendency to respond on each trial. Response inhibition during infrequent nogo trials was therefore likely effortful, which is further confirmed by the

relatively high error rates for nogo trials (39% errors for participants from the ADHD group, and 30% for the control group) (see Figure 2).

Figure_2

fMRI-analysis: successful inhibitions

Table_2

Table 2 lists the areas that showed significant activity when participants successfully inhibited their response on no-go trials (also see Figure 3).

Adolescents with ADHD showed reduced activation, compared with controls, in the right cuneus and in the left inferior parietal cortex.

There were also main effects of genotype, with greater activation for HR compared with LR in the following regions: the right middle frontal gyrus, the ACC, bilateral caudate, the right middle temporal gyrus and the right middle occipital gyrus. One region, the left inferior temporal gyrus showed reduced activation for HR participants.

There were also eight regions where there was an interaction between diagnosis and genotype: the left superior frontal gyrus, the ACC, the left cingulate gyrus, the left lentiform nucleus, the right supramarginal gyrus, the right precuneus, the right cuneus, and the right middle occipital gyrus. In all these regions, there was a larger effect of DAT1 genotype for participants with ADHD than for controls. (see Figure 4).

The matched sample showed the same pattern of results, though the difference in a number of ROIs was no longer significant: there were no longer main effects of diagnosis in the right cuneus and in the left inferior parietal cortex; the main effect of genotype was no longer reliable in the left inferior temporal gyrus, though the other 5 clusters still showed increased activation for the HR group; finally, the right cuneus and left cingulate gyrus no longer showed a reliable interaction between diagnosis and genotype.

Figure_3

Figure_4

fMRI analysis: commission errors

Table_3

Table 3 lists the areas that showed significant activity when participants made a commission error (responded on no-go trials) (also see Figure 5).

Adolescents diagnosed with ADHD showed reduced activation in three regions: the left superior frontal gyrus, the ACC, and the right insula.

Participants with the HR-genotype showed reduced activation compared with those with the LR-genotype in the following regions: the left parahippocampal gyrus, and the left and right postcentral gyri.

Two regions showed an interaction between diagnosis and genotype: the right middle frontal gyrus and the left angular gyrus (see Figure 6). In both regions, activation levels were similar for the HR adolescents with ADHD and LR controls (both t≤.63, p≥.53) with both groups showing activations in the right middle frontal gyrus and deactivations in the left angular gyrus. The other two groups (LR ADHD and HR controls) showed little activation in either region (all t≤1.2, p≥.24).

The matched sample showed the same pattern of results, though the differences in a number of ROIs were no longer significant: the ACC no longer showed a reliable effect of diagnosis; the difference between HR and LR was no longer significant in the right postcentral gyrus; the left angular gyrus no longer showed a significant interaction between diagnosis and genotype.

Figure_5

Figure_6

Discussion

Behavioural data

In line with findings from other studies, we observed significantly more omission errors made by adolescents with ADHD compared with typically developing adolescents, indicative of impaired sustained attention (Marchetta, Hurks, De Sonneville, Krabbendam, & Jolles, 2007) as well as increased response variability (Castellanos et al., 2005; Johnson et al., 2007). While the results were less clear in terms of response inhibition, there was also a trend for a higher number of commission errors, suggestive of impaired response inhibition for adolescents with ADHD, compared with typically developing adolescents. Impaired response inhibition has been proposed as a core deficit in ADHD (Wodka et al., 2007; but see e.g. Banaschewski et al., 2004 for an opposing view) as well as a potential endophenotype for ADHD (Aron & Poldrack, 2005).

No effects of DAT1 genotype on any of the behavioural performance measures were found, despite previous studies showing effects of DAT1 genotype on both response inhibition (Cornish et al, 2005) and response variability (Bellgrove et al., 2005) (though also see Kebir et al., 2009; Rommelse et al., 2008). Given the small effect size associated with the DAT1 gene, our study may simply lack the statistical power to replicate these effects (the HR-group in our sample did make more commission and omission errors, had higher response variability and slower RTs, but none of these differences were significant).

Genetic influence on functional activation patterns

In contrast to the behavioural effects, the neuroimaging data provided a more sensitive measure of the downstream effects of genetic variation on cognition (also see Greene, Braet, Johnson, & Bellgrove, 2008), insofar as they did reveal effects of genotype in the absence of behavioural effects (e.g. Durston et al., 2008; also see Bédard et al., 2009). During successful inhibitions, there was increased activation for the HR-genotype in the right middle frontal gyrus, the ACC and caudate, as well as the right middle temporal and middle occipital gyri, while there was reduced activation in the left inferior temporal gyrus. The right prefrontal cortex, as well as medial brain regions including the ACC and caudate are often implicated in response inhibition (Fassbender et al., 2004), and increased activation in these regions may imply that response inhibition is more effortful or less efficient for the HR-group.

During commission errors, however, the HR-group displayed reduced activation in the left parahippocampal gyrus, as well as in both the left and right postcentral gyri. The (para)hippocampal region has been associated with the regulation of arousal-mechanisms (Gray & McNaughton, 2000), and error-related activation in this region has been associated with subsequent improvements in performance in a task in which one needed to learn from one's errors (Hester, Barre, Murphy, Silk & Mattingley, 2008). Consequently, activation of this region during commission errors may reflect an arousal-mediated response to error-detection required to engage additional top-down control processes and prevent or reduce further errors. Given the role of dopamine in error-monitoring (Krämer et al., 2007) the present result may reflect a reduced error-response

due to the lower tonic levels of dopamine in the HR group, caused by increased expression of the transporter associated with the 10-repeat allele.

Interaction of genotype and diagnosis status on functional activation patterns

There were a number of regions where genotype interacted with diagnosis. During successful inhibitions, such interactions were observed in frontal (the left superior frontal gyrus), medial and subcortical (the ACC, the left cingulate gyrus, and the left caudate), and parietal regions (the right supramarginal gyrus, the right precuneus, and the right cuneus), as well as in the right middle occipital gyrus. For all these regions we observed a similar pattern: DAT1 genotype accounted for significant heterogeneity in neurophysiological networks for response inhibition in children with ADHD (where higher activation was observed in the HR group compared to LR) but not in controls (where there were no differences in activation between the two genotypes). These data demonstrate that the gene had greater penetrance at the level of brain activation associated with response inhibition in the adolescents with ADHD (suggestive of more effortful/ less efficient inhibition) but not in the typically developing adolescents. These data further suggest that physiological heterogeneity in response inhibition networks in the ADHD group might be predicted by variation in the DAT1. This may indicate that the effect that DAT1 genetic variation has on the response inhibition endophenotype in those with ADHD is not "self-contained" insofar as the same effect is not observed in the control participants. Instead, other genetic or enviromental influences particular to the ADHD group must combine with DAT1 status to produce the observed brain activation differences. We also observed interactions between diagnosis and genotype on brain

activation associated with commission errors, in the right middle frontal gyrus and the left angular gyrus, though here the pattern was different, with similar engagement of both regions for the LR control and the HR ADHD groups, while neither of the other groups showed significant activation in these regions. Although the dopaminergic mechanisms underlying these effects are unclear, this type of result indicates that dopaminergic variation in healthy controls can produce some endophenotypic effects that are similar to those with ADHD. This idea is consistent with the notion that ADHD is characterised by a multifactorial polygenetic etiology (see Gizer, Ficks & Waldman, 2009), where the effects of a large number of genes (on e.g. dopaminergic function) combine to form a continuum of phenotypic variation in e.g. response inhibition.

It has been argued that the brain regions that show interactions between diagnosis and genotype may contribute knowledge to aid development of new treatments for ADHD, as a surrogate endpoint in individualized treatments targeting genotype/fMRI activation profiles (Durston et al., 2008). The exact relationship between neurotransmitter levels and changes in fMRI activation however is still unknown, and different studies have often found contradictory effects. For the DAT1 gene, both the 10R and 9R alleles have been associated with increased expression of the transporter (Mill et al., 2002 vs. Van Dyck et al., 2005) and with deficits in sustained attention (Bellgrove et al., 2005 vs. Kim, Kim & Cho, 2006). In a prior study by Durston and colleagues (2008), which also investigated the interaction of ADHD diagnosis and DAT1 genotype using a similar behavioural paradigm, increased activation was observed during successful inhibitions in the vermis of the cerebellum for the 10R compared with the 9R allele. Although we also observed higher activation in this region for the HR compared to LR group, this

difference was not significant in our sample. Durston and colleagues also observed an interaction between diagnosis and genotype in the caudate nucleus in the striatum, but the direction of this interaction effect was opposite to our result. It is unclear why our results differ from those of Durston and colleages. One possibility may be related to differences in the difficulty of the respective go/nogo tasks that were used in both studies: in our paradigm a single trial lasted only 1.4s, compared to 4s, and the proportion of nogo-trials was 10%, compared to 25%. It is therefore likely that in the present study, response inhibition was more difficult, and successful inhibitions more rewarding, leading to significant differences in baseline dopaminergic release in the striatum between the two studies. Similar to our own findings, a recent study by Bédard and colleagues (2009) also found increased striatal activation in individuals diagnosed with ADHD who possess two copies of the 10R allele. Further research will be required to clarify the nature of these interactions, as well as their consistency across different study samples.

There were also a number of regions that showed differences between participants diagnosed with ADHD and typically developing adolescents. During successful inhibitions, the ADHD group showed reduced activation in two parietal regions, the right cuneus and the left inferior parietal cortex, while during commission errors they showed reduced activation of the left superior frontal gyrus, as well as the ACC and the right insula, two key regions for response-monitoring. This confirms previous findings of hypofunction in ADHD in frontal and parietal regions (Durston, 2003). The insula projects to striatal regions, where deactivation during response inhibition has also been observed in ADHD (Vaidya et al., 1998). The present results suggest that these ADHD-

related effects are not attributable to genetic variations in DAT1 but instead may be driven by other genetic or environmental effects.

Conclusions

Imaging individuals with known genetic variation provides us with an exciting opportunity to understand better the functional effects of DNA variation for brain and cognition. Although we lack a full understanding of the pathways leading from gene to changes in BOLD signaling, the current data suggest that fMRI indices may improve our ability to detect subtle genetic effects compared with behavioural measures of cognition alone. Here we have shown that DAT1 genotype influences the physiological substrates of response inhibition and error processing, with effects for response inhibition being most pronounced in children with ADHD. Our data add to a growing body of evidence suggesting that differences in activation in brain regions underlying response inhibition in ADHD are driven by genetic factors. By showing which behavioural effects and which areas of cortical and subcortical activation are influenced by DAT1 genetic variation, ADHD status or the interaction between the two, these results identify the specific contribution of the dopamine transporter to executive functions such as response inhibition and error-monitoring.

Acknowledgements

Legend to the figures and tables:

Figure 1: The Sustained Attention to Response Task (SART): timecourse of a single trial.

Figure 2: Behavioural performance measures. Error bars represent 1 S.E.

Figure 3: Brain regions activated during successful inhibitions. a) superior frontal gyri and ACC, b) ACC, middle frontal gyrus and insulae, c) insulae and parietal lobe, d) parietal lobes. Green: areas activated but showing no diagnosis or genotype differences, Yellow: reduced activation for ADHD participants relative to controls; Dark Blue: increased activation for HR relative to LR; Red: diagnosis x genotype interaction.

Figure 4: Interactions between diagnosis x genotype, in percentage signal change during successful inhibitions.

Figure 5: Brain regions activated during commission errors. a) superior and middle frontal gyri and ACC, b) ACC, middle and superior frontal gyri, and left and right inferior parietal lobes c) insulae and parahippocampal gyrus, d) ACC, left angular gyrus, left and right superior frontal gyri, left and right parietal lobes and postcentral gyri. Green: areas activated but showing no diagnosis or genotype differences; Yellow: reduced activation for ADHD participants relative to controls; Light Blue: increased activation for LR relative to HR; Red: diagnosis x genotype interaction.

Figure 6: Interactions between diagnosis x genotype, in percentage signal change during commission errors.

Table 1: Demographic information on the ADHD and control adolescents. The full sample is displayed on the left, and the matched sample on the right.

Table 2: Brain regions activated during successful inhibitions. From left to right: location, hemisphere, Brodman area(s), cluster-volume, coordinates (MNI), significance levels (*: $<.05$; **:$<.001$) and direction for main effect of diagnosis, main effect of genotype, and the interaction between diagnosis and genotype. Underlined effects were no longer significant in the matched sample.

Table 3: Brain regions activated during commission errors. From left to right: location, hemisphere, Brodman area(s), cluster-volume, coordinates (MNI), significance levels (*: $<.05$; **:$<.001$) and direction for main effect of diagnosis, main effect of genotype, and

the interaction between diagnosis and genotype. <u>Underlined effects</u> were no longer significant in the matched sample.

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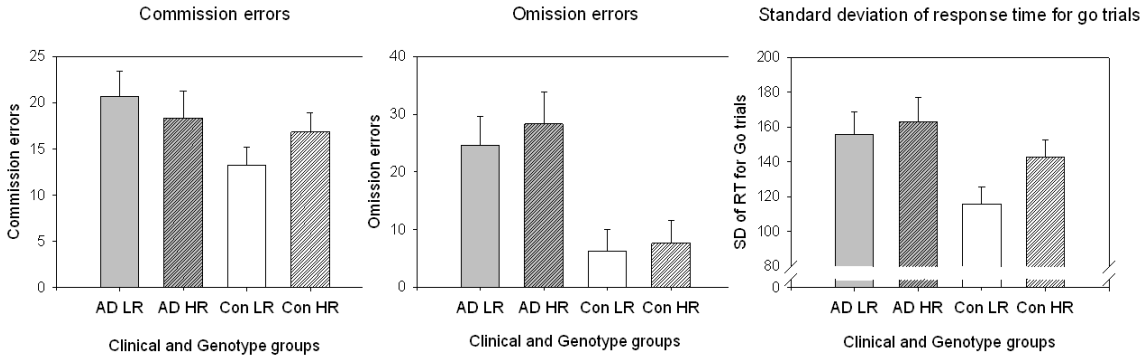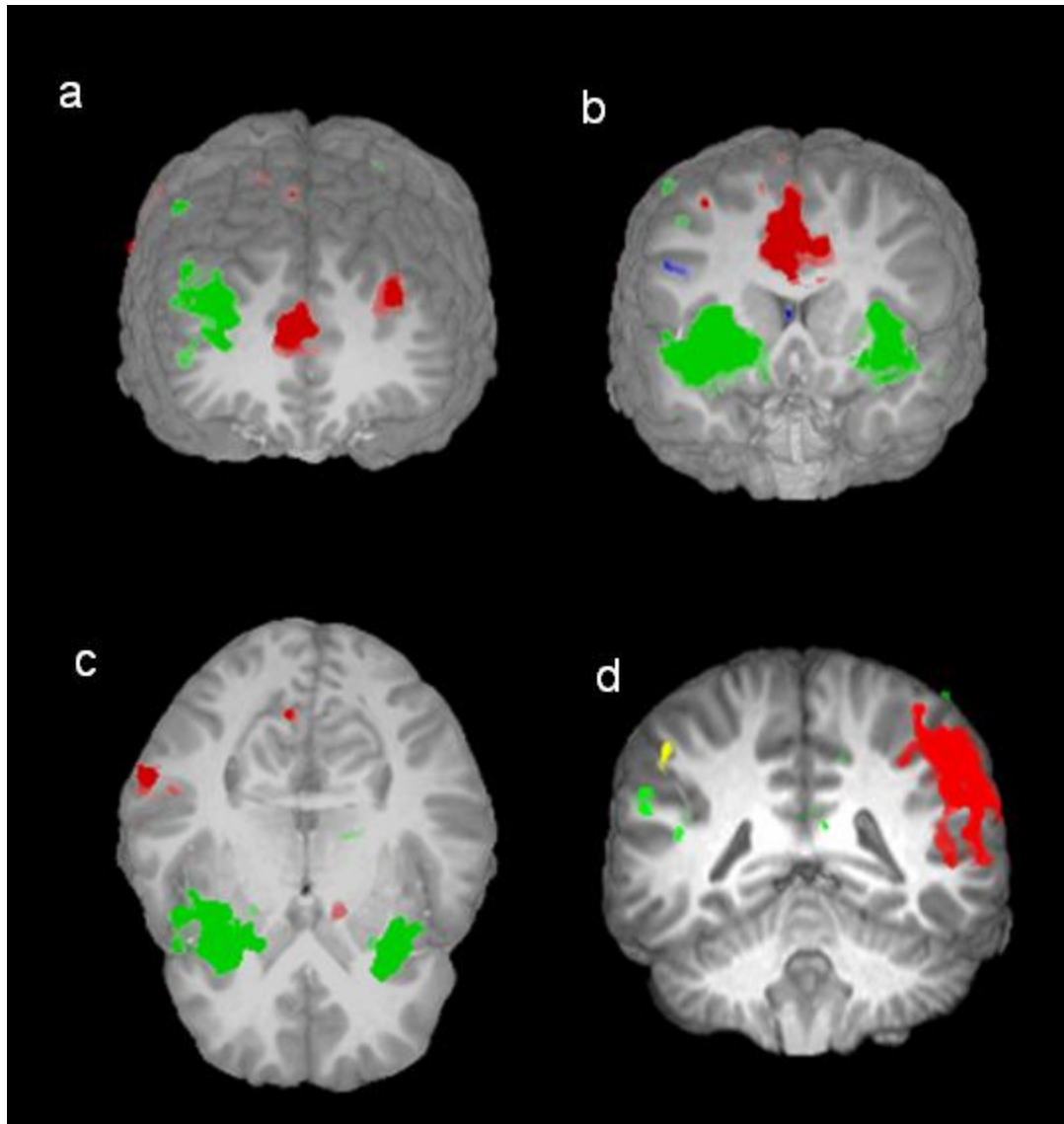
Figure 1

Figure 2
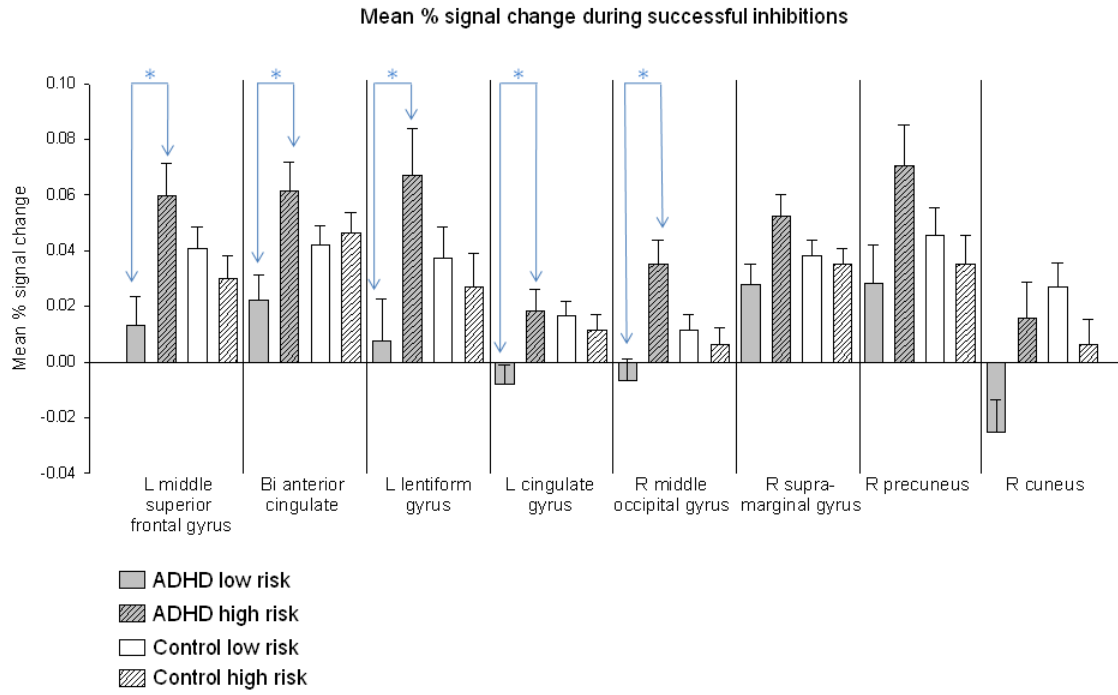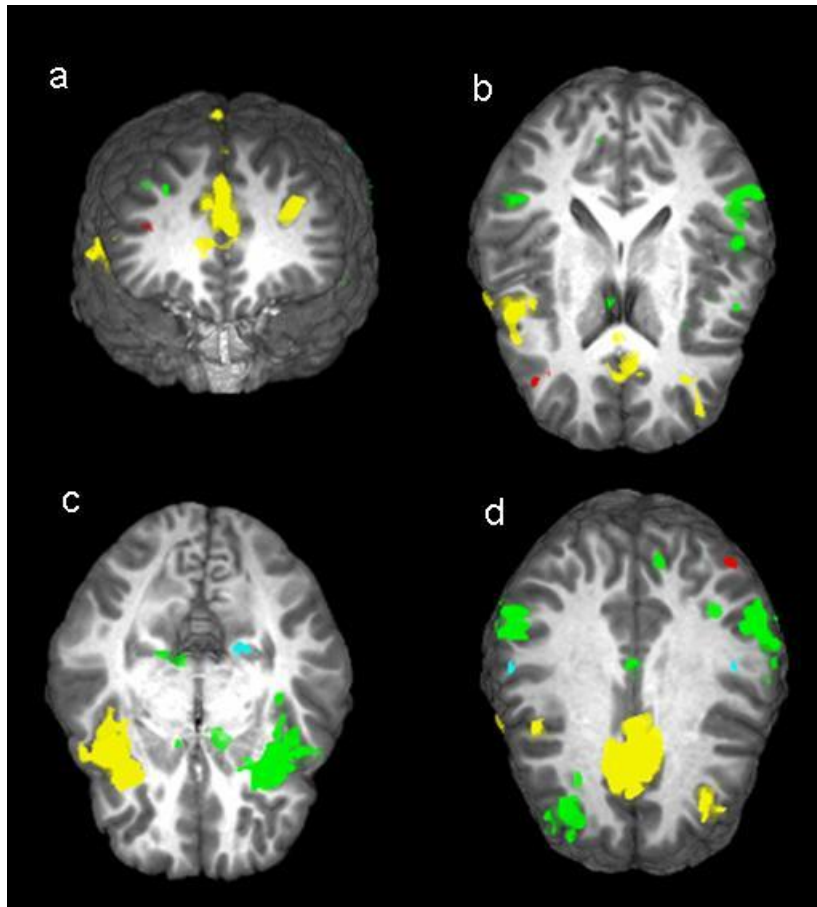
Figure 3

Mean % signal change during successful inhibitions

Figure 4

Figure 5

Mean % signal change during commission errors

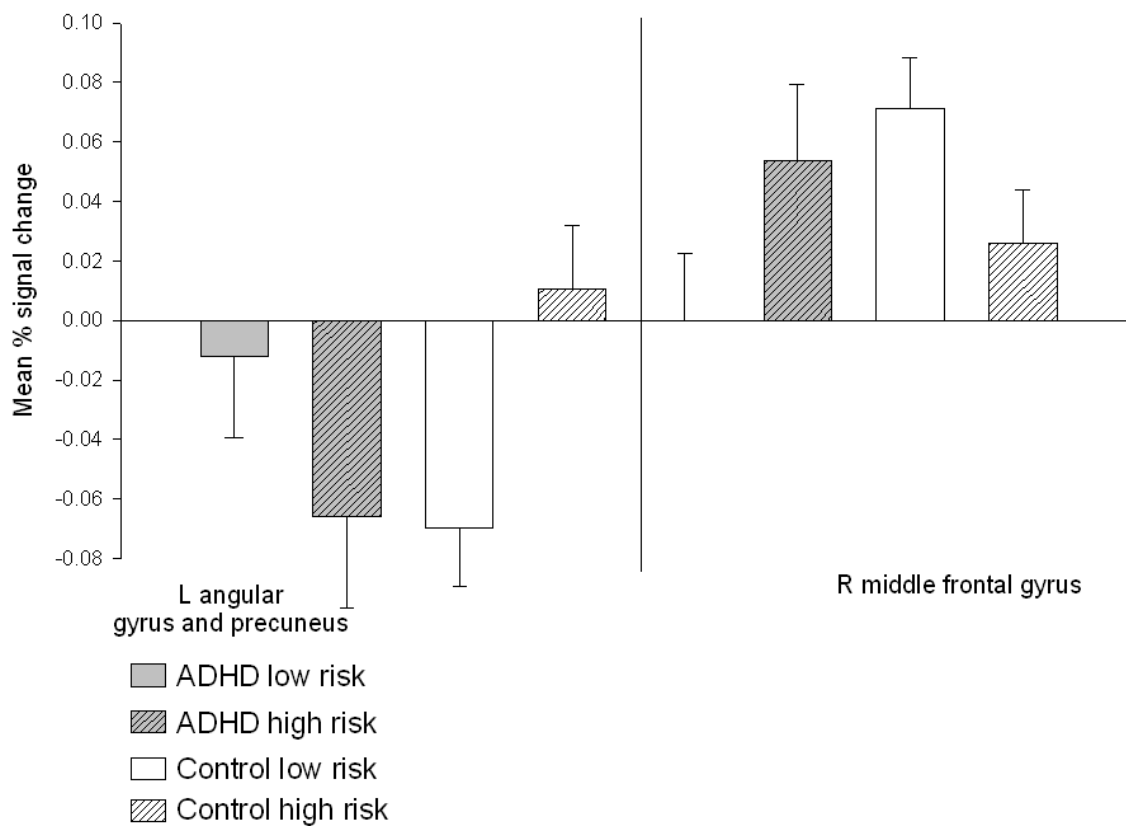Figure 6