ARCHIVAL REPORT

Neural Mechanisms of Attention-Deficit/Hyperactivity Disorder Symptoms Are Stratified by *MAOA* Genotype

Charlotte Nymberg, Tianye Jia, Steven Lubbe, Barbara Ruggeri, Sylvane Desrivieres, Gareth Barker, Christian Büchel, Mira Fauth-Buehler, Anna Cattrell, Patricia Conrod, Herta Flor, Juergen Gallinat, Hugh Garavan, Andreas Heinz, Bernd Ittermann, Claire Lawrence, Karl Mann, Frauke Nees, Angelica Salatino-Oliveira, Marie-Laure Paillère Martinot, Tomas Paus, Marcella Rietschel, Trevor Robbins, Michael Smolka, Tobias Banaschewski, Katya Rubia, Eva Loth, Gunter Schumann, and the IMAGEN Consortium

Background: Attention-deficit/hyperactivity disorder (ADHD) is characterized by deficits in reward sensitivity and response inhibition. The relative contribution of these frontostriatal mechanisms to ADHD symptoms and their genetic determinants is largely unexplored.

Methods: Using functional magnetic resonance imaging and genetic analysis of the monoamine oxidase A (*MAOA*) gene, we investigated how striatal and inferior frontal activation patterns contribute to ADHD symptoms depending on *MAOA* genotype in a sample of adolescent boys ($n = 190$).

Results: We demonstrate an association of ADHD symptoms with distinct blood oxygen level–dependent (BOLD) responses depending on *MAOA* genotype. In A hemizygotes of the expression single nucleotide polymorphism rs12843268, which express lower levels of *MAOA*, ADHD symptoms are associated with lower ventral striatal BOLD response during the monetary incentive delay task and lower inferior frontal gyrus BOLD response during the stop signal task. In G hemizygotes, ADHD symptoms are associated with increased inferior frontal gyrus BOLD response during the stop signal task in the presence of increased ventral striatal BOLD response during the monetary incentive delay task.

Conclusions: Depending on *MAOA* genotype, ADHD symptoms in adolescent boys are associated with either reward deficiency or insufficient response inhibition. Apart from its mechanistic interest, our finding may aid in developing pharmacogenetic markers for ADHD.

Key Words: Attention-deficit/hyperactivity disorder, genetics, inferior frontal gyrus, monoamine oxidase A, neuroimaging, ventral striatum

Attention-deficit/hyperactivity disorder (ADHD) symptoms include impulsivity, hyperactivity, and inattention. The disorder is thought to be dimensional, with the most extreme manifestations clinically diagnosed as ADHD according to DSM-IV (1,2). Evidence from neuroimaging studies suggests that impulsivity and hyperactivity are associated with striatal and frontal activation patterns during reward anticipation and response inhibition, respectively (3–7). Despite the clinical relevance of these neural mechanisms, we know little about how reward anticipation and response inhibition jointly affect ADHD symptoms.

Studies of ADHD patients frequently report reduced blood oxygenation level–dependent (BOLD) response of the ventral striatum (VS) during reward anticipation (6,8–11). This hyporesponsiveness of the VS has been observed during both immediate and delayed rewards (11) in ADHD patients as well as in healthy female subjects (12). However, there is conflicting evidence

From the Medical Research Council—Social, Genetic and Developmental Psychiatry Centre (CN, TJ, SL, BR, SD, GB, AC, PC, EL, GS), Institute of Psychiatry, King's College London, London, United Kingdom; University Medical Centre Hamburg-Eppendorf (CB, FN), Hamburg, Germany; Department of Addictive Behaviour and Addiction Medicine (MF-B, KM), and Department of Cognitive and Clinical Neuroscience (HF), Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany; Department of Psychiatry and Psychotherapy (JG, AH), Charité—Universitätsmedizin Berlin, Berlin, Germany; Departments of Psychiatry and Psychology (HG), University of Vermont, Burlington, Vermont; Physikalisch-Technische Bundesanstalt (BI, TP), Berlin, Germany; School of Psychology (CL), University of Nottingham, Nottingham, United Kingdom; Genetics Department (AS-O), Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Institut National de la Santé et de la Recherche Médicale (M-LPM), INSERM CEA Unit 1000 "Imaging and Psychiatry," Orsay, France; The Rotman Research Institute (TP), Baycrest, University of Toronto, Toronto, Canada; Department of Genetic Epidemiology in Psychiatry (MR), Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany; Behavioural and Clinical Neuroscience Institute (TR), University of Cambridge, Cambridge, United Kingdom (MS), Neuroimaging Center, Department of Psychiatry and Psychotherapy, Technische Universität Dresden, Würzburger, Dresden, Germany; Department of Child and Adolescent Psychiatry (TB), Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; Department of Child Psychiatry (KR), Institute of Psychiatry, King's College London, London, United Kingdom.

Authors EL and GS contributed equally to this work. Authors 6-24 are listed in alphabetical order.

Address correspondence to Charlotte Nymberg, Ph.D., Medical Research Council—Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, United Kingdom; E-mail: charlotte.nymberg@kcl.ac.uk.

Received Oct 17, 2012; revised and accepted Mar 19, 2013.

0006-3223
http://dx.doi.org/10.1016/j.biopsych.2013.03.027

BIOL PSYCHIATRY 2013;74:607–614

showing that there may also be a positive correlation of impulsivity and VS activation during reward anticipation (13,14) and during immediate compared with delayed rewards in the normal population (15).

ADHD has been associated with poor response inhibition resulting either from insufficient activation of the inferior frontal gyrus (IFG) (5,16,17) or from a requirement for larger frontal recruitment for optimal task performance (4,7,18,19). Rubia and colleagues reported that the IFG of ADHD patients is hypoactivated during inhibition trials of the stop signal task (SST) relative to the IFG of healthy control subjects (20), but others have observed a hyperactivation of the IFG during successful inhibition in ADHD patients (4,7). Previous studies suggest that the right IFG in particular is a crucial structure underlying response inhibition (21).

Thus it appears that BOLD responses of the subcortical reward system and inferior frontal inhibitory mechanisms, particularly the right IFG, are crucially related to ADHD symptoms (8,21). However, there is inconsistency regarding whether they are associated with enhanced or decreased BOLD responses in these two systems. Furthermore, because reward processing and response inhibition have never been tested together in adolescents, it is unclear whether ADHD symptoms in the same individuals are associated with abnormalities in either or both systems. We therefore interrogated both systems and investigated potential determinants of brain activity in the regions involved.

Because the mean heritability of ADHD is estimated at 76% (22), we hypothesized that these determinants might include genetic factors (23). ADHD symptoms are more commonly observed in males than females with gender ratios varying from 3:1 to 9:1 (24,25). It has therefore been suggested that genes on the X chromosome may be involved in the development of the disorder. The monoamine oxidase A gene *(MAOA)* is localized on the human X chromosome (26–28). *MAOA* encodes a mitochondrial enzyme, which degrades monoamines, including norepinephrine, dopamine, and serotonin (29), which are thought to underlie neural functions associated with ADHD. Several studies have identified associations between specific *MAOA* polymorphisms and ADHD (26,27,30,31). Most recently, a screen of 23 candidate genes (including *COMT DRD1-DRD4, DAT1, SNAP25, MAOA,* and *MAOB*) reported that *MAOA* was the most promising candidate gene underlying ADHD out of the genes investigated (27). Of 12 *MAOA* polymorphisms that were tested for association with ADHD, rs12843268 showed the strongest association. A haplotype analysis that included the *MAOA* single nucleotide polymorphism (SNP) rs12843268 reported an association with adolescent ADHD and response inhibition in boys (32). Earlier studies showed that a variable number tandem repeat (VNTR) within the promoter of *MAOA*, which is linked to ADHD (33), was also associated with inhibitory control (34) as well as novelty seeking (35,36).

In a sample of 414 adolescents from the IMAGEN study who did not achieve diagnostic criteria for ADHD, we investigated whether *MAOA* genotype might be used for stratification by testing the association between *MAOA* and ADHD symptoms. We then carried out stratified analyses of brain activation in the key reward area of VS and the principal inhibitory frontal area, the right IFG. On the basis of etiologic models just described, we hypothesized that there is 1) a significant association between *MAOA* genotype and ADHD symptoms, 2) a significant association between ADHD symptoms and VS and right IFG BOLD responses, and 3) that the association between ADHD symptoms and brain activation patterns during reward processing and inhibitory control is stratified by *MAOA* genotype.

Methods and Materials

Participants

We used data from the first wave of IMAGEN (n = 648). Individuals who had passed quality controls for genotyping, neuroimaging, and behavioral tests were included in the data set. Four hundred and fourteen adolescents passed the inclusion criteria for further analysis (190 boys, 224 girls). The mean age of the participants was 14.4 years (SD: .4; range: 13.3–15.6 years; Table 1).

Participants were tested at eight IMAGEN assessment centres (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris, and Dresden). Local ethics research committees at each site approved the study. On the day of assessment, written consent was obtained from the parent or guardian, and verbal assent was obtained from the adolescent. A detailed description of recruitment and assessment procedures and inclusion/exclusion criteria have been published elsewhere (37). Three hundred and sixty-seven participants were right-handed, and 47 participants were left-handed or ambidextrous. Individuals with verbal (VIQ) or nonverbal (PIQ) IQ <75 or missing IQ information were excluded (n = 10). Handedness and study site were controlled for in all analyses.

Out of the 190 boys who had completed the monetary incentive delay (MID) task, 143 had also completed the SST (Supplement 1).

Table 1. Demographics Split by Gender and rs12843268 Genotype Groups, Mean ± SD (Range)

	Boys			Girls			
	A (n = 67)	G (n = 123)	Total (n = 190)	AA (n = 16)	AG (n = 100)	GG (n = 108)	Total (n = 224)
Age (years)	14.5 ± .4 (13.6–15.5)	14.5 ± .4 (13.6–15.6)	14.5 ± .4 (13.6–15.6)	14.5 ± .4 (13.9–15.6)	14.4 ± .4 (13.3–15.4)	14.4 ± .5 (13.3–15.5)	14.4 ± .04 (13.3–15.6)
VIQ	117.4 ± 14.9 (83–150)	115.1 ± 14.6 (87–155)	115.9 ± 14.7 (83–155)	110.4 ± 11.8 (88–130)	112.6 ± 15.1 (77–150)	113.1 ± 15.2 (77–152)	112.7 ± 14.9 (77–152)
PIQ	107.2 ± 13.7 (81–149)	107.0 ± 12.5 (79–135)	107.0 ± 12.9 (79–149)	111.8 ± 12.9 (92–141)	111.4 ± 12.1 (86–146)	109.3 ± 12.8 (76–135)	110.9 ± 12.7 (76–147)
ADHD Symptoms	2.7 ± 1.9 (0–7)	3.4 ± 2.6 (0–10)	3.1 ± 2.1 (0–10)	2.7 ± 1.9 (0–7)	2.3 ± 2.1 (0–8)	2.6 ± 2.2 (0–10)	2.4 ± 2.1 (0–10)

We found no significant genotype differences in age, VIQ, PIQ ($p > .05$) in boys or girls after controlling for study site. Boys carry one A allele or one G allele, girls are either AA homozygous, AG heterozygous, or GG homozygous for rs12843268.

ADHD, attention-deficit/hyperactivity disorder; VIQ, verbal IQ; PIQ, nonverbal IQ.

ADHD Symptoms (Strength and Difficulties Questionnaire)

ADHD symptoms were assessed using parental reports of the Strength and Difficulties Questionnaire (SDQ), a brief 25-item behavioral screening tool probing for ADHD-type problems (hyperactivity, inattention, and impulsivity), emotional symptoms, conduct problems, peer problems, and prosocial behavior (38). Our sample of 414 consisted of 28 subjects (20 boys) who "possibly" suffered from ADHD according to the SDQ. The SDQ ADHD symptom scale can be subdivided into two subscales: a hyperactivity/impulsivity subscale, composed of three items, and an inattention subscale, composed of two items (39).

Genotyping

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples preserved in ethylene-eiamine-tetra-acetic acid (EDTA) vacutainer tubes (BD, Becton, Dickinson and Company, Oxford, United Kingdom) using Gentra Puregene Blood Kit (QIAGEN, Valencia, California) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip (Illumina, San Diego, California) as part of a previous genome-wide association study (37) (Supplement 1).

Effect of rs12843268 on *MAOA* Expression

Total RNA was extracted from whole blood cells using the PAXgene Blood RNA Kit (QIAGEN). Labeled complementary RNA (cRNA) from 369 individuals was generated. *MAOA* expression was independently validated in 40 boys using quantitative polymerase chain reaction (Supplement 1). The relative fold change in expression was measured via the comparative method using the formula $2^{-\Delta\Delta Ct}$ (40).

Monetary Incentive Delay Task

The participants performed a modified version of the MID task to study neural responses to reward anticipation and reward outcome. The paradigm has been previously described (41) (Supplement 1).

Stop Signal Task

Participants also performed an event-related SST designed to study neural responses to successful and unsuccessful inhibitory control (5,42). The task has been previously described (5,42) (Supplement 1). The dependent variable of the task is the stop signal reaction time (SSRT), which was calculated by subtracting the mean stop signal delay (the average time between go and stop signal, at which the subject managed to inhibit to 50% of trials) from the mean reaction time to go trials (43). Because of problems in the tracking algorithm, SSRT data was only available for 73 subjects.

Magnetic Resonance Imaging Data Acquisition and Analysis

Structural and functional magnetic resonance imaging (fMRI) data were acquired at eight IMAGEN assessment sites with 3T MRI scanners of different manufacturers (Siemens, Munich, Germany; Philips, Best, The Netherlands; General Electric, Chalfont St Giles, UK; Bruker, Ettlingen, Germany). The scanning variables were specifically chosen to be compatible with all scanners. The same scanning protocol was used at all sites. In brief, high-resolution T1-weighted three-dimensional structural images were acquired for anatomical localization and coregistration with the functional time series. Functional MRI BOLD images were acquired with a gradient-echo, echo-planar imaging sequence. For the MID task, 300 volumes were acquired for each subject. For the SST, 444 volumes were acquired for each subject. For both tasks, each volume consisted of 40 slices aligned to the anterior commission/posterior commission line (2.4-mm slice thickness, 1-mm gap). The echo time was optimized (echo time = 30 msec, repetition time = 2200 msec) to provide reliable imaging of subcortical areas (Supplement 1).

Functional MRI data were analyzed with SPM8 (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm). We extracted regions of interest using the Marsbar toolbox (http://marsbar.sourceforge.net). The IFG opercularis was extracted based on the Montreal Neurological Institute Automated Anatomical Labeling. Because the VS is not available in Automated Anatomical Labeling, it was created using Marsbar based on the peak of the "anticipation high win versus no win" contrast (xyz = ±9, 11, −2, 9-mm radius; Figure S2 and Table S2 in Supplement 1). Averaged beta values based on all voxels in the regions of interest were used for all analyses.

Association Analyses

The general linear model was used to determine associations among the SDQ measure, BOLD responses, and *MAOA* genotype. Correlations between fMRI BOLD-responses and SSRT were derived through Pearson correlations. All analyses were two-sided. Permutations with 100,000 iterations were used to control for hemisphere specific tests of VS BOLD response. Where $p_{corrected}$ is indicated, p values were corrected for statistical tests performed in the left and right hemisphere.

Results

Identification and Characterization of *MAOA* SNP

We extracted eight SNPs covering the *MAOA* locus and identified two haplotypes with a frequency greater than 5%, which accounted for 92.3% of the variance of the gene (Table 2; Figure S4 in Supplement 1). Among the SNPs segregating the two haplotypes was rs12843268, which has previously been associated with ADHD symptoms (27,32). We therefore selected rs12843268 for further analyses. G allele carriers of rs12843268 represent the major haplotype with a frequency of 63.4%, whereas A allele carriers represent the minor haplotype with a frequency of 28.9%. Gene expression data of *MAOA* from peripheral blood were available from 171 boys and 198 girls of the IMAGEN sample. In boys, we found significant differences between genotype groups of rs12843268 ($F_{1,162} = 7.82$, $p = .006$), with higher *MAOA* messenger RNA (mRNA) expression in the G hemizygotes compared with A hemizygotes. The expression analysis was not significant in girls ($F_{1,189} = .58$, $p = .45$). The association was independently validated through quantitative polymerase chain reaction in RNA from 40 boys, which showed a relative fold change in expression between the two genotypes of 6.34 (SE: .296) (Figure S5 in Supplement 1).

Effects of *MAOA* Genotype on ADHD Symptoms

Because of the X-linked nature of *MAOA*, we tested the association between the SNP rs12843268 and ADHD symptoms in boys and girls separately. We found a significant association of SNP rs12843268 and ADHD symptoms in boys ($F_{1,181} = 4.12$, $p = .044$, partial η^2: .022) with G hemizygotes ($n = 123$) showing significantly more ADHD symptoms compared with A hemizygotes ($n = 67$) (Table 1). Rs12843268 genotype accounted for 2.2% of the variance in ADHD symptoms in boys. We did not find

Table 2. Haplotype Analysis of *MAOA* gene

	rs1465108	rs909525	rs1800464	rs12843268	rs6610845	rs2235186	rs2072743	rs1137070	*p* Value	Frequency
Haplotype 1	1	1	1	1	1	1	1	1	.016	.634
Haplotype 2	0	0	1	0	0	0	0	0	.063	.289
Total Frequency										.923

Tagging single nucleotide polymorphism rs12843268 segregates haplotypes with a frequency of >5% and accounts for 92.3% of the variance of the gene.

a significant association between *MAOA* SNP rs12843268 and ADHD symptoms in girls ($F_{1,215} = 1.47$, $p = .23$), suggesting that the effects of *MAOA* are gender specific. On the basis of these findings, we performed imaging genetic analyses in boys only.

Reward Anticipation

In boys, we investigated the effect of rs12843268 genotype on fMRI BOLD response in the VS during reward anticipation using the MID task. G hemizygotes showed significantly higher BOLD response than A hemizygotes in the left VS ($F_{1,180} = 10.87$, $p_{corrected} = .002$, partial η^2: .061; Figure 1A) and in the right VS ($F_{1,180} = 6.80$, $p_{corrected} = .015$, partial η^2: .045; Figure 1B**)**.

We next examined whether individual variability in VS BOLD responses were correlated with ADHD symptoms in the full sample of 190 boys. We found a trend toward a negative correlation between the right VS BOLD response and ADHD symptoms in the full sample of boys ($r = -.16$, $p_{corrected} = .053$). This correlation was particularly driven by the impulsivity/hyperactivity subscale of the SDQ (right VS: $r = -.18$, $p_{uncorrected} = .014$). No significant correlation was identified between VS activation and the inattention subscale (right VS: $r = -.08$, $p_{uncorrected} = .30$; left VS: $r = -.09$, $p_{uncorrected} = .24$).

We carried out an interaction analysis of right VS activation*SNP rs12843268 genotype on ADHD symptoms, which was not significant ($p = .570$) due to multicollinearity for VS*SNP rs12843268, with a corresponding $r = .865$. Orthogonalization was not performed because it would either decompose or integrate variables, thus changing their meaning for this study. We then stratified our analyses by rs12843269 genotype and found that the negative correlation observed between right VS BOLD response and ADHD symptoms was driven by A hemizygotes (right VS: $r = -.29$, $p_{corrected} = .041$; left VS $r = -.22$, $p_{corrected} = .14$). We observed no significant correlation between VS BOLD responses and ADHD symptoms in G hemizygotes (right VS: $r = -.15$, $p_{corrected} = .17$; left VS: $r = -.14$, $p_{corrected} = .21$) (Figure 2). Differences between the correlation coefficients were not significant when separated by genotype for the right VS ($z = .95$, $p = .34$) or the left VS ($z = .53$, $p = .60$). No significant differences were found in reaction times (RT) during the MID task (Table S4 in Supplement 1). Although we observed an association of rs12843268 genotype and dorsal striatal activation, this was not correlated with ADHD symptoms (Table S5 in Supplement 1).

The amount of variance of ADHD symptoms explained by neural responses in the right VS without rs12843268 stratification accounted for 2.6%. After stratification by genotype, the variance in ADHD symptoms accounted for in A hemizygous boys increased to 8.4%.

Although the negative correlations between the right VS BOLD response and ADHD symptoms is consistent with a blunted reward system (6,10), the absence of a significant association between VS BOLD response and ADHD symptoms in G hemizygous boys suggested that brain regions other than the VS might mediate the effect of rs12843268 in this genotype group. We hypothesized that G hemizygous boys may show an association between ADHD symptoms and brain activation measured during response inhibition (5,21). Therefore, we investigated genotype-specific BOLD response of the right IFG during successful response inhibition.

Successful Response Inhibition

We measured BOLD response of the right IFG using the SST in 143 of the 190 boys (Supplement 1). Whereas we did not observe a significant association between right IFG and *MAOA* rs12843268 ($F_{1,133} = .02$, $p = .88$), we found a significant interaction between *MAOA* genotype and right IFG activation on ADHD symptoms ($F_{1,131} = 6.24$, $p = .014$). Upon stratification by *MAOA* rs12843268 genotype, we found a positive correlation between right IFG

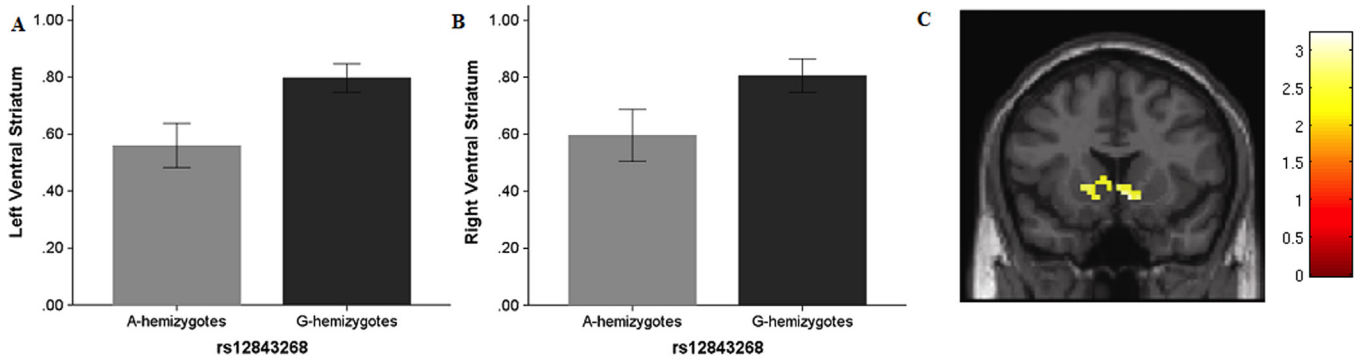

Figure 1. (A) Associations between *MAOA* genotype and left activation: *MAOA* rs12843268 is associated with left ventral striatum (VS) activation ($F_{1,180} = 10.87$, $p = .001$, partial η^2: .061). **(B)** Associations between *MAOA* genotype and right VS activation: *MAOA* rs12843268 is associated with right VS activation ($F_{1,180} = 6.80$, $p = .007$, partial η^2: .045) during reward anticipation. **(C)** Coronal section showing genotype differences in VS activation during reward anticipation, suggesting that G hemizygotes of *MAOA* rs12843268 show higher activation of bilateral VS compared with A hemizygotes (xyz: ±9, 11, −2, $p < .01$, uncorrected).

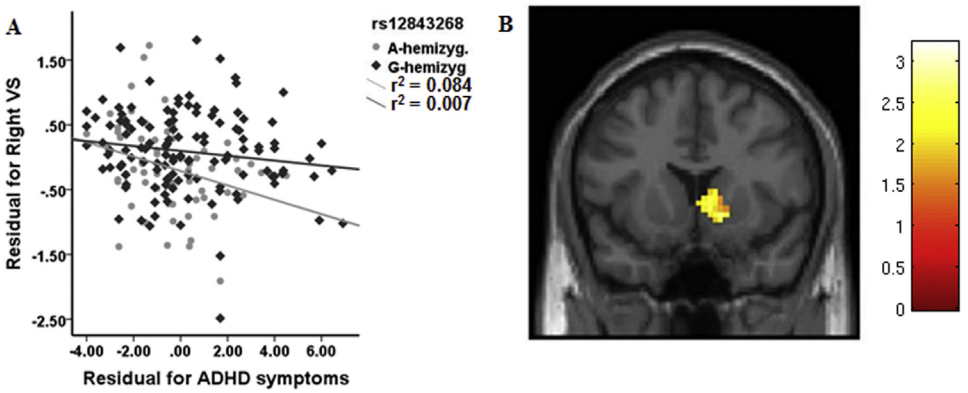

Figure 2. **(A)** Correlation of ventral striatum (VS) activation and attention-deficit/hyperactivity disorder (ADHD) symptoms: the correlation between right VS activation and ADHD symptoms were driven by A hemizygotes ($r = -.29$, $p = .025$). No significant association was found in the left VS or among G hemizygotes in either left or right VS. However, the difference between correlation coefficients were not significant (right VS: $z = .95$, $p = .34$; left VS: $z = .53$, $p = .60$). **(B)** Coronal section showing the correlation between right VS activation and ADHD symptoms during reward anticipation in A hemizygotes (xyz: 9, 11, −2, $p < .05$, uncorrected).

BOLD response and ADHD symptoms ($r = .26$, $p = .017$) among G hemizygotes, whereas in A hemizygotes, we found a negative correlation between right IFG BOLD response and ADHD symptoms ($r = -.30$, $p = .049$; Figure 3). The difference be tween the correlation coefficients was significant ($z = 3.23$, $p = .001$). Without *MAOA* rs12843268 stratification, the neural responses in the right IFG accounted for 1.9% of the variance in ADHD symptoms, whereas after stratification, the variance in ADHD symptoms accounted for in G hemizygotes increased to 6.8% and the variance in ADHD symptoms accounted for in A hemizygotes increased to 9%.

There was no significant difference between A hemizygotes and G hemizygotes in SSRT ($n = 73$) or Mean reaction time to go trials, and no genotype-differences in the number of successfully completed stop trials or go trials (Tables S6 and S7 in Supplement 1). However, we found a negative correlation between SSRTs and right IFG BOLD response ($r = -.28$, $p = .02$), indicating that higher BOLD response of the right IFG during successful inhibition trials was associated with lower SSRT. This association was significant in G hemizygotes ($r = -.36$, $p = .02$) but not in A hemizygotes ($r = -.15$, $p = .44$; Table S6 in Supplement 1).

To determine combined variance accounted for by right VS and right IFG activation on ADHD symptoms, we performed additional analyses in the sample of 143 boys, for whom both MID and SST information was available. We found that right VS activation alone accounts for 3.7% of the variance in ADHD symptoms ($r^2 = .037$, $p = .021$). When the right IFG was added to the model, an additional 1.9% of variance in ADHD symptoms was accounted for (combined $r^2 = .056$). This change in r^2 was not significant ($p = .095$), indicating that the contribution of the IFG to ADHD symptoms is indeed dependent on *MAOA* genotype as previously suggested by the significant interaction term (Figure 3).

Discussion

In a population-based sample of 414 adolescent boys and girls, we demonstrate that *MAOA* is associated with ADHD symptoms in boys only. On the basis of these findings, we investigated whether neural mechanisms thought to underlie ADHD are affected by *MAOA* in boys. We found that ADHD symptoms were correlated with distinct frontostriatal activation patterns, depending on rs12843268 genotype.

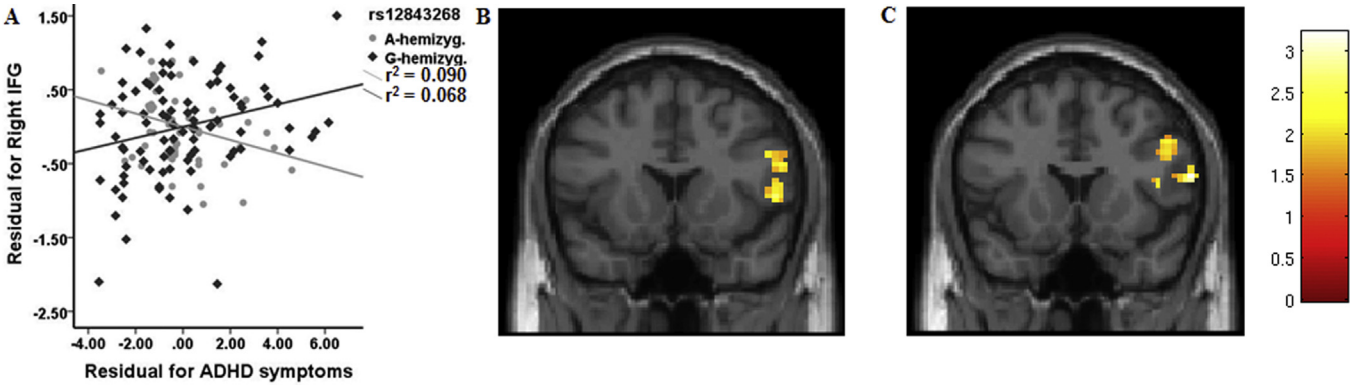

Figure 3. **(A)** Correlation between right inferior frontal gyrus (IFG) activation and attention-deficit/hyperactivity disorder (ADHD) symptoms: right IFG activation was positively correlated with ADHD symptoms in G hemizygotes ($r = .26$, $p = .017$). In A hemizygotes, a negative correlation was found between right IFG activation and ADHD symptoms ($r = -.30$, $p = .049$). The difference between the correlation coefficients was significant ($z = 3.23$, $p = .001$). **(B)** Coronal section showing the positive correlation between right IFG opercularis and ADHD symptoms during successful stop trials in G hemizygotes (xyz: 55, 15, 10, $p < .05$, uncorrected). **(C)** Coronal section showing the negative correlation between right IFG opercularis and ADHD symptoms during successful stop trials in A hemizygotes (xyz: 57, 16, 17, $p < .05$, uncorrected).

In A hemizygotes of rs12843268, which express lower levels of *MAOA*, ADHD symptoms are associated with lower VS BOLD response, indicating lower reward-related activity in this region, as well as reduced inhibition as measured by right IFG BOLD response. This may suggest that ADHD symptoms in this group arise from a blunted reward response coupled with lower prefrontal inhibitory control, as postulated by the reward deficiency syndrome hypothesis (44).

Conversely, in G hemizygotes, who express higher levels of *MAOA*, ADHD symptoms were associated with increased right IFG BOLD response in the presence of increased VS BOLD response. The observed negative correlation of SSRT and right IFG BOLD response in G hemizygotes indicates that higher VS BOLD responses alone may not be a risk factor for ADHD symptoms in this group but must be considered together with the requirement for larger frontal recruitment for optimal task performance (7).

Theories based on neuroimaging studies suggest two alternative mechanisms underlying ADHD symptoms: the impulsivity hypothesis suggests that insufficient inhibitory control underlies the disorder, whereas the reward deficiency syndrome hypothesis proposes that impulsive behaviors compensate for blunted sensitivity of the reward system (3,47,48). Our results suggest that both mechanisms contribute to ADHD symptoms, depending on *MAOA* genotype. Thus, while supporting recent reports that provide genetic evidence for the contribution of dopaminergic mechanisms to reward anticipation and ADHD (45,46), our study is first to dissociate distinct neurobehavioral mechanisms of ADHD symptoms by applying stratification by genotype. This *MAOA*-dependent stratification may contribute to the resolution of seemingly contradictory findings in the ADHD literature, which report both over- or underactivation of the inhibitory control network (4,5,7,16,18).

To understand how *MAOA* rs12843268 affects BOLD response in our sample, we investigated the expression levels of *MAOA* in A hemizygotes and G hemizygotes. We find allele specific gene expression differences in peripheral blood with G hemizygotes showing a sixfold increase in *MAOA* expression, compared with A hemizygotes. This suggests that G hemizygotes have higher *MAOA* mRNA levels, which might result in increased degradation of monoamines and lower baseline levels of serotonin, dopamine, and noradrenaline. A *cis*-acting effect of the VNTR promoter polymorphism on a yet to be identified functional variant that is in linkage disequilibrium (LD) with rs1137070 has recently been reported (49). In our sample rs1137070 is in high linkage disequilibrium ($D' = 1$; $r^2 > .9$) with rs12843268. The increased *MAOA* mRNA levels might reflect changes in transcriptional activity due to an interaction of the promoter VNTR and rs12843268 and may therefore result in differential activation in the context of ADHD.

Reduced levels of serotonin are known to enhance premature responding and are associated with higher impulsiveness (50). Accordingly, G hemizygotes showed more ADHD symptoms than A hemizygotes. In G hemizygotes, we found an association of increased right IFG BOLD response and high ADHD symptoms as well as a negative correlation between right IFG BOLD response and shorter SSRTs. These results might suggest a requirement for higher brain activity in the key inhibitory region to achieve similar synaptic serotonin concentrations in G hemizygotes compared with A hemizygotes and to inhibit inappropriate responses in the SST to obtain similar behavioral results (4,7). Lower *MAOA* levels in A hemizygotes might result in increased baseline levels of monoamines in the VS relative to G hemizygotes. Because the motivational salience of a reward stimulus depends on the relative increase in dopamine (51), as opposed to the absolute level, an increased baseline might result in a smaller relative increase due to a ceiling effect in dopamine response.

Our results indicate that stratification of neuroimaging phenotypes by *MAOA* genotype notably increases the amount of variance explained. For example, BOLD response during reward anticipation in the right VS of A hemizygotes accounts for 8.4% of the variance in ADHD symptoms. This contrasts with 2.2% of the variance accounted for by genotype on ADHD symptoms and 2.6% of the variance accounted for by right VS BOLD response on ADHD symptoms, when both genotypes are considered jointly. In the case of right IFG BOLD response, we found that associations only became apparent upon stratification by *MAOA* genotype. This might explain recent results (8) that, in the absence of genetic analyses, failed to identify an association between ADHD and IFG BOLD response during inhibition trials. However, our findings also suggest a sizeable proportion of unexplained variance, which can probably be accounted for by the influence of multiple genes as well as additional brain functions underlying ADHD symptoms.

It is noteworthy that this is one of the first studies to investigate the association between neural responses and ADHD symptoms, rather than ADHD. The mean number of ADHD symptoms in our population-based sample is approximately 50% below the threshold for clinical ADHD (2.7 vs. 5). Although this does not affect the interpretation of the association of ADHD symptoms and neurobiological functions observed, it indicates the normative character of our data, and the need for validation in ADHD patients to fully assess their clinical applicability.

In conclusion, through stratification of ADHD symptoms by *MAOA* genotype, we identified two distinct frontostriatal mechanisms that determine the manifestation of ADHD symptoms in adolescent boys: one of blunted reward and inhibitory control and another characterized by increased reward processing coupled with enhanced efforts to recruit the top down frontal inhibitory system.

This work was supported by the European Union–funded FP6 Integrated Project IMAGEN (Reinforcement-Related Behaviour in Normal Brain Function and Psychopathology; Grant No. LSHM-CT-2007-037286), the FP7 projects ADAMS (Genomic Variations Underlying Common Neuropsychiatric Diseases and Disease Related Cognitive Traits in Different Human Populations) (Grant No. 242257), and the Innovative Medicine Initiative Project EU-AIMS (Grant No. 115300-2), as well as the United Kingdom National Institute for Health Research Biomedical Research Centre Mental Health and the Medical Research Council Programme Grant "Developmental Pathways into Adolescent Substance Abuse" (Grant No. 93558) and the Swedish Research Council.

The authors report no biomedical financial interests or potential conflicts of interest.

IMAGEN consortium: Steven Charles Rees Williams, Laurence John Reed, Lauren Topper, Lindsey Smith, Catherine Morgan, David Stacey, Cybele Peng Wong, Fabiana Carvalho, Simone Kühn, Ines Häke, Nicole Strache, Lisa Albrecht, Nikolay Ivanov, Jan Reuter, Carla Palafox, Christina Schilling, Katharina Lüdemann, Alexander Romanowski, Andreas Ströhle, Elisabeth Wolff, Michael Rapp, Rüdiger Brühl, Albrecht Ihlenfeld, Bernadeta Walaszek, Florian Schubert, Jennifer Jones, Edmund Lalor, Eithne McCabe, Aisling Ní Shiothcháin, Robert Whelan, Rainer Spanagel, Fernando Leonardi-Essmann, Wolfgang Sommer, Maren Struve, Sabine Steiner, Sabine Vollstedt-Klein, Eva Stolzenburg, Christine Schmäl, Penny Gowland, Nadja

Heym, Craig Newman, Zdenka Pausova Amir Tahmasebi, Melkaye G. Melka, Thomas Hübner, Stephan Ripke, Eva Mennigen, Katrin Müller, Veronika Ziesch, Uli Bromberg, Stefan Meindl, Jan Peters, Sophia Schneider, Tahmine Fadai, Lisa Lueken, Juliana Yacubian, Jürgen Finsterbusch, Eric Artiges, Jean-Luc Martinot, Hervé Lemaitre, Nadège Bordas, Zuleima Bricaud, Fanny Gollier Briand, Jessica Massicotte, Ruben Miranda, Jani Penttilä, Nathalie Boddaert, Alexis Barbot, Yannick Schwartz, Christophe Lalanne, Vincent Frouin, Virgile Fritsch, Jeff Dalley, Adam Mar, Naresh Subramaniam, David Theobald, Nicola Richmond, Mischa de Rover, Anna Molander, Emily Rose Jordan, Emma Robinson, Margarita Moreno, Mercedes Arroyo, Dai Stephens, Tamzin Ripley, Hans Crombag, Yolanda Pena, Mark Lathrop, Diana Zelenika, Simon Heath, Dirk Lanzerath, Bert Heinrichs, Tade Matthias Spranger, Beate Fuchs, Claudia Speiser, Arno Klaassen, Ivo Klaassen, Patrick Constant, Xavier Mignon, Tormod Thomsen, Stephan Zysset, Anders Vestboe, James Ireland, John Rogers. CN is currently affiliated with the Karolinska Institute, Department of Neuroscience, Stockholm, Sweden.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2013.03.027.

1. Coghill D, Sonuga-Barke EJ (2012): Annual research review: Categories versus dimensions in the classification and conceptualisation of child and adolescent mental disorders: Implications of recent empirical study. *J Child Psychol Psychiatry* 53:469–489.
2. Shaw P, Malek M, Watson B, Sharp W, Evans A, Greenstein D (2012): Development of cortical surface area and gyrification in attention-deficit/hyperactivity disorder. *Biol Psychiatry* 72:191–197.
3. Hommer DW, Bjork JM, Gilman JM (2011): Imaging brain response to reward in addictive disorders. *Addiction Rev* 1216:50–61.
4. Pliszka SR, Glahn DC, Semrud-Clikeman M, Franklin C, Perez Iii R, Xiong J, *et al.* (2006): Neuroimaging of inhibitory control areas in children with attention deficit hyperactivity disorder who were treatment naive or in long-term treatment. *Am J Psychiatry* 163:1052–1060.
5. Rubia K, Smith AB, Brammer MJ, Toone B, Taylor E (2005): Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry* 162:1067–1075.
6. Scheres A, Milham MP, Knutson B, Castellanos FX (2007): Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biol Psychiatry* 61:720–724.
7. Schulz KP, Fan J, Tang CY, Newcorn JH, Buchsbaum MS, Cheung AM, *et al.* (2004): Response inhibition in adolescents diagnosed with attention deficit hyperactivity disorder during childhood: An event-related fMRI study. *Am J Psychiatry* 161:1650–1657.
8. Carmona S, Hoekzema E, Ramos-Quiroga JA, Richarte V, Canals C, Bosch R, *et al.* (2012): Response inhibition and reward anticipation in medication-naive adults with attention-deficit/hyperactivity disorder: A within-subject case-control neuroimaging study. *Hum Brain Mapp* 33:2350–2361.
9. Hoogman M, Aarts E, Zwiers M, Slaats-Willemse D, Naber M, Onnink M, *et al.* (2011): Nitric oxide synthase genotype modulation of impulsivity and ventral striatal activity in adult ADHD patients and healthy comparison subjects. *Am J Psychiatry* 168:1099–1106.
10. Strohle A, Stoy M, Wrase J, Schwarzer S, Schlagenhauf F, Huss M, *et al.* (2008): Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. *Neuroimage* 39:966–972.
11. Plichta MM, Vasic N, Wolf RC, Lesch KP, Brummer D, Jacob C, *et al.* (2009): Neural hyporesponsiveness and hyperresponsiveness during immediate and delayed reward processing in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry* 65:7–14.
12. Stark R, Bauer E, Merz CJ, Zimmermann M, Reuter M, Plichta MM, *et al.* (2011): ADHD related behaviors are associated with brain activation in the reward system. *Neuropsychologia* 49:426–434.
13. Hahn T, Dresler T, Ehlis AC, Plichta MM, Heinzel S, Polak T, *et al.* (2009): Neural response to reward anticipation is modulated by Gray's impulsivity. *Neuroimage* 46:1148–1153.
14. Forbes EE, Brown SM, Kimak M, Ferrell RE, Manuck SB, Hariri AR (2009): Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. *Mol Psychiatry* 14:60–70.
15. Hariri AR, Brown SM, Williamson DE, Flory JD, de Wit H, Manuck SB (2006): Preference for immediate over delayed rewards is associated with magnitude of ventral striatal activity. *J Neurosci* 26:13213–13217.
16. Dickstein SG, Bannon K, Castellanos FX, Milham MP (2006): The neural correlates of attention deficit hyperactivity disorder: An ALE meta-analysis. *J Child Psychol Psychiatry* 47:1051–1062.
17. Rubia K (2011): "Cool" inferior frontostriatal dysfunction in attention-deficit/hyperactivity disorder versus "hot" ventromedial orbitofrontal-limbic dysfunction in conduct disorder: A review. *Biol Psychiatry* 69: e69–e87.
18. Ma J, Lei D, Jin X, Du X, Jiang F, Li F, *et al.* (2011): Compensatory brain activation in children with attention deficit/hyperactivity disorder during a simplified go/no-go task. *J Neural Transm* 119:613–619.
19. Schulz KP, Newcorn JH, Fan J, Tang CY, Halperin JM (2005): Brain activation gradients in ventrolateral prefrontal cortex related to persistence of ADHD in adolescent boys. *J Am Acad Child Adolesc Psychiatry* 44:47–54.
20. Rubia K, Halari R, Smith AB, Mohammed M, Scott S, Giampietro V, *et al.* (2008): Dissociated functional brain abnormalities of inhibition in boys with pure conduct disorder and in boys with pure attention deficit hyperactivity disorder. *Am J Psychiatry* 165:889–897.
21. Hampshire A, Chamberlain SR, Monti MM, Duncan J, Owen AM (2010): The role of the right inferior frontal gyrus: Inhibition and attentional control. *Neuroimage* 50:1313–1319.
22. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, *et al.* (2005): Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57:1313–1323.
23. Gizer IR, Ficks C, Waldman ID (2009): Candidate gene studies of ADHD: A meta-analytic review. *Hum Genet* 126:51–90.
24. Arnold LE (1996): Sex differences in ADHD: Conference summary. *J Abnorm Child Psychol* 24:555–569.
25. Gaub M, Carlson CL (1997): Gender differences in ADHD: A meta-analysis and critical review. *J Am Acad Child Adolesc Psychiatry* 36: 1036–1045.
26. Domschke K, Sheehan K, Lowe N, Kirley A, Mullins C, O'Sullivan R, *et al.* (2005): Association analysis of the monoamine oxidase A and B genes with attention deficit hyperactivity disorder (ADHD) in an Irish sample: Preferential transmission of the MAO-*A* 941G allele to affected children. *Am J Med Genet B Neuropsychiatr Genet* 134B:110–114.
27. Guan L, Wang B, Chen Y, Yang L, Li J, Qian Q, *et al.* (2009): A high-density single-nucleotide polymorphism screen of 23 candidate genes in attention deficit hyperactivity disorder: Suggesting multiple susceptibility genes among Chinese Han population. *Mol Psychiatry* 14:546–554.
28. Wargelius HL, Malmberg K, Larsson JO, Oreland L (2011): Associations of *MAOA*-VNTR or 5HTT-LPR alleles with attention-deficit hyperactivity disorder symptoms are moderated by platelet monoamine oxidase B activity. *Psychiatr Genet* 22:42–45.
29. Shih JC (2004): Cloning, after cloning, knock-out mice, and physiological functions of MAO A and B. *Neurotoxicology* 25:21–30.
30. Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, *et al.* (2006): The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. *Mol Psychiatry* 11:934–953.
31. Das M, Das Bhowmik A, Sinha S, Chattopadhyay A, Chaudhuri K, Singh M, *et al.* (2006): *MAOA* promoter polymorphism and attention deficit hyperactivity disorder (ADHD) in Indian children. *Am J Med Genet B Neuropsychiatr Genet* 141B:637–642.
32. Rommelse NNJ, Altink ME, Arias-Vasquez A, Buschgens CJM, Fliers E, Faraone SV, *et al.* (2008): Differential association between *MAOA*, ADHD and neuropsychological functioning in boys and girls. *Am J Med Genet B Neuropsychiatr Genet* 147B:1524–1530.
33. Manor I, Tyano S, Mel E, Eisenberg J, Bachner-Melman R, Kotler M, *et al.* (2002): Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): Preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). *Mol Psychiatry* 7:626–632.
34. Meyer-Lindenberg A, Buckholtz JW, Kolachana B, Hariri AR, Pezawas L, Blasi G, *et al.* (2006): Neural mechanisms of genetic risk for impulsivity and violence in humans. *Pro Natl Acad Sci U S A* 103:6269–6274.

35. Bodi N, Keri S, Nagy H, Moustafa A, Myers CE, Daw N, *et al.* (2009): Reward-learning and the novelty-seeking personality: A between- and within-subjects study of the effects of dopamine agonists on young Parkinsons patients. *Brain* 132:2385–2395.
36. Shiraishi H, Suzuki A, Fukasawa T, Aoshima T, Ujiie Y, Ishii G, *et al.* (2006): Monoamine oxidase A gene promoter polymorphism affects novelty seeking and reward dependence in healthy study participants. *Psychiatric Genet* 16:55–58.
37. Schumann G, Loth E, Banaschewski T, Barbot A, Barker G, Buchel C, *et al.* (2010): The IMAGEN study: Reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry* 15: 1128–1139.
38. Herjanic B, Reich W (1997): Development of a structured psychiatric interview for children: Agreement between child and parent on individual symptoms (reprinted from *J Abnorm Child Psychol* 10:307–324, 1982). *J Abnorm Child Psychol* 25:21–31.
39. Carroll JM, Maughan B, Goodman R, Meltzer H (2005): Literacy difficulties and psychiatric disorders: Evidence for comorbidity. *J Child Psychol Psychiatry* 46:524–532.
40. Livak KJ, Schmittgen TD (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 25:402–408.
41. Nees F, Tzschoppe J, Patrick CJ, Vollstädt-Klein S, Steiner S, Poustka L, *et al.* (2012): Determinants of early alcohol use in healthy adolescents: The differential contribution of neuroimaging and psychological factors. *Neuropsychopharmacology* 37:986–995.
42. Rubia K, Smith AB, Taylor E, Brammer M (2007): Linear age-correlated functional development of right inferior fronto-striato-cerebellar networks during response inhibition and anterior cingulate during error-related processes. *Hum Brain Mapp* 28:1163–1177.
43. Logan GD, Schachar RJ, Tannock R (1997): Impulsivity and inhibitory control. *Psychol Sci* 8:60–64.
44. Blum K, Cull JG, Braverman ER, Comings DE (1996): Reward deficiency syndrome. *Am Scientist* 84:132–145.
45. Hahn T, Heinzel S, Dresler T, Plichta MM, Renner TJ, Markulin F, *et al.* (2011): Association between reward-related activation in the ventral striatum and trait reward sensitivity is moderated by dopamine transporter genotype. *Hum Brain Mapp* 32:1557–1565.
46. Paloyelis Y, Mehta MA, Faraone SV, Asherson P, Kuntsi J (2012): Striatal sensitivity during reward processing in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 51:722–732.
47. Bechara A (2005): Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. *Nat Neurosci* 8: 1458–1463.
48. Comings DE, Blum K (2000): Reward deficiency syndrome: Genetic aspects of behavioral disorders. *Prog Brain Res* 126:325–341.
49. Zhang JX, Chen YB, Zhang KR, Yang H, Sun Y, Fang Y, *et al.* (2010): A cis-phase interaction study of genetic variants within the *MAOA* gene in major depressive disorder. *Biol Psychiatry* 68:795–800.
50. Robbins TW, Crockett MJ (2010): Role of central serotonin in impulsivity and compulsivity: Comparative studies in animals and humans. In: Muller CP, Jacobs B, editors. *Handbook of the Behavioral Neurobiology of Serotonin*. London: Elsevier BV, 415–427.
51. Samaha AN, Robinson TE (2005): Why does the rapid delivery of drugs to the brain promote addiction? *Trends Pharmacol Sci* 26:82–87.