Neural correlates of gene-environment interactions in ADHD

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Chapter 4

Brain correlates of the interaction between 5-HTTLPR and psychosocial stress mediating ADHD severity


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Abstract

Introduction: Serotonin transporter 5-HTTLPR genotype has been found to moderate the effect of stress on severity of attention-deficit/hyperactivity disorder (ADHD), with stronger effects of stress in carriers of the short allele than in individuals homozygous for the long allele. The underlying neurobiological mechanism of this gene-environment interaction in ADHD is unknown. This study aimed to determine whether 5-HTTLPR moderates the effect of stress on brain grey matter volume and, if so, which brain regions mediate the effect of this gene-environment interaction on ADHD severity.

Methods: Structural magnetic resonance imaging, 5-HTTLPR genotype, and stress exposure questionnaire data were available for 701 adolescents and young adults participating in the multicentre ADHD cohort study NeuroIMAGE (from 385 families; 291 with ADHD, 78 with subthreshold ADHD, 332 healthy controls; 55.8% males; average age 17.0 years). ADHD symptom count was determined through multi-informant questionnaires. For the analysis, we combined a whole-brain voxel-based morphometry approach with mediation analysis.

Results: Stress exposure was associated with significantly less grey matter volume in the precentral gyrus, middle and superior frontal gyrus, frontal pole, and cingulate gyrus for S-allele carriers than for participants homozygous for the L-allele. The association of this gene-environment interaction with ADHD symptom count was mediated by grey matter volume in the frontal pole and anterior cingulate gyrus.

Conclusions: 5-HTTLPR genotype moderates the effect of stress on brain regions involved in social cognitive processing and cognitive control. Specifically regions important for cognitive control link this gene-environment interaction to ADHD severity.
Introduction

Attention-deficit/hyperactivity disorder (ADHD) results in most cases from the combined influence of multiple genetic and environmental risk factors of small effect size (Gizer, Ficks & Waldman, 2009; Banerjee, Middleton & Faraone, 2007). Research into the aetiology of ADHD is further complicated by gene-environment interactions, whereby a person’s genetic make-up in part determines reactivity to environmental influences (Nigg, Nikolas & Burt, 2010).

The serotonin transporter gene (SERT; also known as SLC6A4) is a gene implicated in ADHD (Gizer, Ficks & Waldman, 2009). This gene contains a variable number tandem repeat polymorphism in its promoter region (5-HTTLPR), consisting of a 14-repeat, short variant (S-allele) and a 16-repeat, long variant (L-allele, Lesch et al., 1996). There is a large body of literature documenting that 5-HTTLPR may moderate the effects of stress exposure on mood disorders (Caspi et al., 2010). Animal models have provided evidence of a causal relation between this gene-environment interaction and a range of pathological behaviours (Spinelli et al., 2007). It has also been shown to be involved in ADHD; we recently reported a stronger positive association between stress exposure and severity of ADHD in individuals carrying an S-allele than in those homozygous for the L-allele, and found that this was independent of comorbid internalizing problems (Van der Meer et al., 2014). In the present paper we aimed to further our understanding of these findings by investigating brain correlates of this gene-environment interaction in the same study cohort.

ADHD is characterized by a delay in brain maturation (Shaw et al., 2007), and both 5-HTTLPR genotype and stress exposure have been shown to influence brain maturation (Daubert & Condron, 2010; Lupien et al., 2009). Magnetic resonance imaging (MRI) studies of both healthy individuals and those with internalizing problems have reported interaction effects between 5-HTTLPR genotype and stress exposure on limbic and frontal brain regions involved in (the regulation of) social and emotional behaviour, including the amygdala and anterior cingulate cortex (Lemogne et al., 2011; Canli et al., 2006; Frodl et al., 2010). S-allele carriers have been shown to have less connectivity between these regions, which was associated with higher levels of anxiety, suggesting that less top-down control of frontal regions over subcortical structures underlies part of the behavioural correlates of the 5-HTTLPR genotype (Pezawas et al., 2005). Hypofunctioning of frontal regions and connected subcortical structures is also a hallmark of both stress exposure (Cohen et al., 2006) and ADHD (Arnsten & Rubia, 2012), indicating overlap in neurobiological correlates of 5-HTTLPR, stress, and ADHD.

Knowledge of how 5-HTTLPR moderates environmental risk factors for ADHD may eventually lead to prevention and treatment being better adjusted to patients’ individual characteristics. The present study therefore aimed to determine: 1) whether the interaction between stress and 5-HTTLPR genotype also affects brain grey matter volume and 2) which brain regions would mediate the effect of this gene-environment interaction on ADHD severity. To accomplish these aims we performed a whole-brain voxel-based morphometry mediation analysis, with the
gene-environment interaction as predictor, grey matter volume as a mediator, and ADHD symptom count as outcome, see Figure 1. Based on previous literature (Hariri & Holmes, 2006), we expected that paralimbic regions would be most prominently involved in this gene-environment interaction. However, we are the first to study the brain correlates of this gene-environment interaction in relation to ADHD severity; for this reason, a whole-brain analysis was chosen above a region-of-interest approach, allowing for the identification of different or previously overlooked brain regions. The analyses were carried out in an adolescent and young adult sample (mean age 17.0 years) of individuals with ADHD, their unaffected siblings, and healthy controls, thus enabling analysis within a wide range of ADHD severity, in accordance with the continuous distribution of ADHD within the population (Levy et al., 1997).

**Figure 1.** The mediation model. Path A represents the association between the gene-environment interaction and grey matter volume, consistent with aim 1; path B represents the association between grey matter volume and ADHD symptom count, which, in combination with path A, is used to assess how grey matter volume mediates the effect of the gene-environment interaction on ADHD symptom count (aim 2). Path C represents the effect of the gene-environment interaction on ADHD symptom count, which we previously
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Methods

Participants were selected from the NeuroIMAGE study, a follow-up of the Dutch part of the International Multicentre ADHD Genetics (IMAGE) study (von Rhein et al., 2014). NeuroIMAGE included 365 families with at least one child with ADHD and at least one biological sibling (regardless of ADHD diagnosis) and 148 control families with at least one child, without any formal or suspected ADHD diagnosis in any of the first-degree family members. ADHD families were recruited through ADHD outpatient clinics in the regions Amsterdam, Groningen, and Nijmegen (the Netherlands). Control families were recruited through primary and high schools in the same geographical regions. To be included in NeuroIMAGE, participants had to be of European Caucasian descent, between ages 5 and 30, have an IQ ≥ 70, and no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, or known genetic disorders. More information on the NeuroIMAGE study and its participants is available elsewhere (von Rhein et al., 2014).

All measurements were part of a comprehensive assessment protocol. Testing was carried out either at the VU University Amsterdam and VU University Medical Centre or at the Radboud University Nijmegen Medical Centre and Donders Institute for Brain, Cognition, and Behaviour in Nijmegen. Participants were motivated with short breaks, and received €50 and a copy of their MRI scan at the end of the day. The study was approved by the regional ethics committee (CMO Regio Arnhem – Nijmegen; 2008/163; ABR: NL23894.091.08) and the medical ethical committee of the VU University Medical Centre. All participants signed informed consent (parents signed informed consent for participants under 12 years of age).

Assessment of ADHD

We constructed an ADHD symptom count based on the Conners ADHD Rating Scales questionnaires (Conners et al., 1998). These questionnaires were filled in by the parents and either a teacher (for children <18 years) or the participants themselves (for those ≥18 years old). The Conners Rating Scales provide operational definitions of each of the 18 ADHD symptoms defined by the Diagnostic and Statistical Manual (DSM) IV-TR. In this sample, the symptom count ranged from 0 to 18 with an average of 5.4. Crohnbach’s α for this measure was .91.

The 701 participants who met the inclusion criteria and had structural MRI data available came from 385 families; 291 participants from 233 families had a diagnosis of ADHD, 78 participants had subthreshold ADHD (i.e., had ADHD symptoms without meeting the criteria for a full ADHD diagnosis; 56 being siblings of participants with ADHD), and 284 were healthy controls (of which 154 were unaffected siblings of participants with ADHD). ADHD diagnoses were made in accordance with DSM IV-TR criteria on the basis of a combination of a semi-structured diagnostic interview, the Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime version (Kaufman et al., 1997), and the Conners Rating Scales. In this sample, 97 participants had an oppositional defiant disorder or conduct disorder, 23 an internalizing disorder, and 79 reading disorder.
An extensive description of the diagnostic algorithm for ADHD and comorbid disorders is provided in the Supplementary Information (SI).

Assessment of stress exposure

Two questionnaires were used to assess the amount of exposure to psychosocial stress. Parents filled in the Long-Term Difficulties (LTD) questionnaire (Bosch et al., 2012; Oldehinkel, Verhulst & Ormel, 2008), which contained thirteen items measuring whether their children have been exposed to chronic stressors such as a handicap, being bullied, having financial difficulties, or other persisting problems at home or school. They were asked to only report chronic, ongoing difficulties. In addition, participants themselves filled in a Stressful Life Events questionnaire (Oldehinkel, Verhulst & Ormel, 2008; Bosch et al., 2012), which contained eleven items on exposure to specific major stressful events in the past five years, such as death or serious illness of a loved one, physical or sexual abuse, or failure at something important to them. For the composite stress measure, the scores on the questionnaires were transformed to Z-values and averaged according to common practice for aggregating similar measures, as previously described elsewhere (Van der Meer et al., 2014). See the SI for further information on both questionnaires and an overview of the items.

Genotyping

Genotyping was performed as described in (Brookes et al., 2006). Briefly, DNA was extracted from blood samples at Rutgers University Cell and DNA Repository, New Jersey, USA. Standard polymerase chain reaction protocols were used for the determination of 5-HTTLPR genotype.

Socio-economic status

As a measure of socio-economic status, the highest, successfully completed education level of the parents was recoded into a measure reflecting years of education. This scale contained nine levels, ranging from 0 (no formal education) to 17 (university) years of education (Buis, 2010). The average of both parents was used, which, in this sample, ranged from 5 to 17 with an average of 12.1.

MRI data acquisition and pre-processing

Both scanning locations used two identical 1.5 Tesla scanners. Of each participant, two high-resolution T1-weighted MP-RAGE anatomical scan were obtained (176 sagittal slices, repetition time = 2730 ms, echo time = 2.95 ms, voxel size = 1.0 x 1.0 x 1.0 mm, field of view = 256 mm). Before processing, raw scans were manually evaluated for motion artefact and scan quality. Only scans with no or mild motion artefact were selected for further analysis. To increase signal-to-noise, scans from the same participant were averaged if they both contained no or mild motion. Three participants were excluded for further analysis due to severe motion in both scans and seventeen participants were excluded due to incidental morphologic abnormalities (e.g., enlarged ventricles).
Pre-processing of the sMRI data was carried out with Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, United Kingdom; [http://www.fil.ion.ucl.ac.uk/spm/software/spm8/] implemented in MATLAB 7.9 (Mathworks Inc., Sherborn, Massachusetts), using the VBM8 toolbox with standard settings. This included normalization to Montreal Neurological Institute (MNI) space, segmentation into tissue specific maps, modulation by dividing the images through the non-linear component of the Jacobian determinant of the warp, and smoothing with an 8 mm full width at half maximum Gaussian kernel.

**Statistical analysis**

This study investigated a dominant genetic model of the 5-HTTLPR S-allele, wherein S-allele carriers were coded as ‘1’ and L-allele homozygotes were coded as ‘0’. This is in accordance with the majority of studies investigating this gene-environment interaction (Caspi *et al.*, 2010) and is based on the functional effects of the S- and L-alleles (Lesch *et al.*, 1996). In addition, L-alleles with the rs25531 C-G single nucleotide polymorphism were recoded as a functional S-allele, in accordance with prior studies (Hu *et al.*, 2006). This led to 59 L-allele homozygotes being recoded to S-allele carriers. Compliance of genotype distribution with Hardy-Weinberg equilibrium was checked using standard methods.

All behavioural data was analysed using R (v3.1.1) (R Core Team, 2012). Differences between genotypes in sample demographics were checked with Pearson’s Chi-squared tests for categorical variables and with one-way ANOVA for continuous variables. The model investigating the effect of the gene-environment interaction on ADHD symptom count consisted of 5-HTTLPR genotype, stress exposure, and their interaction, as well as age, sex, socio-economic status, and location as covariates. In order to account for the within-family correlation due to the inclusion of siblings in the sample, we analysed the data with linear mixed effects models with family as a random factor, estimating a random intercept. The p-values of the mixed models results were estimated through a Markov-Chain Monte Carlo algorithm, included in the LanguageR package.

**Whole-brain voxel-based morphometry mediation analysis**

We employed Mediation Effect Parametric Mapping (Wager *et al.*, 2008) to determine the relationship between the gene-environment interaction, grey matter volume, and ADHD symptom count. This analysis technique is based on a standard three-variable mediation model, as depicted in Figure 1. Here, path A represents the association of the predictor X with the mediator M, path B represents the effect of the mediator M on the dependent variable Y, and path C represents the total effect of the predictor X on the dependent variable Y. The mediation effect, i.e. the effect of X on Y mediated by M, is the product of path A and path B, the significance of which is determined through bootstrapping. This approach to mediation analysis is in line with the currently most accepted approach to mediation, which deviates from the classic ‘causal steps’ approach to mediation, as the latter has been shown to be less powerful and rest on false assumptions (Hayes, 2013).
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Our whole-brain mediation model consisted of 5-HTTLPR genotype, amount of stress exposure, and their interaction as predictors, grey matter volume as a mediator, and ADHD symptom count as dependent variable. Sex, age, socio-economic status, and scanner location were added as covariates. All continuous predictors were mean-centred.

The whole-brain mediation analysis on the voxel-based morphometry data was performed in MATLAB with the Multilevel Mediation and Moderation Toolbox (Wager et al., 2008). As a mask, we used the average grey matter image of the sample with an absolute threshold value of 0.2 (number of voxels: 463,956). The toolbox performed a bootstrap test (5000 samples), to estimate the significance of the effect on each voxel included in the mask, resulting in p-value maps for path A, B, and AB. Family-wise error correction was applied through the use of FSL’s EasyThresh, which carries out cluster-based thresholding. A Z-value of 2.6 was used to define contiguous clusters and subsequently, each cluster’s significance level was estimated on the basis of Gaussian Random Field theory. Those clusters surviving a significance threshold of p=.001 are reported. Localization was done with the Harvard-Oxford atlas. All reported coordinates are in MNI-space and in millimetre.

In order to further probe the interaction and mediation effects, as well as to correct for the non-independence of the data, mean grey matter volume from significant clusters was extracted and analysed with linear mixed effects models in R, as described above for the behavioural data. Significance of the mediation effect was determined through bootstrapping with 5000 samples. We further calculated Cohen’s $f^2$, suitable for mixed models (Selya et al., 2012), as a measure of additional percentage variance explained by the gene-environment interaction term. For the mediation effects, we report $\kappa^2$ as a ratio measure of the indirect effect compared to its maximal possible value (Preacher & Kelley, 2011).

**Sensitivity analyses**

We conducted sensitivity analyses to check whether the findings were not biased due to methodological choices. We checked whether the findings were not driven by either a diagnostic subgroup or testing location, by rerunning the analyses with an interaction term between the gene-environment interaction and either diagnosis or testing location, and checking whether these interaction terms had significant effects on grey matter volume. More information on the methods for these analyses can be found in the SI. Furthermore, given the large age-range, we checked whether age plays a significant role in the association of the gene-environment interaction with ADHD symptom count, by adding a three-way interaction to the model.
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\section*{Results}

\subsection*{Demographic characteristics}

No significant differences in sex distribution, age, stress exposure, socio-economic status, or testing location were found between S-allele carriers and L-allele homozygotes, as summarized in Table 1. Genotyping frequencies did not deviate from Hardy-Weinberg Equilibrium (p=.11).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
\textbf{Variable} & \textbf{S-allele carriers} & \textbf{SD} & \textbf{L-allele homozygotes} & \textbf{SD} & \textbf{Test-statistic} & \textbf{DF} & \textbf{P-value} \\
\hline
Participants & 456 & 245 & & & & & \\
Amsterdam location & 51.5\% & 51.4\% & $\chi^2=0.0007$ & 1 & .98 & & \\
Male sex & 53.9\% & 59.2\% & $\chi^2=1.57$ & 1 & .21 & & \\
Age in years & 16.93 & 3.56 & 17.14 & 3.63 & F=0.54 & 700 & .47 \\
Parents’ years of education & 12.00 & 2.51 & 12.18 & 2.46 & F=0.78 & 700 & .38 \\
Stress Z-score & -0.04 & 0.99 & 0.07 & 1.01 & F=2.09 & 700 & .15 \\
Stressful live events & 2.01 & 1.51 & 2.19 & 1.54 & F=2.09 & 700 & .15 \\
Long-term difficulties & 1.09 & 1.36 & 1.27 & 1.50 & F=2.61 & 688 & .11 \\
\hline
\end{tabular}
\caption{Demographics table of the participants, split by genotype.}
\end{table}

\textit{Note: Differences between genotypes in the categorical variables ‘location’ and ‘sex’ were analysed with a Chi-square test; for the other, continuous variables we performed an analysis of variance. A variant of this demographics table, providing the statistics for homozygotes and heterozygotes separately, is given in the supplementary information. SD= standard deviation. DF= degrees of freedom.}

\subsection*{Association of 5-\textit{HTTLPR} and stress with ADHD symptom count}

There was no evidence of an association of genotype or stress with ADHD symptom count, as previously reported in a sample highly overlapping with the current sample (Van der Meer \textit{et al.}, 2014). The interaction effect was significant (B=0.80, SE=0.38, p = .03), indicating that 5-\textit{HTTLPR} genotype moderated the effect of stress exposure on ADHD symptom count. Within-group analysis confirmed that stress was highly significantly correlated with ADHD symptom count in S-allele carriers (B=0.80, SE=0.24, p<.001), but not in L-allele homozygotes (B=0.06, SE=0.31, p=.85).

\subsection*{Association of 5-\textit{HTTLPR} and stress with grey matter volume}

The association between stress and grey matter volume was moderated by 5-\textit{HTTLPR} genotype in the precentral gyrus, middle and superior frontal gyrus, frontal pole, and paracingulate gyrus, shown in Figure 2. In these regions, S-allele carriers had a more pronounced negative correlation between stress and grey matter volume than L-allele homozygotes. Information on the clusters is presented in Table 2. Given our focus on the gene-environment interaction, significant clusters from the conditional effects are presented in the SI.
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The whole-brain mediation analysis

The mediation analysis revealed two clusters, one in the anterior cingulate and paracingulate gyrus, and one in the frontal pole. These clusters are shown in Figure 2. Further inspection of our results revealed that the pattern was the same across both clusters: there was a negative correlation between the interaction term and grey matter volume (path A), and a negative correlation between grey matter volume and ADHD symptom count (path B), leading to a significant positive (path A x path B) mediation effect. That is, the stronger positive correlation between stress and ADHD symptom count found in S-allele carriers compared to L-allele homozygotes was in part statistically explained by less volume in these frontal brain regions. This, and further information on the clusters, is displayed in Table 3.

Table 2. Summary of the clusters where the gene-environment interaction is significantly correlated with grey matter volume.

<table>
<thead>
<tr>
<th>Location (peak, other regions in cluster)</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Cluster size</th>
<th>Coefficient</th>
<th>P-value</th>
<th>Cohens f'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal pole, middle frontal gyrus</td>
<td>-51</td>
<td>21</td>
<td>33</td>
<td>1232</td>
<td>-0.022</td>
<td>.0003</td>
<td>.007</td>
</tr>
<tr>
<td>Paracingulate gyrus, superior frontal gyrus</td>
<td>0</td>
<td>32</td>
<td>50</td>
<td>334</td>
<td>-0.015</td>
<td>.001</td>
<td>.001</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>5</td>
<td>-23</td>
<td>54</td>
<td>1097</td>
<td>-0.017</td>
<td>.00001</td>
<td>.008</td>
</tr>
</tbody>
</table>

Note: X, Y, Z coordinates are in MNI-space in mm, and represent the peak of the cluster. The anatomical labels are according to the Harvard-Oxford atlas. MNI=Montreal Neurological Institute

Figure 2. Visualization of the location of the clusters where grey matter volume was significantly associated with the interaction between 5-HTTLPR and stress exposure (red), and those where grey matter volume mediated the effect of this gene-environment interaction on ADHD symptom count (blue). Overlap between the clusters is shown in purple. The thresholded Z-value maps are overlain on the sample’s average grey matter image. The image is depicted in neurological convention, in MNI-space, at the coordinates X=0, Y=23, and Z=-9 (in mm).
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Sensitivity analyses
Age was not significantly correlated with symptom count ($r=.06$, $p=.11$). It was, however, associated with stress, with older participants having experienced more stress ($r=.21$, $p<.0001$), and with total grey matter volume, with younger participants having more grey matter ($r=-.18$, $p<.0001$), as would be expected given that pruning of grey matter takes place with increasing age. We have added a three-way interaction between the gene-environment interaction and age on ADHD symptom count, to check whether this played a significant role in our findings. The gene-environment interaction effect remained significant ($B=0.92$, $SE=0.39$, $p=.02$), whereas none of the other two-way interactions or the three-way interaction was significant (all $p>.16$). Results from the other sensitivity analyses can be found in the SI. Briefly, we found no evidence that diagnosis or testing location influenced the effects of the gene-environment interaction on grey matter volume in any of the significant clusters from the main analysis.

Table 3. Summary of the clusters where there was a significant mediation effect of grey matter volume.

<table>
<thead>
<tr>
<th>Location (peak, other regions in cluster)</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Cluster size</th>
<th>Path A</th>
<th>Path B</th>
<th>P-value</th>
<th>$\kappa^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal pole, middle frontal gyrus</td>
<td>-30</td>
<td>54</td>
<td>20</td>
<td>390</td>
<td>-0.019</td>
<td>-8.46</td>
<td>.004</td>
<td>.025</td>
</tr>
<tr>
<td>Anterior cingulate gyrus, paracingulate gyrus</td>
<td>0</td>
<td>35</td>
<td>44</td>
<td>363</td>
<td>-0.019</td>
<td>-9.65</td>
<td>.003</td>
<td>.029</td>
</tr>
</tbody>
</table>

Note: $X$, $Y$, $Z$ coordinates are in MNI-space in mm, and represent the peak of the cluster. The anatomical labels are according to the Harvard-Oxford atlas. MNI=Montreal Neurological Institute.
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Discussion

We aimed to identify brain grey matter volume correlates of the interaction between 5-HTTLPR and stress exposure, and to examine whether grey matter volume mediates the effect of this gene-environment interaction on ADHD severity. To achieve this, we combined a whole-brain voxel-based morphometry approach with mediation analysis. We found that stress exposure was associated with significantly less grey matter volume in the precentral gyrus, middle and superior frontal gyrus, frontal pole, and paracingulate gyrus in S-allele carriers than in participants homozygous for the L-allele. The association of this gene-environment interaction with ADHD symptom count was mediated by grey matter volume in the frontal pole and anterior cingulate gyrus.

Assuming that less grey matter volume is unfavourable, our findings would indicate that S-allele carriers are more sensitive to stress, in accordance with our previous findings at the behavioural level on ADHD severity (Van der Meer et al., 2014), as well as with the majority of studies into the link between this gene-environment interaction with anxiety and depression (Caspi et al., 2010). Because of the reported association between this gene-environment interaction and internalizing disorders, neuroimaging studies of 5-HTTLPR and its moderation of stress effects have mostly employed a region-of-interest approach focusing on limbic regions such as the amygdala (Hariri & Holmes, 2006). However, whole-brain structural and functional MRI studies have linked this gene-environment interaction to a brain network involved more broadly in social cognitive processing and emotion regulation (Canli et al., 2006; Canli et al., 2005). This network includes the precentral gyrus and anterior cingulate and paracingulate gyrus, regions also reported in the current study. We further found an association with grey matter volume in the frontal pole and superior and middle frontal gyrus. These regions, together with the anterior cingulate gyrus, are essential for cognitive control, such as suppressing automatic emotional reactions in favour of more flexible goal-directed behaviour (Volman et al., 2011). This includes control over the amygdala (Volman et al., 2011), which is in line with reports that top-down control of the anterior cingulate over the amygdala is central to the association of 5-HTTLPR, and its moderation of effects of stressors, with anxiety and depression (Pezawas et al., 2005).

The current findings speak to the idea that, in the context of ADHD, 5-HTTLPR, and its moderation of the effects of stress, is more broadly involved in self-regulation problems above and beyond the regulation of anxiety and sad affect. We found that the stronger negative association between stress and grey matter volume in the frontal pole and anterior cingulate gyrus in S-allele carriers compared to L-allele homozygotes mediated the association between this gene-environment interaction and ADHD severity. Prefrontal cortex dysfunction, and associated problems with cognitive control, is a hallmark of ADHD (Arnsten & Rubia, 2012); neuroimaging studies have repeatedly reported less volume and lower activity across the frontal lobes of individuals with ADHD compared to healthy controls (Dickstein et al., 2006). The results from the current study suggest that the interaction between 5-HTTLPR and stress exposure may contribute to these structural and functional deficits of the prefrontal cortex in ADHD. As described above, 5-HTTLPR and stress...
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have been linked to social cognitive processing and cognitive control, both anterior cingulate and frontal pole are important for cognitive control in socio-emotional situations (Volman et al., 2011; Amodio & Frith, 2006), and cognitive control problems in ADHD manifest in academic (Thorell, 2007), social (Nijmeijer et al., 2008), or emotional (Shaw et al., 2014) contexts. We therefore hypothesize that this gene-environment interaction may be linked to ADHD through its effect on broadly defined self-regulation problems.

Strengths of this study include a large sample size, use of multiple informants to determine ADHD phenotype, and the application of a whole-brain moderated mediation analysis that allowed for assessment of the neural pathways coupling the gene-environment interaction to ADHD severity. Limitations are the retrospective assessment of stress exposure and the observational, cross-sectional design, the latter preventing strong inferences about causality. For instance, it could be the case that the reported grey matter volume differences are a causal factor in maladaptive behaviour, which in turn may lead to the experience of more stressful life events. While animal studies have provided causal evidence that the brain of S-allele carriers is more affected by exposure to stress, longitudinal studies or studies making use of ‘natural experiments’ (Rutter, 2007), are needed to confirm this causality in humans.

To summarize, we demonstrated that 5-HTTLPR moderates the effects of stress at the neural level, such that S-allele carriers show a more negative association between stress and grey matter volume than L-allele homozygotes. The implicated brain regions have been linked to social cognition, emotion regulation, and more broadly defined cognitive control functions. The anterior cingulate gyrus and frontal pole, regions important for cognitive control, statistically mediated the association between the gene-environment interaction and ADHD severity in adolescence and young adulthood. Our findings suggest that the interaction between 5-HTTLPR and stress may render individuals vulnerable to broadly defined self-regulation problems, and that this mechanism is not only relevant for internalizing symptoms of anxiety and depression, but also for ADHD symptoms. These findings have implications for both clinicians and researchers. Clinicians may eventually use information on the moderating effects of patients’ genotypes to shape their prevention and treatment strategies to individual patients’ needs; S-allele carriers may benefit more from preventing stressful experiences and treatments in order to better regulate their behaviour, although more research is needed to confirm this. For researchers, these findings underline the fact that genetic and environmental factors do not operate in isolation and need to be studied in context. Such approaches are a step forward in resolving heterogeneity of ADHD and its underlying mechanisms. Our findings also suggest that future research may need to consider a broader role for this gene-environment interaction in shaping behaviour than previously assumed, with effects on cognitive control. As the brain regions reported in this study are complex and serve multiple functions, future studies should further specify which and how neurocognitive functions are affected by the interaction between 5-HTTLPR and stress exposure, and how this relates to ADHD.
References


Buis, M.L. (2010) *Scaling levels of education*, Faculty of Social Sciences, VU-University Amsterdam.


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