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Neural response to working memory load varies by dopamine transporter genotype in children

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ABSTRACT

Inheriting two (10/10) relative to one (9/10) copy of the 10-repeat allele of the dopamine transporter genotype (DAT1) is associated with Attention Deficit Hyperactivity Disorder, a childhood disorder marked by poor executive function. We examined whether functional anatomy underlying working memory, a component process of executive function, differed by DAT1 in 7–12 year-old typically developing children. 10/10 and 9/10 carriers performed a verbal *n*-back task in two functional magnetic resonance imaging (fMRI) runs varying in working memory load, high (2-back vs. 1-back) and low (1-back vs. 0-back). Performance accuracy was superior in 9/10 than 10/10 carriers in the high but not low load runs. Examination of each run separately revealed that frontal–striatal–parietal regions were more activated in 9/10 than 10/10 carriers in the high load run; the groups did not differ in the low load run. Examination of load effects revealed a DAT1 × Load interaction in the right hemisphere in the caudate, our *a priori* region of interest. Exploratory analysis at a more liberal threshold revealed this interaction in other basal ganglia regions (putamen, and substantial nigra/subthalamic nuclei – SN/STN) and in medial parietal cortex (left precuneus). The striatal and parietal regions were more activated in 9/10 carriers under high than low load, and DAT1 differences (9/10>10/10) were evident only under high load. In contrast, SN/STN tended to be more activated in 10/10 carriers under low than high load and DAT1 differences (10/10>9/10) were evident only under low load. Thus, 10-repeat homozygosity of DAT1 was associated with reduced performance and a lack of increased basal ganglia involvement under higher working memory demands.

Introduction

Individual differences in executive function, the ability to control thoughts and actions in a goal-directed manner, are attributable at least in part, to genetic variation (reviewed in Goldberg and Weinberger, 2004). One approach to identifying sources of genetic variation has been to examine association with heritable disorders that include executive dysfunction. This approach has been productive in a common disorder of childhood, Attention Deficit Hyperactivity Disorder (ADHD), that is defined by symptoms of inattention, impulsivity, and hyperactivity that significantly impede executive function [e.g., Tower of London (Nigg et al., 2002)] and its component processes such as working memory (Nigg et al., 2002) and response inhibition (DeVito et al., 2009). Molecular genetic studies of ADHD found a small [<4% (Waldman et al., 1998)] but significant association with a variable number of tandem repeat sequences (VNTR) in the 3′-untranslated region (UTR) of the gene (SLC6A3) coding for the dopamine transporter (DAT1) (first reported by Cook et al., 1995, and meta-analysis by Yang et al., 2007). ADHD was more prevalent in homozygous carriers of the 10-repeat allele (10/10) relative to heterozygous carriers (9/10) and number of hyperactive-impulsive symptoms increased with the number of 10-repeat alleles (Waldman et al., 1998). Furthermore, 10/10 typically developing children exhibited more hyperactivity symptoms (Mill et al., 2005). Thus, a functional polymorphism of DAT1 contributes to variability in phenotypic expression that is relevant to executive function.

The effect of the DAT1 VNTR on executive task performance is not conclusively known at present. As ADHD is associated with poor executive function and 10-repeat homozygosity is associated with ADHD, 10/10 carriers ought to perform worse on tasks of executive function or its component processes than 9/10 carriers. Indeed, typically developing 10/10 children had worse inhibitory performance [TEA-Ch Opposite Worlds task (Cornish et al., 2005), more errors of commission on the Continuous Performance Test (Loo et al., 2003)] relative to 9/10 children. Furthermore, 10/10 adults made more errors of commission on the Continuous Performance Test relative to 9

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carriers [combined 9/9 and 9/10 carriers (Caldu et al., 2007)]. However, in a study of ADHD children, 10/10 carriers performed better on various tasks tapping working memory (e.g., Self-ordered pointing, Arithmetic and Digit Span subtests of the WISC-III) relative to 9/10 carriers (Karama et al., 2008). In contrast to findings showing DAT1 differences, 10/10 and 9-carrier adults did not differ in working memory performance (e.g., *n*-back task) (Bertolino et al., 2006, 2009; Caldu et al., 2007). Results from neuropsychological tasks of executive function also reveal a mixed picture. While 10/10 ADHD children performed better on the Tower of London than 9/10 carriers (Karama et al., 2008), performance on the Wisconsin Card Sorting Task did not differ by DAT1 (Barkley et al., 2006). These mixed findings cannot be explained by developmental stage (adults vs. children), diagnosis (ADHD vs. controls), or type of task. Thus, although 10-repeat homozygosity ought to be disadvantageous for executive function, task performance does not reveal a consistent pattern of results.

Three lines of evidence suggest that the influence of DAT1 VNTR on executive function is mediated by the striatum and its cortical projections. First, the dopamine transporter (DAT) reuptakes dopamine from extracellular space following release, and therefore, plays a role in regulating synaptic dopamine levels. It is expressed most abundantly in the striatum and other basal ganglia regions where it is the primary means of dopamine clearance from the synapse (Madras et al., 2005). Relative to the basal ganglia, DAT is found in lower concentrations in other regions such as parietal cortex (Lewis et al., 2001), hippocampus (Lewis et al., 2001), and cerebellum (Dahlin et al., 2007). It is minimally expressed in the prefrontal cortex where the main mechanism for dopamine clearance is the catechol-*O*-methyltrasferase (COMT) enzyme rather than DAT (Karoum et al., 1994). Thus, functional effects of individual variability in DAT expression are associated with the striatum most directly, relative to any other brain region.

Second, striatal DAT expression differs as a function of DAT1 alleles. *In vitro* studies found that higher DAT expression was associated with the 10-repeat allele (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005). Indeed, DAT expression in the caudate measured by ligand-based *in vivo* imaging was higher in children with ADHD who were 10/10 carriers relative to 9/10 carriers (Cheon et al., 2005). However, findings from *in vivo* studies of typically developing subjects are mixed, showing greater (Heinz et al., 2000), reduced (Jacobsen et al., 2000; van de Giessen et al., 2009; van Dyck et al., 2005), or no different (Krause et al., 2006; Martinez et al., 2001) DAT availability in the caudate in 10/10 relative to 9/10 carriers. Higher striatal DAT expression may be functionally deleterious because it is likely to result in reduced dopamine signaling due to enhanced clearance. Indeed, higher DAT expression in the caudate has been observed in ADHD relative to control subjects [reviewed in (Spencer et al., 2005)] and is related to reduced attentional function mediated by parietal cortex (Tomasi et al., 2009). Together, these findings suggest that effects of DAT1 alleles on DAT expression are observed in the caudate and furthermore, differences in striatal DAT expression are functionally relevant.

Third, functional magnetic resonance imaging (fMRI) studies found that prefrontal–striatal activation during tasks tapping component processes of executive function was reduced in 10/10 relative to 9 carriers. During response inhibition, 10/10 ADHD children and their unaffected siblings had reduced activation in the caudate relative to 9/10 carriers (Durston et al., 2008). This difference was not observed in control children. In normal adults, prefrontal regions showed reduced activation in 10/10 relative to 9 carriers during *n*-back working memory performance (Bertolino et al., 2006, 2009). As DAT expression is minimal in the prefrontal cortex, these results may reflect differences associated with striatal projections to the prefrontal cortex. Parallel measurement of functional activation by fMRI and dopamine signaling by ligand-based positron emission tomography indicated that prefrontal–striatal activation during working memory relates to dopaminergic activity (Landau et al., 2009). Thus, prefrontal–striatal functional activation revealed by fMRI is sensitive to DAT1 allelic differences.

In the present study, we used fMRI to examine whether activation during the *n*-back working memory task differs by the DAT1 VNTR in 7–12 year-old typically developing children. This study fills two gaps in current knowledge about the role of DAT1 in executive function: First, it is not known whether DAT1 differences influence brain activation during typical development. Caudate activation differences observed in ADHD children and their unaffected siblings in Durston et al.'s (2008) study appear to reflect disorder-related and inherited alterations in DAT expression because they were not observed in control children. However, the small sample size of each genotype group in controls ($n=4$) could have limited the ability to reveal activation differences in typical development. Furthermore, DAT1 differences observed in typically developing adults may not extend to childhood as they could depend upon maturation of prefrontal–striatal circuits through adolescence. Indeed, developmental functional imaging studies of working memory indicate that involvement of prefrontal and associated regions increases from childhood to adulthood (Crone et al., 2006; Klingberg et al., 2002). Second, it is not known whether neural response to added working memory load differs by DAT1 VNTR. fMRI studies show that prefrontal and striatal involvement increases with working memory demands (Chang et al., 2007; Wager and Smith, 2003). Studies in animals and humans suggest that those functional changes are mediated by load-dependent changes in dopaminergic signaling (Aalto et al., 2005; Floresco and Phillips, 2001). Thus, it is possible that individual differences in load-dependent functional activation relate to DAT1 allelic status.

Children in the present study performed a verbal *n*-back task under high and low working memory loads. The *n*-back task requires a response to the current trial if the stimulus letter matches that on a trial occurring *n* trials ago (e.g., 0, 1, 2). Thus, performance accuracy depends upon maintaining and updating of task-relevant stimuli and inhibiting interference from irrelevant stimuli, as trials proceed. Furthermore, varying *n* provides a manipulation for working memory load as maintenance, updating, and inhibitory demands increase with the size of *n* (e.g., low for 0, medium for 1, and high for 2). The low load fMRI run included $n=1$ (termed 1-back) relative to $n=0$ (termed 0-back) conditions and the high load fMRI run included $n=2$ (termed 2-back) relative to 1-back conditions. We selected the *n*-back task for three reasons: (1) its requirements evoke working memory processes relevant to executive function; (2) it has been used successfully with fMRI in children as young as 8 years (Libertus et al., 2009); and (3) it is sensitive to DAT1 differences in past fMRI studies of adults reviewed above. We hypothesized that caudate activation would be lower in 10/10 than 9/10 children based upon Durston et al.'s (2008) findings, and furthermore, this difference would be enhanced under high working memory load (DAT1 × Load interaction) as more demanding cognition ought to be more sensitive to reduced dopaminergic signaling in those with higher DAT expression. While our hypothesis focused on the caudate because it is a striatal region with high DAT expression that is reliably engaged during working memory, we also explored DAT1 × Load interaction in the rest of the brain. Based upon the association of DAT1 VNTR and ADHD, we expected *n*-back accuracy to be worse in 10/10 than 9/10 children.

Methods

Participants

Twenty typically developing children between 7 and 12 years of age were recruited through advertisements in the Washington DC area and were paid for participation. Ten children were carriers of two copies of the 10-repeat allele (termed "10/10") and 10 were carriers of one copy of the 10-repeat and 9-repeat allele (termed "9/10").

Children were genotyped following recruitment and consecutively enrolled into the study until equal samples of the two genotypes were obtained. The 9/10 group (7 males; age: M = 10.4, SD = 1.2; IQ: M = 113, SD = 14) did not differ from the 10/10 group (7 males; age: M = 10.4, SD = 1.7; IQ: M = 112, SD = 6) in age ($p = 0.90$), IQ ($p = 0.80$), or gender ($p = 0.70$). Furthermore, both groups had more right-handed children (9/10: 9 RH, 1 LH; 10/10: 8 RH; 2 LH) ($p = 0.5$). Parents and children gave informed consent and assent, respectively, in accordance with guidelines of the Georgetown University Institutional Review Board.

Exclusion criteria were (a) full-scale IQ below 85 [measured by Wechsler Abbreviated Scale of Intelligence, WASI, (Wechsler, 1999)], (b) parent reported history of psychiatric or neurological disorder, (c) reading problems [scores below 85 on the Woodcock-Johnson III Letter Word Identification and Word Attack subtests; (Woodcock et al., 2001)] and (d) a sibling with diagnosis of ADHD. Participants were screened for psychiatric conditions such as mood disorder with the Behavioral Assessment System for Children (BASC) (Reynolds and Kamphaus, 1992) and ADHD with the ADHD rating scale (DuPaul et al., 1998).

DAT1 3′ UTR VNTR genotyping

DNA was extracted from whole blood with a PureGene kit from 10 ml of whole blood or taken from cheek swab or Oragene saliva kits (DNA Genotek Inc., Ottawa, Ontario, Canada). Genotyping was performed in the following manner. PCR was carried out in a 10 µl volume containing 50 ng of genomic DNA, 0.5 µM of each primer 5′-NED-TGTGGTGTAGGGAACGGCCTGAG-3′ and 5′-(GTTTCTT) CTTCCTGGAGGTCACGGCTCAAGG-3′, 800 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 1 × EXT PCR buffer (including 1.5 mM MgCl2), GC melt, and 0.3 units DyNAzyme EXT polymerase. Samples were amplified on a 9700 thermal cycler with an initial 12-min step to heat-activate the enzyme, 45 cycles consisting of a denaturation step of 95 °C for 30 s, an annealing step of 68 °C for 45 s, and an extension step of 72 °C for 3 min. Products were injected onto an ABI 3730XL multi-capillary array genetic analyzer. Alleles were called with GeneMapper software, blind to all phenotypic information.

Task procedure

Each child performed two fMRI runs, Low and High working memory (WM) load, in counterbalanced order, each lasting 5 min 10 s. Each run consisted of 12 blocks, each comprising 9 trials preceded by an instruction stating the type of trial in the block, e.g., “0-back” or “1-back” or “2-back.” For all conditions, one letter was presented on the screen at a time (for 1 s followed by a 1.5 s inter-trial interval) and the participant was instructed to press a button on a box held in the right hand, when the letter on the screen was the same as the one presented *n* trials previously. The Low WM Run consisted of alternating blocks of 1-back and 0-back trials. In the 0-back condition, children were instructed to press the button for the letter “Q”; in the 1-back condition, they were to press the button if the letter was the same as the one before it (e.g., ‘M’ followed by ‘M’). The High WM Run consisted of alternating blocks of 1-back and 2-back trials. In the 2-back condition, children were instructed to press the button if the letter was the same as 2 before it (e.g., ‘R’ then ‘L’ then ‘R’). The number of target responses was identical across trial conditions. Stimuli comprised consonants only; vowels were omitted to prevent encoding series of letters as pronounceable strings. All children practiced the task prior to scanning.

fMRI data acquisition

Imaging data were acquired using a 3T Siemens magnet (Siemens Magnetom Trio, Erlangen, Germany). Head movement was minimized by foam padding that held the subject's head in the coil firmly and comfortably. Participants viewed the stimuli via a mirror mounted on the coil that reflected the images that were projected onto a screen (209 × 279 cm) at the back of the bore of the magnet approximately 950 mm from the mirror. Stimuli were generated in E-prime (Version 1.1, Psychology Software Tools Inc., 2002) and viewed via a magnet-compatible projector. Functional images (122/run) were acquired using a T2*-sensitive gradient EPI sequence. Forty-two axial slices (4.0 × 4.0 × 3.7 mm) were positioned to cover the whole brain (TR = 2500 ms, TE = 30 ms, 256 × 256 mm FOV, 90° flip angle). After functional imaging, a high resolution sagittal T_1-weighted structural scan was acquired using a 3D MPRAGE sequence with a scan time of 6:51 min and the following parameters: TR = 1600 ms, TE = 4.4 ms, 256 × 256 mm FOV, 160-mm slab with 1-mm-thick slices, 256 × 256 × 160 matrix (effective resolution of 1.0 mm^3), 1 excitation and a 15° flip angle.

fMRI processing and data analysis

Images were analyzed in SPM5 (www.fil.ion.ucl.ac.uk/spm). All participants displayed less than 4 mm of motion in *x*, *y*, and *z* directions throughout the course of each scan. Functional images were normalized into MNI standardized space and aligned to the high-resolution T1 structural image for the individual subject. Normalized images were smoothed (8 mm full width at half-maximum Gaussian kernal) (Turner et al., 1998). fMRI responses were modeled by a canonical hemodynamic response function. At the individual subject level, activation maps were generated using linear contrasts identifying regions that were more active during 2-back relative to 1-back blocks (for the High WM Run), and 1-back relative to 0-back (for the Low WM Run).

Three second-level analyses were performed: (1) To test our hypothesis of a DAT1 × Load interaction in the caudate, a 2 × 2 mixed analysis of variance (ANOVA) with DAT1 (9/10, 10/10) as a between-subject factor and Load (Low WM Run, High WM Run) as a within-subject factor was performed using the caudate mask from the AAL library (Tzourio-Mazoyer et al., 2002) with $p < 0.05$ FDR-corrected for small volume of 8 mm sphere. (2) To identify whether voxels in the rest of brain exhibited a DAT1 × Load interaction, an ANOVA similar to that in (1) was performed without any mask with height threshold $p < 0.005$ uncorrected, extent threshold 5 voxels. For both (1) and (2), contrast estimates were extracted from activated clusters using MARSBAR (Brett et al., 2002) and examined for genotype and load differences with *t*-tests. (3) To visualize DAT1 differences separately for each working memory load, we compared 9/10 and 10/10 groups using two-sample *t*-tests with height threshold of $p < 0.005$ uncorrected, extent threshold 5 voxels, separately for each fMRI run. All reported coordinates are converted from MNI to Talairach space using the algorithm mni2tal (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).

Results

Behavioral results

For each run, mean percent accuracy [percent hits (number of correct targets/total number of targets) minus percent false alarms (number of presses for non-targets/total number non-targets)] and mean reaction time (in ms) for hits were computed for each subject. Data from each run were analyzed separately to match the fMRI contrasts of interest, with a mixed ANOVA including DAT1 (9/10, 10/10) as a between-subject factor and Condition (Low WM Run: 1-back vs. 0-back; High WM Run: 2-back vs. 1-back) as a within-subject factor.

Accuracy

For the High WM Run, a main effect of DAT1 [$F(1,18) = 4.62$, $p = 0.05$] revealed that accuracy was higher in 9/10 (M = 89.3%, SD = 7.7) than 10/10 (M = 69.8%, SD = 27.6) children. Furthermore, a

marginally significant main effect of Condition [F(1,18) = 4.107, $p = 0.06$] suggested higher accuracy in the 1-back (M = 84.3% SD = 26.3) than 2-back (M = 74.8%, SD = 22.3) blocks. However, the effect of working memory condition did not differ between 9/10 (2-back: M = 83.0%, SD = 11.4; 1-back: M = 95.5%, SD = 6.8) and 10/10 (2-back: M = 66.5%, SD = 27.8; 1-back: M = 73.2%, SD = 33.7) children, as the DAT1 × Condition interaction was not significant ($p = 0.54$). For the Low WM Run, accuracy did not differ between 9/10 and 10/10 children, as the main effect of DAT1 was not significant ($p = 0.16$). A main effect of Condition [$F(1,18) = 7.202$, $p = 0.02$] indicated higher accuracy in the 0-back (M = 95.1%, SD = 0.09) than 1-back (M = 88.3%, SD = 0.14) blocks. Similar to the High WM Run, the DAT1 × Condition interaction was not significant ($p = 0.40$) indicating that the effect of Condition did not differ between 9/10 (1-back: M = 92.7%, SD = 8.9; 0-back: M = 97.4%, SD = 2.7) and 10/10 (1-back: M = 83.8%, SD = 17.7; 0-back: M = 92.9%, SD = 12.4) children. Thus, while genotype differences did not depend upon working memory condition within either WM run, overall performance was worse in 10/10 than 9/10 children in the fMRI run with the higher WM load.

Reaction time

For the High WM Run, reaction time did not differ by DAT1 ($p = 0.11$) or working memory condition ($p = 0.72$) as both main effects did not reach significance. Furthermore, the effect of Condition did not differ between 9/10 (2-back: M = 652, SD = 112; 1-back: M = 651, SD = 124) and 10/10 (2-back: M = 765, SD = 198; 1-back: M = 750; SD = 146) children, as the DAT1 × Condition interaction was not significant ($p = 0.76$). For the Low WM Run, while there was no main effect of DAT1 ($p = 0.35$), a main effect of Condition [$F(1,18) = 15.7$, $p = 0.001$] indicated that children were faster in the 0-back (M = 619, SD = 95) than the 1-back (M = 670, SD = 126) condition. However, the effect of condition did not differ between 9/10 (1-back: M = 638, SD = 102; 0-back: M = 604, SD = 91) and 10/10 (1-back: M = 701, SD = 144; 0-back: M = 634; SD = 101) children, as the DAT1 × Condition interaction was not significant ($p = 0.21$). Thus, response speed did not differ by DAT1 for either working memory load and similar to results for accuracy, working memory condition did not influence genotype differences within each run.

fMRI results

DAT1 × working memory load interaction

Using the caudate mask, a DAT1 × Load interaction was observed in one cluster in the right hemisphere ($x,y,z = 10, 1, 11$; $Z = 2.5$, $k = 34$ voxels, $p < 0.05$ FDR-corrected). Fig. 1 shows the activated region and corresponding graph. Contrast estimates revealed that activation was greater for the High than Low WM load in 9/10 children ($t(9) = 3.25$, $p = 0.01$) but did not differ in 10/10 children ($p = 0.46$). Furthermore, activation was significantly greater in 9/10 than 10/10 children for the High WM load ($t(18) = 3.52$, $p = 0.002$) but did not differ for the Low WM load ($p = 0.96$). Thus, the right caudate was sensitive to working memory demands in 9/10 but not 10/10 children and DAT1 differences (9/10 > 10/10) were evident only under higher working memory demands.

Whole brain analysis revealed a DAT1 × Load interaction in three clusters, in the right putamen (18, 5, −9; $Z = 2.73$, $k = 7$ voxels), left precuneus (BA 7, −12, −68, 48; $Z = 2.67$, $k = 12$ voxels), and in a region below the thalamus corresponding to the substantia nigra and subthalamic nuclei (SN/STN) ($x,y,z = 12, -12, -6$; $Z = 2.81$, $k = 13$ voxels). Fig. 2 shows each region and the corresponding graph. Comparisons of contrast estimates showed that the pattern of results in the right putamen and left precuneus was similar to that in the right caudate reported above. In these two regions, activation was greater for the High than Low WM load (putamen $t(9) = 3.09$, $p = 0.01$; precuneus $t(9) = 3.12$, $p = 0.01$) in 9/10 children but not in 10/10 children (putamen $p = 0.20$; precuneus $p = 0.49$). Furthermore, activation was greater in 9/10 than 10/10 children during High WM Load (putamen $t(18) = 3.25$, $p = 0.004$; precuneus $t(18) = 2.48$, $p = 0.02$), but did not differ during Low WM Load (putamen $p = 0.32$; precuneus $p = 0.14$). Thus, similar to the right caudate, the right putamen and left precuneus were sensitive to working memory demands in 9/10 but not 10/10 children and DAT1 differences (9/10 > 10/10) were evident only under higher working memory demands.

In contrast, comparison of contrast estimates in the SN/STN cluster suggested a different pattern of load and DAT1 differences than that in the striatum and precuneus in the 10/10 children. In 9/10 children, activation tended to be greater for the High than Low WM load ($p = 0.09$); while this comparison was marginally significant, it is notable that the direction of the load effect is similar to that in striatum and parietal cortex. In contrast to those regions, activation in 10/10 children tended to be greater for Low than High WM load ($t(9) = 2.20$, $p = 0.06$). Furthermore, activation was greater in 10/10 than 9/10 children during Low WM Load ($t(18) = 2.28$, $p = 0.04$); groups did not differ for the High WM load ($p = 0.11$). Thus, in contrast to the striatum and precuneus, the right SN/STN was sensitive to working memory load in 10/10 children, in a direction opposite to that in the 9/10 children, and DAT1 differences (10/10 > 9/10) were evident only under low working memory demands.

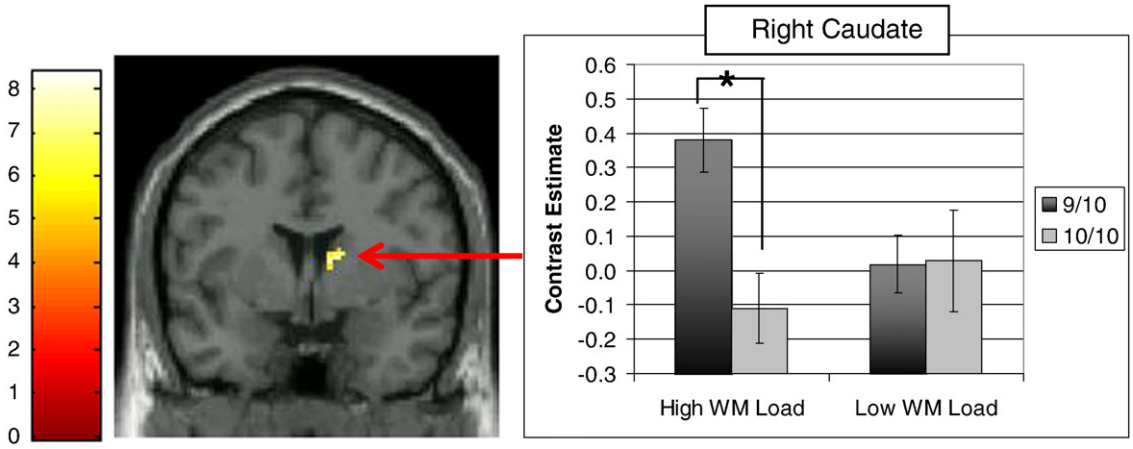

Fig. 1. Interaction between DAT1 (9/10, 10/10) and working memory load (High WM Run, Low WM Run) in the right caudate ($y = 1$), $p < 0.05$ FWE corrected for small volume. Graph shows mean contrast estimates (± standard error) in the activated cluster by DAT1 and working memory load (*$p < 0.01$).

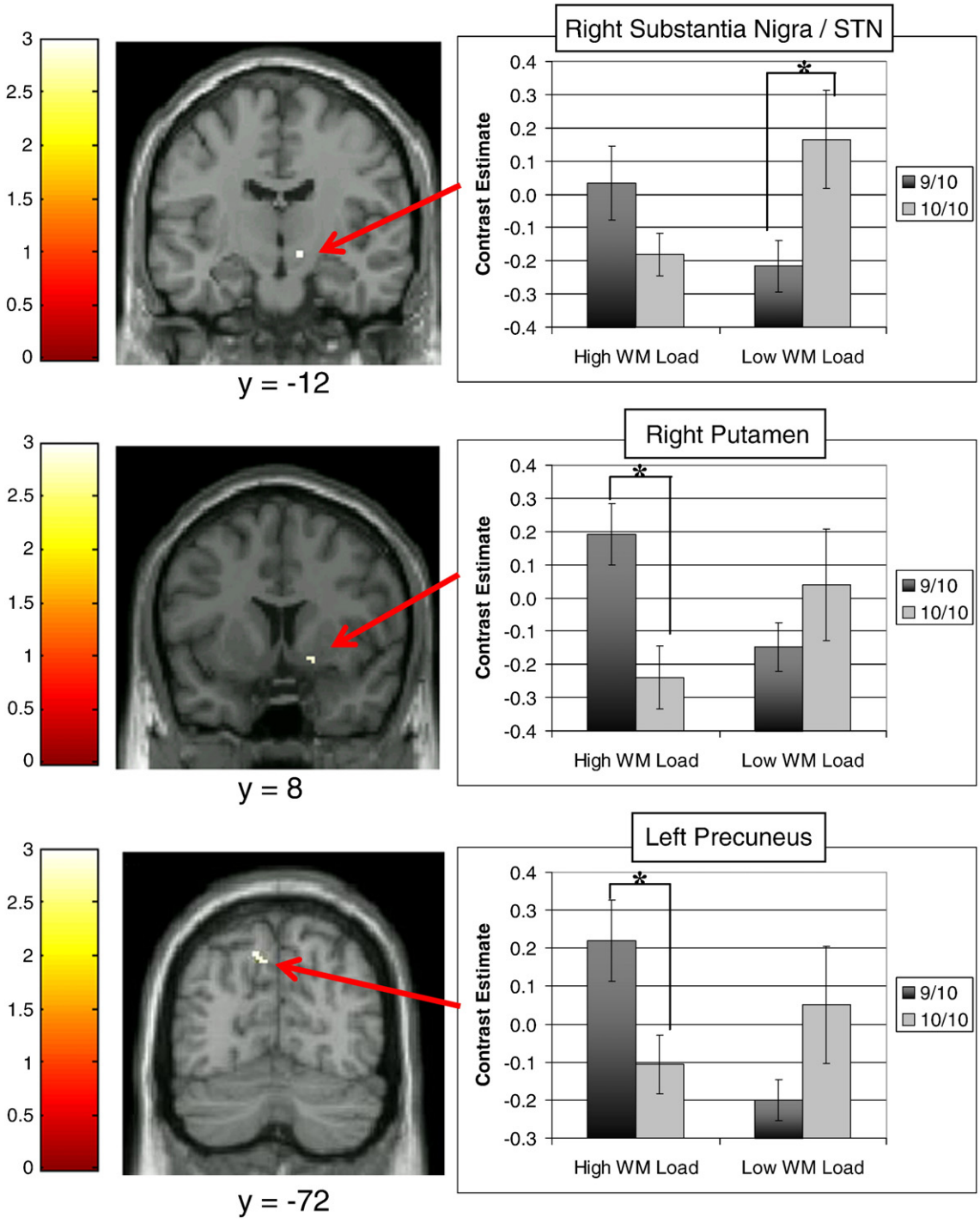

Fig. 2. Regions showing the interaction of DAT1 (9/10, 10/10) and working memory load (High WM Run, Low WM Run) in whole brain analysis ($p<0.005$ uncorrected, extent threshold 5 voxels). Graph shows mean contrast estimates (±standard error) for each activated cluster by DAT1 and load (*$p<0.05$).

DAT1 differences at each load

Separate examination of each fMRI run revealed genotype differences for the High WM Run. 9/10 children activated a network of subcortical and cortical regions often observed during working memory, including the bilateral caudate and right putamen subcortically, and premotor, parietal, and insula/inferior prefrontal cortices bilaterally, to a greater extent than 10/10 children (see Table 1). Furthermore, no regions were more activated in 10/10 than 9/10 children. For the Low WM Run, activation in the two groups did not differ in any region.

Discussion

The present results showed that the neural response to working memory load varied by DAT1 VNTR in typically developing 7–12 year-old children. *n*-back accuracy was superior in 9/10 than 10/10 carriers under high but not low working memory demands. Examination of each run separately revealed that a prefrontal–striatal–parietal network of regions was more activated in 9/10 than 10/10 carriers in the high load run; no DAT1 differences were observed in the low load run. Examination of load effects revealed a

Table 1
Group comparison between 9/10 and 10/10 children in regional activation during the High WM Run (2-back vs. 1-back) and Low RM Run (1-back vs. 0-back), $p<0.005$ uncorrected.

Comparison and region of activation	Brodmann's area	Talairach coordinates			Voxels	*Z*-score	*p*-value
		x	*y*	*z*			
Group differences at high working memory load							
9/10>10/10							
Right caudate	n/a	10	−1	11	311	3.46	<0.001
Left caudate	n/a	−10	−2	7		3.06	0.001
Right putamen	n/a	18	5	−10		3.28	0.001
Left premotor	6	−22	−3	59	41	3.41	<0.001
Right premotor	6	16	7	57	7	2.81	0.002
Right parietal	7/40	36	−44	43	202	3.16	0.001
Left parietal	7	−16	−69	51	22	2.91	0.002
Right inferior frontal/insula	47	30	17	−4	45	3.00	0.001
Left inferior frontal/insula	47	−26	17	−9	21	2.95	0.002
10/10>9/10	No significant voxels						
Group differences at low working memory load							
9/10>10/10	No significant voxels						
10/10>9/10	No significant voxels						

DAT1 × Load interaction in the right hemisphere in the caudate, our *a priori* region of interest. Furthermore, exploratory analysis at a liberal threshold revealed this interaction in other basal ganglia regions (putamen and substantial nigra/subthalamic nuclei — SN/STN) and in medial parietal cortex (left precuneus). The striatal and parietal regions were more activated in 9/10 carriers under high than low load, and DAT1 differences (9/10>10/10) were evident only under high load. In contrast, SN/STN tended to be more activated in 10/10 carriers under low than high load and DAT1 differences (10/10>9/10) were evident only under low load. Thus, 10-repeat homozygosity of DAT1 was associated with reduced performance and lack of increased basal ganglia involvement under demanding working memory conditions.

Our behavioral results provide some insight into the lack of consistency in past findings of DAT1 effects on component processes of executive function. Superior working memory in 9/10 carriers is consistent with findings from inhibitory tasks in typically developing children (Cornish et al., 2005; Loo et al., 2003). Together, these findings support the notion that 10-repeat homozygosity is disadvantageous for executive function as suggested by its association with ADHD. However, this disadvantage depended upon working memory demands in the present study, as DAT1 differences were evident under higher but not lower working memory load. The load-dependent findings suggest that the lack of DAT1 effects in 0-back and 2-back performance in past adult studies (Bertolino et al., 2006, 2009; Caldu et al., 2007) may owe to insufficient working memory burden. The 2-back condition may not be sufficiently demanding for adults as it was for children in the present study. However, performance of adults was not at ceiling (<~85%) in some studies (Bertolino et al., 2006, 2009) and therefore, it is possible that DAT1 effects on working memory depend upon developmental stage such that immature working memory is more sensitive to DAT1 effects than when mature. Inclusion of a higher load such as a 3-back condition in adult studies is needed to determine whether load-dependency is unique to childhood.

The present behavioral findings stand in contrast to one study in ADHD children showing worse performance in 9/10 than 10/10 carriers on neuropsychological tasks dependent on working memory [Self-ordered pointing, Arithmetic and Digit Span subtests of the WISC-III (Karama et al., 2008)]. While these tasks draw upon working memory, they are not pure measures as they are sensitive to global intellectual function (e.g., WISC-III subtests). Furthermore, the self-ordered pointing task is insensitive to individual differences in prefrontal dopamine levels associated with the COMT genotype whereas another task drawing upon working memory and inhibitory function was sensitive to COMT-related differences in typically developing children (Diamond et al., 2004). Thus, Diamond et al. (2004) posited that sensitivity to dopaminergic function depends upon the extent to which working memory tasks also require inhibitory control. Indeed, the *n*-back task requires continuous inhibition of stimuli and associated response that were targets on prior trials but are irrelevant on the current trial. Nevertheless, these task differences cannot account for *superior* performance in 10/10 children in Karama et al.'s study. Clearly, studies using common tasks in both ADHD and typically developing children are needed for definitive conclusions about DAT1 effects on working memory performance.

Load-dependent DAT1 effect in functional activation

Our fMRI results indicated that basal ganglia involvement varied by DAT1 VNTR depending upon working memory demands. Regions showing DAT1 × Load interaction in the present study, caudate, putamen, and SN/STN, show high DAT expression (Lewis et al., 2001) and form a circuit mediating inhibitory control that is linked via reciprocal projections between striatum and SN/STN and to the prefrontal cortex through the thalamus. The striatum (caudate and putamen) was more engaged with higher working memory load in 9/10 children. This response to increased processing load was not observed in 10/10 children. While the reported interaction analysis revealed right-sided striatal activation, left-sided regions were evident at a lower threshold suggesting involvement of that hemisphere, albeit weak. Increased demand on working memory is associated with greater dopaminergic signaling (Floresco and Phillips, 2001), and therefore, our results suggest that functional consequences of individual differences in DAT expression due to the VNTR became apparent following increased dopamine release. If striatal expression of DAT is greater in 10/10 carriers as suggested by some studies (Heinz et al., 2000), it would result in weaker dopaminergic signals due to enhanced clearance, that ought to be more disadvantageous under higher cognitive demands. Load-dependency in the SN/STN in 9/10 children was similar to that in the striatum, although the load difference was marginally significant. Human fMRI studies show that the STN contributes to inhibitory control (Aron and Poldrack, 2006), a process that was more demanding under higher working memory load. In 10/10 children, however, SN/STN involvement tended to be higher for the low than high load, contributing to a DAT1 difference at the low load (10/10>9/10). Thus, in contrast to the striatum that was insensitive to working memory load in 10/10 children, SN/STN was load-sensitive such that its engagement was higher under lower processing demands. This pattern of differences suggests that relative contributions of basal ganglia regions involved in inhibitory function differ by DAT1 VNTR.

Only one cortical region, the precuneus in medial parietal cortex, showed load-dependent DAT1 differences. The precuneus is specifically involved in visual attention but is often activated in a variety of cognitively demanding tasks requiring enhanced voluntary attention (Cavanna, 2007). It has connections to lateral parietal and prefrontal cortices as well as subcortical structures such as the striatum (Cavanna and Trimble, 2006). Indeed, DAT availability in the caudate was associated with precuneus activation during visual attention measured by fMRI (Tomasi et al., 2009). In the present study, the pattern of load and DAT1 differences in the precuneus was similar to that in the caudate and putamen. Thus, precuneus engagement increased with load in 9/10 children but not in 10/10 children. Differences in precuneus activation as a function of DAT1 may relate directly to differences in DAT expression in the precuneus (Lewis et al., 2001). Alternately, they may reflect a secondary influence of striatal regions via thalamocortical projections during cognitively demanding conditions.

The prefrontal cortex did not show reliable load-dependent DAT1 effects in the present study. Although DAT expression is low in the prefrontal cortex, past studies of adults have shown DAT1 differences in activation that may relate to cortical striatal projections (Bertolino et al., 2006, 2009). In the present study, separate examination of each fMRI run revealed greater prefrontal involvement in 9/10 than 10/10 children in the high but not in the low load run. However, those regions did not exhibit a DAT1 × Load interaction statistically. A possible reason for this is that prefrontal involvement varied widely across children. In secondary analysis (see Supplementary Fig. 3), we examined whether variability of prefrontal recruitment was related to performance on the *n*-back task. We performed this analysis for the high load fMRI run because performance was highly variable, especially for the 10/10 group (standard deviation 2-back = 27.8%; 1-back = 33.7%). Both genotype groups showed that greater prefrontal activation was related to higher performance accuracy. Thus, high individual variability may have prevented the detection of load-dependent DAT1 differences in the prefrontal cortex. This variability could relate to the presence of other genotypes with functional polymorphisms influencing prefrontal functioning. In adults, the effect of the DAT1 VNTR was additive with the COMT val108/158met genotype (Bertolino et al., 2006; Caldu et al., 2007) and interactive with DRD2 (Bertolino et al., 2009). Distribution of the other genotypes is unknown in the present samples and ought to be controlled in future studies.

Developmental differences in DAT1 effects

Relative to past findings in adults, the present pattern of functional activation suggests two developmental differences in DAT1 effects. First, while the direction of DAT1 effects (greater activation in 9/10 than 10/10 children) was consistent with that in adults, the present loci included a wider network of regions than those reported in adults. Specifically, in the high load fMRI run, 9/10 children activated prefrontal, premotor, parietal and striatal regions to a greater extent than 10/10 children. In contrast, in studies with adults, DAT1 differences were limited to the prefrontal cortex including left/right middle and inferior frontal gyri and anterior cingulate (Bertolino et al., 2006, 2009). Perhaps the different results reflect differences in manipulation of processing load in the *n*-back task (e.g., 2-back vs. 0-back in adults but 2-back vs. 1-back in children). Furthermore, inclusion of a wider network of regions in children could also represent the diffuse, less focused functional engagement typical of less mature cognition (Casey et al., 2000).

Second, DAT1 differences in the context of performance similarity differed in children from those in adult studies. In the studies by Bertolino et al. (2006, 2009), *n*-back performance did not differ between adult DAT1 groups and thus, reduced prefrontal activation in the 10/10 carriers was interpreted as a more focused neural response. In contrast, we observed reduced activation in 10/10 children in the context of lower performance (e.g., high load fMRI run) suggesting inadequate engagement. The correlational analysis also indicated that 10/10 children with higher accuracy engaged prefrontal–striatal–parietal regions (Supplemental materials). During the low load fMRI run in which both genotype groups had similar accuracy, direct comparison did not reveal activation differences between 9/10 and 10/10 children. However, the SN/STN region that was also sensitive to load, was more activated in 10/10 than 9/10 children. Therefore, rather than a more focused neural response observed in 10/10 adults, 10/10 children showed a less focused response relative to 9/10 children in the context of matched performance.

For a full understanding of developmental differences, it is necessary to examine genetic effects on the full complement of relationships between working memory load, activation, and performance across developmental stages. Functional imaging of adults performing an *n*-back task with parametric load increases (0-back to 3-back) revealed that dorsolateral prefrontal activation increased with earlier load increments but decreased with later increments (e.g., 2-back to 3-back); performance accuracy also decreased following the higher load increment (Callicott et al., 1999). Such load–response relationships may be rooted in characteristics of dopaminergic stimulation that show an inverted-U response, with very low and very high endogenous dopamine release having deleterious effects on prefrontal neuronal activity in behaving monkeys (Vijayraghavan et al., 2007). Adult individual variation in the nature of the load–response relationship relates to a functional polymorphism for COMT, the enzyme that regulates prefrontal dopamine levels (Mattay et al., 2003). Most importantly, these genetic effects are moderated by developmental changes as COMT activity increases from childhood to adulthood, and by interaction with other functional polymorphisms such as BDNF, that are instrumental in structural maturational changes (reviewed in Dickinson and Elvevag, 2009). Consequently, genetic effects may be magnified or attenuated at different developmental stages.

Conclusion

Functional anatomy mediating working memory varied by DAT1 VNTR depending upon processing load in 7–12 year-old typically developing children. Future studies in adults that vary working memory load are needed to determine whether the observed load-dependency is selective to childhood development when cortical–subcortical circuits important for working memory are still undergoing maturation (Toga et al., 2006). 10-repeat homozygosity was associated with reduced performance and lack of greater basal ganglia involvement under higher working memory demands. An implication of these findings is that 10-repeat homozygosity may mediate susceptibility to ADHD via diminished basal ganglia function. However, our sample sizes were rather small ($n = 10$/genotype group), and thus, the observed DAT1 differences need to be replicated in larger samples. Nevertheless, the present findings suggest a nuanced view of the effects of DAT1 VNTR on executive function that is useful to models of normal cognition, cognitive development and developmental psychopathology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2009.12.104.

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