Variation in serotonin neurotransmission genes affects neural activation during response inhibition in adolescents and young adults with ADHD and healthy controls

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ORGANAL INVESTIGATION

Variation in serotonin neurotransmission genes affects neural activation during response inhibition in adolescents and young adults with ADHD and healthy controls

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Abstract

Objective. Deficits in response inhibition have been associated with attention-deficit/hyperactivity disorder (ADHD). Given the role of serotonin in ADHD and impulsivity, we postulated that genetic variants within the serotonin pathway might influence response inhibition. Methods. We measured neural activation during stop-signal task performance in adolescents with ADHD (N = 185), their unaffected siblings (N = 111), and healthy controls (N = 124), and investigated the relationship of two serotonin gene polymorphisms (the rs6296 SNP of the HTRIB gene and HTTLPR variants of the 5-HTT gene) with the neural correlates of response inhibition. Results. The whole-brain analyses demonstrated large scale neural activation differences in the inferior and medial frontal and temporal/parietal regions of the response inhibition network between the different variants of both the HTRIB and 5HTT genes. Activation in these regions was significantly associated with stop-task performance, but not with ADHD diagnosis or severity. No associations were found between HTRIB and 5HTT variants and ADHD or ADHD-related neural activation. Conclusions. These results provide novel evidence that serotonin may play an important role in the neurobiology of response inhibition. Although response inhibition is strongly linked to ADHD, serotonin linked genetic variants associated with response inhibition and its neural correlates do not explain variance of the ADHD phenotype.

Key words: ADHD; response inhibition; 5HT; siblings; endophenotype

Introduction

Serotonin neurotransmission has a link with both cognitive control and impulsivity, one of the defining characteristics of attention-deficit/hyperactivity disorder (ADHD; see for a review Cools et al. 2008). A main cognitive control function is the process of response inhibition, or the ability to withhold, delay, or alter an already initiated response. Response inhibition is associated with impulsivity (Nigg 2000) and has therefore been extensively studied in relation to ADHD (Goos et al. 2009; Crosbie et al. 2013). Recently, neural correlates of response inhibition have been reported as potential endophenotypes for ADHD, going beyond purely behavioural measures (Durston et al. 2006; Van Rooij et al. 2015a).
On one hand, evidence for the link between serotonin and impulsivity stems from studies of tryptophan (the 5-HT precursor) depletion. Testing the effects of acute tryptophan depletion in healthy human volunteers demonstrated that tryptophan depletion increased impulsive behaviour (Walderhaug et al. 2002; Finger et al. 2007), but did not alter stop-signal response inhibition performance (Clark et al. 2005). However, tryptophan depletion was shown to be associated with decreased neural activation in the response inhibition network even in the absence of altered behavioural response inhibition performance (Rubia et al. 2005). Thus, these results suggest that neural measures may offer more insight into the mechanisms underlying the influence of serotonin neurotransmission on response inhibition.

Also, genetic studies have indicated that impaired serotonergic transmission is associated with increased impulsivity (Winstanley et al. 2006). A meta-analysis (Gizer et al. 2009) has indicated two serotonin-related gene variants as risk factors for ADHD. The first is the HTTLPR long allele of the 5-HTT (or SLC6A4) serotonin transporter gene. On the other hand, it is the S allele that has been associated with lower serotonin availability (Heinz et al. 2000). Genetic association studies have also shown inconsistent results for this polymorphism. The S allele of HTTLPR has been linked with heightened impulsivity in healthy participants (Walderhaug et al. 2010). However, other studies have reported no association between the HTTLPR S allele and impulsivity (Baca-Garcia et al. 2004), or even the opposite effect, with increased impulsivity for carriers of the L allele (Lee et al. 2003).

The second serotonergic genetic polymorphism implicated in ADHD in the meta-analysis of Gizer et al. (2009) is the rs6296 single nucleotide polymorphism (SNP) G allele in the HTR1B serotonin receptor gene. This G allele is part of a haplotype block causing decreased HTR1B expression (Duan et al. 2003), leading to decreased serotonergic transmission (Sanders et al. 2001; Conner et al. 2010). The rs6296 G allele has been implicated in both trait impulsivity (Varga et al. 2012) and psychiatric disorders like depression, bipolar disorder, and substance abuse (Huang et al. 2003; Conner et al. 2010; Murphy et al. 2011), suggesting a role for HTR1B in both cognition and psychiatric disease phenotypes.

Polymorphisms of the serotonin transporter and receptor genes have also been linked to response inhibition performance in healthy participants (i.e., the HT1A C-1019G polymorphism and rs6296, respectively) (Stoltenberg et al. 2006; Beste et al. 2011), and, importantly, have been shown to influence both impulsivity and response inhibition performance in individuals with ADHD (Oades et al. 2008). So far, studies are lacking on the role of HTR1B or SHTT in the neural correlates of response inhibition, both in healthy controls and individuals with ADHD.

Given the previously found associations between serotonin genes, impulsivity, and ADHD, the goal of the current study was to investigate the role of SHTT and HTR1B variants on the neural correlates of response inhibition, behavioural performance, and the clinically defined ADHD phenotype in a sample of adolescents with ADHD, their unaffected siblings, and healthy controls. Inclusion of unaffected siblings enabled us to examine the role of familiality in the distribution of genetic risk factors as well as neural activation patterns. Particularly, we aimed to assess the role of the HTTLPR and rs6296 polymorphisms in this sample using three methods: first, we investigated whole-brain neural activation during response inhibition in relation to these polymorphisms. Second, we investigated if the HTTLPR and rs6296 polymorphisms were associated with ADHD diagnosis and response inhibition performance. Last, we tested if these variants could explain the differences in neural activation in regions that exhibit differential brain responses in ADHD. We expected that the rs6296 and HTTLPR variants associated with higher impulsivity might also influence response inhibition, reflected in decreased activation in the response inhibition network, which in turn might explain variance in the ADHD phenotype.

Methods

Participants

Participants were part of the NeuroIMAGE study, the Dutch follow up of the International Multicenter ADHD Genetics (IMAGE) study. Details concerning informed consent, recruitment, demographics, diagnostics, and testing procedures can be found in the NeuroIMAGE methods publication (Von Rhein et al. 2014). Within the current sample, we included participants with ADHD (N = 184), their unaffected siblings (N = 111), and healthy controls (N = 124). Participant demographics for our study are listed in Table I.

Stop-Signal task

Response inhibition was measured using a version of the Stop-Signal task (Logan et al. 1984) adapted for functional magnetic resonance imaging (fMRI; Van Meel et al. 2007). Participants were instructed to respond as quickly as possible to a go-signal by choosing the correct response out of two possibilities, unless the go-signal was followed after a short
interval by a stop-signal (25% of trials), in which case they were instructed to withhold their response. By varying the delay between go- and stop-signal, it was possible to derive the main outcome measure of the task, the Stop-Signal Reaction Time (SSRT), which reflects the time necessary for a participant to successfully inhibit their response in 50% of the stop-trials. Secondary outcome measures were the number of omission and commission (i.e., a wrong button response) errors on go-trials (errors) and the Intra-individual component of variation (ICV), derived by dividing the reaction time variability by the mean reaction time over all go-trials. The task consisted of a total of four blocks of 60 trials, separated by 1-min intervals.

Task outcome analyses were performed in SPSS (version 19.0, SPSS Inc.), General Estimated Equations (GEE) regression models were used to correct for familial relations between siblings. Separate regression models were executed for SSRT, ICV Errors, and MRT, with age, gender, and IQ added as covariates. A significance threshold of 0.05 was entailed for all analyses.

Genotyping

An extensive description of DNA extraction and genotyping of the HTTTLPR VNTR in IMAGE is provided elsewhere (Brookes et al. 2006). The rs6296 SNP was genotyped using KASPar analysis at the Radboud University Medical Center, details can be found in the Supplementary Information (SI) available online at http://dx.doi.org/10.3109/15622975.2015.1067371.

fMRI acquisition and analysis

fMRI data were collected at two sites using similar Siemens Scanners and identical coils and protocols, and were processed using FSL FEAT (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl; version 6.0). Details regarding acquisition, preprocessing and first-level analysis can be found in the SI available online at http://dx.doi.org/10.3109/15622975.2015.1067371.

Genetic effects on ADHD diagnosis and task performance

The diagnostic group factor consisted of three groups of interest, i.e., participants with ADHD, unaffected siblings, and unrelated controls. The effects of diagnosis and behavioural response inhibition were investigated using chi-squared statistics and analysis of variance respectively (see Tables II and III).

Role of genetic variants on whole-brain activation in the combined ADHD-control sample

To investigate the effect of each genetic variant on task activation at the whole brain level, two separate higher-level analyses were conducted. An F-contrast was constructed for each polymorphism, treating the three possible rs6296 genotypes or the three HTTTLPR genotypes as primary between-participant factor. ADHD diagnosis was entered as a second factor in order to investigate possible mediation or interaction effects. Age, IQ, gender, and scan site were added as nuisance regressors in all group-level analyses. Statistical inference was done after correction for multiple comparisons, thresholding at a
voxl-level (Z<2.3) using Gaussian random field (GRF) theory-based cluster statistics at P<0.05 (FSL cluster; Woo et al. 2014). Post-hoc tests were performed for beta values from clusters showing significant main effects of genetic variants to specify the direction of the genetic effects and to investigate potential effects of diagnostic group. Post-hoc tests were performed using GEE analyses in SPSS, correcting for familial dependency between siblings. Additionally, correction for multiple comparisons between nodes was done using Bonferroni–Holm (Holm 1979) correction.

Additional models were run to associate the extracted beta-values with stop-task performance as well as with the number of ADHD symptoms. Besides the above-mentioned covariates, family membership was added as a between-participant factor in all above-mentioned models to account for the family structure of our data.

Sensitivity analyses
Sensitivity analyses were performed using similar GEE models to investigate any potential confounding effects of age, gender, IQ, and scanner-site on whole-brain activation, together with tests investigating the potential effects of stimulant medication use and duration (as measured by self-report questionnaire and pharmacist prescription data), as well as the potential effects of comorbid oppositional defiant disorder and conduct disorder.

Genetic effects on diagnosis-sensitive task responses
To investigate genetic effects on regions that exhibit differential brain responses in ADHD, we applied region of interest (ROI) analyses. For the three main task contrasts, namely failed stop–go, successful stop–go and failed–successful stop trials, ROIs were defined functionally by calculating an F-contrast for the diagnostic group × task effects on neural activation across all participants (see Supplementary I available online at http://dx.doi.org/10.3109/15622975.2015.1067371, or Van Rooij et al. 2015a). Beta values from these ROIs were exported from the individual contrast maps and subsequently used to test the effect of the three possible HTTLPR or rs6296 variants. We used GEE models for each ROI separately with the same predictors as mentioned above. Likewise, familial relatedness was entered as a random factor to correct for non-independence of the data. Gender, age, IQ, and scanner were added as covariates. P-values were corrected for multiple comparisons using Bonferroni–Holm correction (Holm 1979).

Results
Genetic effects on diagnostic status and task outcome measures
The distribution of the risk variants did not differ significantly between participants with ADHD, their unaffected siblings, and healthy controls (see Table II). No significant relations between any of the risk variants and task outcome measures were observed, nor were there any main effects of (or interactions with) age, gender, or IQ (see Table III).

Genetic effects on whole-brain fMRI activation
Both HTTLPR and rs6296 genotype significantly influenced the neural activation in the successful

### Table II. Distribution of genotypes per diagnostic group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Risk genotype</th>
<th>MAF</th>
<th>HWE P value</th>
<th>ADHD Risk</th>
<th>No risk</th>
<th>Siblings Risk</th>
<th>No risk</th>
<th>Control Risk</th>
<th>No risk</th>
<th>Odds ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HTT</td>
<td>HTTLPR</td>
<td>LL</td>
<td>0.37</td>
<td>0.78</td>
<td>65</td>
<td>94</td>
<td>43</td>
<td>58</td>
<td>30</td>
<td>71</td>
<td>1.637 (0.962–2.78)</td>
<td>0.217</td>
</tr>
<tr>
<td>HTRIB</td>
<td>rs6296</td>
<td>CC</td>
<td>0.26</td>
<td>0.29</td>
<td>67</td>
<td>90</td>
<td>44</td>
<td>54</td>
<td>42</td>
<td>52</td>
<td>1.085 (0.648–1.816)</td>
<td>0.517</td>
</tr>
</tbody>
</table>

Note: MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium. *Odds Ratio illustrate the relative distribution of genotypes between participants with ADHD and healthy controls.

### Table III. Relationships between gene variants and stop-task outcome measures.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Risk genotype</th>
<th>SSRT</th>
<th>ICV</th>
<th>Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chi²</td>
<td>P value</td>
<td>Chi²</td>
</tr>
<tr>
<td>5-HTT</td>
<td>HTTLPR</td>
<td>LL</td>
<td>0.751</td>
<td>0.687</td>
<td>0.685</td>
</tr>
<tr>
<td>HTRIB</td>
<td>rs6296</td>
<td>CC</td>
<td>1.016</td>
<td>0.602</td>
<td>1.696</td>
</tr>
</tbody>
</table>

Note: SSRT, stop-signal reaction time; ICV, intra-individual coefficient of variance; Errors, number of omission and commission errors on go-trials. Bold values indicate significant outcomes. *Gene effects on the stop-task outcome measures were derived from generalized estimating equation model corrected for familiality, age, gender and IQ.
Role of genetic effects in whole-brain fMRI activation, stop-task performance, and ADHD severity

During successful response inhibition, the right inferior/orbitofrontal area that was differentially activated for the different HTTLPR genotypes was also associated with SSRTs ($\beta = -0.113$, $\chi^2 = 9.511$, $P = 0.002$), indicating better response inhibition with increased activation in this node. Both the right inferior/orbitofrontal area and left frontal pole were additionally associated with error rates ($\beta = 0.921$, $\chi^2 = 6.986$, $P = 0.008$; $\beta = 0.95$, $\chi^2 = 9.217$, $P = 0.002$, respectively), both indicating increased error rates with higher neural activation in these clusters (see Supplementary I available online at http://dx.doi.org/10.3109/15622975.2015.1067371).

Rs6296 genotype was associated with differential activation in anterior cingulate, occipital, inferior temporal, and cerebellar regions during successful stop trials. During failed stops, inferior and superior frontal gyrus, superior parietal gyrus, occipital cortex, and precuneus were differentially active (see Figure 2). Post-hoc tests indicated that these group effects were mainly driven by the difference between the CC genotype and CG and/or GG genotype. However, the direction of these effects was inconsistent, with both increased and decreased activation for the CC genotype being observed in frontal and posterior nodes (see Table IV).

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Figure 1. Effects of the HTTLPR variant on neural activation during successful-stop contrast (A) and failed-stop contrast (B). Right side of the image depicts right side of the brain.
Table IV. Role of HTTLP PR genotypes in brain activation during the Stop Signal Task.

<table>
<thead>
<tr>
<th></th>
<th>Side</th>
<th>#Voxels</th>
<th>P</th>
<th>F</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Group effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Successful-stop contrast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal pole</td>
<td>L</td>
<td>113</td>
<td>&lt;0.001</td>
<td>2.89</td>
<td>-36</td>
<td>62</td>
<td>16</td>
<td>SS &lt; SL = LL</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td>89</td>
<td>&lt;0.01</td>
<td>3.71</td>
<td>-58</td>
<td>-40</td>
<td>SS &gt; SL = LL</td>
<td></td>
</tr>
<tr>
<td>Inferior/Orbitofrontal gyrus</td>
<td>R</td>
<td>90</td>
<td>&lt;0.01</td>
<td>3.95</td>
<td>46</td>
<td>20</td>
<td>-14</td>
<td>SS &lt; SL = LL</td>
</tr>
<tr>
<td><strong>Failed-stop contrast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>R</td>
<td>195</td>
<td>&lt;0.0001</td>
<td>3.81</td>
<td>48</td>
<td>8</td>
<td>4</td>
<td>SS &lt; SL = LL</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>R</td>
<td>140</td>
<td>&lt;0.001</td>
<td>3.29</td>
<td>58</td>
<td>0</td>
<td>SS &lt; SL = LL</td>
<td></td>
</tr>
<tr>
<td>Cingulate gyrus</td>
<td>R</td>
<td>113</td>
<td>&lt;0.01</td>
<td>3.69</td>
<td>-46</td>
<td>-2</td>
<td>SS &gt; SL = LL</td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>L/R</td>
<td>113</td>
<td>&lt;0.01</td>
<td>4.09</td>
<td>-32</td>
<td>-20</td>
<td>SS &gt; SL = LL</td>
<td></td>
</tr>
<tr>
<td><strong>Effects of rs6296 on neural activation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful-stop contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>246</td>
<td>&lt;0.0001</td>
<td>3.52</td>
<td>-32</td>
<td>-48</td>
<td>-28</td>
<td>CC &gt; GC = GG</td>
</tr>
<tr>
<td>Lateral occipital cortex</td>
<td>L</td>
<td>190</td>
<td>&lt;0.0001</td>
<td>3.4</td>
<td>-42</td>
<td>-90</td>
<td>-4</td>
<td>CC &gt; GC</td>
</tr>
<tr>
<td>Anterior cingulate gyrus</td>
<td>R</td>
<td>183</td>
<td>&lt;0.0001</td>
<td>3.73</td>
<td>16</td>
<td>42</td>
<td>8</td>
<td>CC &lt; GC = GG</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>R</td>
<td>146</td>
<td>&lt;0.0001</td>
<td>3.61</td>
<td>26</td>
<td>-38</td>
<td>-50</td>
<td>CC &gt; GC = GG</td>
</tr>
<tr>
<td>Lateral occipital cortex</td>
<td>L</td>
<td>121</td>
<td>&lt;0.001</td>
<td>3.31</td>
<td>-42</td>
<td>-72</td>
<td>-16</td>
<td>CC &lt; GC</td>
</tr>
<tr>
<td>Inferior temporal gyrus</td>
<td>L</td>
<td>90</td>
<td>&lt;0.05</td>
<td>3.09</td>
<td>-48</td>
<td>-70</td>
<td>26</td>
<td>CC &lt; GC</td>
</tr>
<tr>
<td>Failed-stop contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus</td>
<td>L/R</td>
<td>348</td>
<td>&lt;0.0001</td>
<td>3.7</td>
<td>-2</td>
<td>-62</td>
<td>16</td>
<td>CC &lt; GC &lt; GG</td>
</tr>
<tr>
<td>Lateral occipital cortex</td>
<td>R</td>
<td>223</td>
<td>&lt;0.0001</td>
<td>3.45</td>
<td>54</td>
<td>-68</td>
<td>32</td>
<td>CC &lt; GC = GG</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>L</td>
<td>162</td>
<td>&lt;0.0001</td>
<td>3.39</td>
<td>-6</td>
<td>54</td>
<td>28</td>
<td>CC &lt; GC = GG</td>
</tr>
<tr>
<td>Superior parietal lobe</td>
<td>L</td>
<td>131</td>
<td>&lt;0.001</td>
<td>3.93</td>
<td>-24</td>
<td>-54</td>
<td>48</td>
<td>CC = GG &gt; GC</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>R</td>
<td>116</td>
<td>&lt;0.01</td>
<td>2.98</td>
<td>60</td>
<td>20</td>
<td>32</td>
<td>CC &gt; GC = GG</td>
</tr>
</tbody>
</table>

*Activation clusters derived from the F-contrasts testing differences in task activation as a function of HTTLP PR genotype (SS vs. SL vs. LL) or rs6296 genotype (CC vs. CG vs. GG) over all participants, including gender, IQ, age and scan-site as covariates. †Correction for multiple comparisons in FSL FEAT was done using a cluster threshold of Z > 2.3 and a significance threshold of P < 0.05 corrected. ‡Group effects are derived from post-hoc analyses, corrected for familiality.

Figure 2. Effects of the rs6296 variant on neural activation from the successful-stop contrast (A) and failed-stop contrast (B). Right side of the image depicts right side of the brain.
performed to further explore whether the association between the neural activation and SST performance described above was mediated by the genetic variants. No evidence of mediation effects was detected. No significant associations were found between the activation in neural nodes indicated in the whole-brain analysis and either ADHD diagnosis (ADHD, unaffected sibling, or control), nor the number of ADHD symptoms, indicating no relation between ADHD status and the effects of rs6296 and HTTLP on neural activation.

Influence of covariates on whole-brain fMRI activation

To investigate whether the whole-brain activation was influenced by age, gender, IQ, scan site, medication use, and comorbid disorders, several post-hoc analyses were performed. No main or interaction effects of IQ, gender, or scan-site were detected, indicating that these variables did not influence the reported genetic effects on fMRI activation. The activation in the right inferior frontal and frontal pole areas where differential effects of the HTTLP genotypes were observed during failed inhibitions also showed a main effect of age ($\beta = -1.197$, $P = 0.011$; $\beta = 2.637$, $P = 0.005$, respectively), indicating a general decrease of activation in these nodes with increased age. However, there was no interaction with the gene effect in the same location, indicating that both effects occur independently. No other effects of age were observed.

The effects of medication were assessed by incorporating both medication use and duration of use in post-hoc analyses, as were the effects of comorbid diagnoses of oppositional defiant disorder and conduct disorder. None of the medication and comorbidity factors showed main effects or interaction with Genetic effects on differential fMRI activation between diagnostic groups

The main diagnostic group contrast on neural activation during the stop-task indicated differential activation between participants with ADHD, unaffected siblings, and controls in a range of nodes in inferior and superior frontal, anterior cingulate, and temporal/parietal areas. Details regarding these ROIs can be found in Van Rooij et al. 2015a) as well as in the SI. None of the multivariate tests indicated main (neither with nor without incorporation of the diagnostic effect) or interaction effects with group of rs6296 or HTTLP genotypes on the neural activation in these ROIs (see Supplementary I available online at http://dx.doi.org/10.3109/15622975.2015.1067371).

Discussion

In the present study, we investigated the effects of two genetic variants, HTTLP in the serotonin transporter gene and rs6296 in the serotonin receptor gene HTRIB on response inhibition performance and its underlying neural activation patterns in a cohort consisting of participants with and without ADHD. We provide for the first time direct evidence for a genetically driven effect of serotonin transmission on the neural correlates of response inhibition.

The first part of this study was to test the effects of two genetic variants within SHTT and HTRIB on whole-brain activation during response inhibition. These analyses indicated effects of HTTLP in the frontal nodes of the response inhibition network, as well as in more posterior nodes like the cerebellum and cingulate cortex. Specifically, decreased neural activation was observed in individuals with the SS genotype in the right inferior frontal gyrus and frontal poles; the former is recognized as an essential node of the response inhibition network (Aron and Poldrack 2006; Chambers et al. 2009). On the other hand, increased neural activation in individuals with the SS genotype was observed in the cerebellum, cingulate cortex, and brainstem. Lower activation in the right inferior frontal region was associated with decreased response inhibition performance during successful stop-trials, although lower activation in the same region as well as in the frontal pole were also associated with lower error rates on go-trials, suggesting a possible deficit in response inhibition, but an increase in general attention performance on go-trials in individuals with the SS genotype (Esterman et al. 2014).

Our results indicate that the effect of HTTLP on neural activation is driven by the SS genotype, which showed less activity in the frontal response inhibition nodes and more in the posterior areas. The relations between neural activation and stop-task performance further indicate that these frontal areas are directly involved in response inhibition and attentional performance; although no direct effect of HTTLP genotype on performance was observed. Taken together, these findings might indicate a posterior shift of neural activation in individuals with the SS genotype, possibly compensating for decreased activation of the main response inhibition network. Previous studies show decreased serotonin transporter expression in individuals with the SS genotype, signaling higher serotonin availability (Lesch et al. 1996; Heinz et al. 2000). These findings may indicate a relation between serotonin availability, decreased response inhibition, and increased impulsivity (Walderhaug et al. 2010). However, this would be in conflict with the meta-
analytic findings marking the L carriers of the HTTLPR as an ADHD risk-group, given that decreased neural activation in frontal nodes during response inhibition has usually been associated with ADHD severity, including in the current sample (Van Rooij et al. 2015a), although a study also reported an association between the S allele of the HTTLPR and adults with ADHD (Landaas et al. 2010). Another study compared different types of impulsivity paradigms in rats, demonstrating that tryptophan depletion may influence reactivity on go/no-go trials, but not stop-signal reaction times in a go/no-go task. This indicates that while the delay discounting aspect of impulsivity may have been affected, response inhibition was not (Eagle et al. 2009). These findings may explain our current effects of HTTLPR on neural activation, which showed opposite effects on neural activation levels, error rates, and SSRTs. This dissociation between different aspects of impulsivity will need to be further investigated to fully understand the relation between serotonin, impulsivity, and ADHD.

The effect of rs6296 showed a similar distribution across frontal-parietal, occipital, and cerebellar nodes, equally indicating relatively widespread differential activation. The GG genotype, considered an ADHD risk factor (Gizer et al. 2009), showed increased activation in occipital, temporal/parietal, superior frontal, and cingulate regions, with decreased activation in cerebellar and inferior frontal areas. Of those, activation in anterior-cingulate regions was significantly associated with SSRT length, indicating higher activation levels correlated with better inhibition performance. Temporal parietal, superior frontal, and cingulate regions have all been implicated in attentional control and action monitoring processes necessary for response inhibition (Bekker et al. 2005; Fassbender et al. 2006; van Meel et al. 2007). In addition, previous studies have suggested the involvement of a separate frontal-thalamo-cerebellar pathway involved in inhibitory control (Rubia et al. 2007). The current results may suggest decreased activation in the frontal-cerebellar pathway in G allele carriers, compensated by increased activation in attentional and top-down control areas. We postulate that the utilization of compensatory or alternative strategies using attention resources may explain the lack of direct effects of rs6296 on stop-task performance.

In the second part of the study, we investigated whether variants in the 5HTT and HTR1B genes were associated with ADHD diagnosis, response inhibition, or whether previous outcomes detailing the influence of ADHD on neural activation during response inhibition were related to variants in the 5HTT and HTR1B genes. We found that the HTTLPR and rs6296 variants were not associated with ADHD diagnosis, nor did they influence the ADHD effect on behavioural or neural measures of response inhibition. These findings therefore suggest there is no direct causal pathway between the genetic variants investigated, response inhibition, and the ADHD phenotype. Results fit within the mediational endophenotype model as discussed in the conceptual review by Kendler and Neale (2010). In this model a relation exists between the genes and endophenotype, as well as between the endophenotype and disease, without a necessary relation between genes and disease. This is specifically true in case of relatively limited effect sizes, in which case the direct association between gene variance and phenotype may be overshadowed by noise, while associations with the neural endophenotype can still be observed. While in our previous publications we found support for the endophenotype to disease relation (Van Rooij et al. 2015a, 2015b), the current results support the relation between genes and the endophenotype. This illustrates how the incorporation of a neural endophenotype allows us to study potential relations between genes and disease phenotype that would otherwise be invisible.

Limitations

There may be limitations to the current study that should be taken into consideration when interpreting the results. First, we may not have missed a possible relation between serotonin-related gene variants and the ADHD phenotype since we tested only a small part of the functional variants related to the serotonin system. Recent studies have suggested that the cumulative variance across a large number of genes within a single pathway may offer additional explanatory power over the single gene variant approaches (Bralten et al. 2013). Future research should consider a broader scope of functional gene variants across neurotransmitter systems that may be required to fully establish or dissociate the genetic links between response inhibition and ADHD. Second, response inhibition and the variants HTTPLR and rs6296 from the 5-HTT and HTR1B genes have been implicated in a wide range of psychiatric disorders including depression, bipolar disorder, anxiety, and substance abuse disorder (Lesch et al. 1996; Huang et al. 2003; Cho et al. 2005). This may indicate possible shared genetic and neural underpinnings of different psychiatric disorders. The abovementioned findings suggest that diagnostic boundaries between psychiatric disorders may not necessarily represent underlying genetic mechanisms (Lee et al. 2013); and the current findings suggest that the use of neurobiological constructs may provide more specific targets for genetic studies than diagnostic phenotypes.

Future studies should take these limitations into consideration, and aim to broaden the scope of both
genetic variants and phenotypes incorporated in these studies.

Conclusions

To summarize, whole-brain analysis of neural activation indicated a broad pattern of differential neural activation in frontal-parietal, cerebellar, and occipital areas during response inhibition associated with HTTLPR and rs6296. Activation in these nodes was related to response inhibition performance, but independent of ADHD diagnosis and severity. These results demonstrate the effect of the HTTLPR and rs6296 variants on the behavioural and neural correlates of response inhibition. Since there were no direct associations between the genetic variants and task performance, neural correlates may be a more sensitive measure of genotype effects than solely behavioural or clinically defined phenotypes.

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References


Supplementary materials available online

Supplementary methods

Determining diagnostic status in the NeuroIMAGE sample
Participant inclusion
Genotyping
fMRI acquisition and preprocessing
fMRI single subject analysis

Supplementary results

fMRI task activation
Between group differences in fMRI activation
Genetic effects on between-group differences in fMRI activation
Role of genetic effects in whole-brain fMRI activation and stop-task performance

Supplementary Tables 1–5.