Effect of MAOA Genotype on Resting-State Networks in Healthy Participants

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Up to now, it remains unclear how monoamine oxidase A (MAOA), which has been repeatedly linked to aggression, affects brain activity within resting-state networks (RSN). Here, we used functional magnetic resonance imaging (fMRI) to test whether the MAOA genotype might influence activity within the common RSN. Our results demonstrate that during rest, participants with the low-activity genotype (MAOA-L) exhibit more activity within frontal-parietal and temporal parts of the default mode network (DMN) and the cerebellum. The executive control and salience RSN revealed reduced activity for the MAOA-L group in several areas related to executive control, namely the right middle frontal gyrus (BA 6 and BA 9), and the dorsal part of the anterior cingulate cortex. Participants with the high-activity genotype (MAOA-H) showed increased activity in the posterior cingulate part of the DMN. Taken together, we found widespread hyperactivity within the DMN and reduced activity in brain areas related to executive and inhibitory control for the MAOA-L group. We discuss how these first results examining the influence of MAOA on the resting brain might be related to previous findings regarding the genetics of aggression, while acknowledging that this is an exploratory study which needs further confirmation.

Keywords: aggression, functional magnetic resonance imaging, genotype, monoamine oxidase A, resting-state networks

Introduction

The influence of genes on behavior is often subtle and exerted in a complex interaction with environmental factors, and thus challenging to study. Examining brain activity during rest allows for a relatively environmentally free investigation of subtle genetic influences. Recently, using functional magnetic resonance imaging (fMRI) to investigate resting-state networks (RSN) became quite popular in the neuroscience community, with the number of publications increasing steadily (Fox and Raichle 2007; Lindenberg et al. 2006). These studies generally concern activity within RSN that are functionally related to antisocial behavior and aggression in animal (Cases et al. 1995) and human studies (Brunner et al. 1993; McDermott et al. 2009; Gallardo-Pujol et al. 2013; Kuepper et al. 2013).

Recent genetic studies have revealed that one of the polymorphisms of the MAOA gene, the variable-number tandem repeat polymorphism, results in a relatively higher (MAOA-H) or lower (MAOA-L) expression (Brunner et al. 1993; McDermott et al. 2009; Gallardo-Pujol et al. 2013; Kuepper et al. 2013). Specifically, the low-activity genotype (MAOA-L) has been related to increased aggression and abnormal behavior in humans (McDermott et al. 2009; Gallardo-Pujol et al. 2013). In 1993, Brunner et al. (1993) provided the first evidence that the MAOA gene might be related to aggression by studying a male Dutch family cohort with an increased prevalence of abnormal behavior (e.g., exhibitionism, attempted rape) and a missense mutation (i.e., selective and complete deficiency) in the MAOA gene. Up to now, several studies revealed interactions between genetic and environmental determinants such as childhood abuse (Caspi et al. 2002; Kim-Cohen et al. 2006; Eisenberger et al. 2007; McDermott et al. 2009; Gallardo-Pujol et al. 2013). Moreover, it has been shown that the MAOA genotype can influence patterns of brain activity during cognitive or emotional tasks (Fan et al. 2003; Eisenberger et al. 2007; Buckholtz and Meyer-Lindenberg 2008). In addition, behavioral studies with healthy participants revealed that those carrying the MAOA-L genotype demonstrated more aggressive behavior in experimental settings inducing social exclusion or stress (McDermott et al. 2009; Gallardo-Pujol et al. 2013; Kuepper et al. 2013). A neuroimaging study in a large sample of healthy participants showed that the MAOA-L genotype was related to increased risk of violent behavior, as well as increased amygdala and decreased prefrontal cortex activity during emotional arousal (Meyer-Lindenberg et al. 2006). These studies generally confirm epidemiologic findings showing that MAOA interacts with aggressive and antisocial behavior (Caspi et al. 2002; Beitchman et al. 2004; Frazzetto et al. 2007; Ducci et al. 2008).

Here, we used fMRI to examine the most common RSN in a sample of healthy participants, for which we determined whether they carry the high-activity (MAOA-H) or the low-activity (MAOA-L) allele. The systematic investigation of RSN began in 1995, when Biswal et al. (1995) showed that functionally connected brain networks, which are also present in the absence of a cognitive task, can be identified from temporal correlations observed in spontaneous low-frequency fluctuations (<0.1 Hz) of blood oxygen level-dependent (BOLD) data. Using either seed-based correlational analysis or independent component analysis (ICA), different RSN have been identified based on their functional
and/or anatomical resemblance with well-known brain circuits (Beckmann et al. 2005; Damoiseaux et al. 2006; Smith et al. 2009; Rosazza and Mirati 2011). RSN have been found in the visual (Cordes et al. 2001; Kiviniemi et al. 2004; Stevens et al. 2010), auditory (Cordes et al. 2000; Koyama et al. 2010), and sensorimotor domain (De Luca et al. 2005; Fox et al. 2006). Additionally, RSN focusing on the attention system (Fox et al. 2005; Filippi et al. 2012), the executive control network (Seeley et al. 2007; Filippi et al. 2012), and the salience network (Seeley et al. 2007; Filippi et al. 2012) have been identified. Another RSN that has been studied extensively is the default mode network (DMN), comprising a connected set of brain areas exhibiting greater BOLD activity during rest than during cognitive tasks (Shulman et al. 1997; Raichle et al. 2001; Greicius et al. 2003; Raichle and Snyder 2007; Buckner et al. 2008). Increased interest in RSN is also due to recent studies claiming that RSN, especially the DMN, might serve as an indicator for dysfunctional brain activity or connectivity in psychiatric (e.g., schizophrenia) and neurological (e.g., Alzheimer’s disease) diseases (Greicius et al. 2004; Sorg et al. 2007; Buckner et al. 2008; Jafri et al. 2008; Supkar et al. 2008; Calhoun et al. 2009; Whitfield-Gabrieli et al. 2009; Mingoga et al. 2012, 2013).

Based on previous findings and due to the fact that some RSN comprise brain regions potentially related to aggressive behavior, we expected to find genotype-related functional activity differences in the following RSN. Due to the crucial involvement of the prefrontal cortex in emotion regulation, impulse control, and psychopathologies associated with increased aggressive behavior (Raine et al. 1994, 1998; Grafman et al. 1996; Pietrini et al. 2000; Soderstrom et al. 2002; Yang and Raine 2009; Coccaro et al. 2011), we expected to find group differences in the RSN involving frontal brain regions (executive control and frontoparietal RSN). This hypothesis was also based on previous findings (Meyer-Lindenberg et al. 2006), demonstrating that functional differences in prefrontal cortex are influenced by MAOA. Moreover, it was shown that the anterior cingulate cortex (ACC) is differentially activated depending on the MAOA genotype (Fan et al. 2003; Passamonti et al. 2006; Eisenberger et al. 2007; Buckholtz and Meyer-Lindenberg 2008). Additionally, the ACC is involved in pathologies related to aggression (Hazlett et al. 2005; Yang and Raine 2009). Therefore, we expected to find differences also in the salience network, which contains the ACC as a crucial node (Seeley et al. 2007). Based on previous research, differences in other RSN were not expected, but were also examined in an explorative manner due to the novelty of the present topic. We have to acknowledge that, due to the exploratory nature of the present study (i.e., the first fMRI study investigating differences in RSN due to MAOA), we cannot derive more specific hypothesis. Thus, we would like to emphasize the highly explorative character of the present study, and at the same time point out that the proposed hypotheses are relevant as they allow us to discuss the present results in the context of previous findings regarding MAOA-related differences in fMRI studies. The reader should thus keep in mind that, although some limited hypotheses can be derived from previous findings, the present study has an explorative character.

Materials and Methods

Participants

All data presented here were acquired in the context of a joint research project aimed at evaluating the neural and genetic correlates of aggression in healthy participants and psychiatric patients. In the present study, we present resting-state data of healthy participants. All healthy participants were recruited via public announcement from the community around Aachen (Germany), and all had normal or corrected vision, no contraindications against MR measurements, and no history of traumatic brain injury, psychiatric or neurological illness. According to the Edinburgh Handedness Inventory (Oldfield 1971), all participants were fully right handed. Blood samples were routinely taken from all healthy participants enrolled in the joint research project. Because female participants must be homozygote for optimal identification of the MAOA genotype, we only included resting-state measurements of homozygous females. Inspection of the data revealed 32 male and 16 female participants. The analysis of the MAOA genotype revealed 18 participants with the MAOA-L genotype and 30 participants with the MAOA-H genotype (for a detailed description of MAOA genotype analyses see MAOA Genotyping). It should be noted that several participants enrolled in the joint research project underwent fMRI measurements without resting-state scans being recorded (due to time constraints). In order to have a (more) equal number of male and female participants, we asked 6 homozygote female participants to come once more in order to assess resting-state fMRI. This was done to balance the factor of gender. In order to also increase the number of participants with the MAOA-L genotype and thus create a more balanced sample, all 6 female participants who were scanned once more had the MAOA-L genotype. Their genotype was known due to the genotype analysis routinely conducted in the context of the joint research project. Overall, 54 healthy volunteers (mean age = 27.12 years; SD = 8.92) were included in the present study, comprising 22 female and 32 male participants. Experimental procedures were approved by the Ethics Committee of the Medical Faculty of the RWTH Aachen University and were performed in compliance with the latest version of the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave their written informed consent and received compensatory payment.

MAOA Genotyping

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral lymphocytes by a simple salting-out procedure. For MAOA genotyping, standard polymerase chain reaction (PCR) amplification was performed in a 25 µL volume containing 80-ng genomic DNA, 1 unit recombinant TaqPolymerase (Invitrogen, Germany), PCR buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl2, pH 8.3), 200 mM dNTPs, and 20 pmol of each primer. MAOA primer sequences were obtained from the literature (Sabol et al. 1998). The forward primer was FAM-labeled. PCR reaction was amplified through 35 cycles on a MJ PTC200 Temperature Cycler (Biosym, Germany) and each cycle consisted of a 95° denaturation step for 45 s, a 62° (MAOA) annealing step for 30 s and finally a 72° elongation step for 90 s. Electropherograms were analyzed using the Genemapping software (Applied Biosystems, USA). After determining the genotype for each participant, 2 different experimental groups were created: Based on their individual genotype, 24 participants were assigned to the MAOA-L group and 30 participants to the MAOA-H group. The groups included 11 females homozygous for MAOA-L and 11 females homozygous for MAOA-H.

Behavioral Analysis

Using the SPSS 20.0 software package (IBM, Armonk, NY, USA), we employed two-sample t-tests to compare demographic factors (age, intelligence) between the 2 experimental groups. Premorbid intelligence was assessed using the WMT-B (German estimation of verbal crystallized intelligence) (Lehr 2005). Furthermore, all participants completed the Buss-Perry Aggression Questionnaire (AQ) before the fMRI measurement (Buss and Perry 1992), and a two-sample t-test was used to compare the mean AQ score between the 2 experimental groups. The distribution of gender between the 2 experimental groups was compared using the χ2 test as implemented in SPSS.

Image Acquisition

fMRI measurements were performed at the RWTH Aachen University Hospital employing a Siemens 3T Trio scanner (Siemens AG; Erlangen, Germany) using a 12-channel head matrix coil. To stabilize the position...
of the head during scanning, foam pads were used for immobilization of participant’s heads. In order to acquire resting-state data, each participant underwent one functional run, which was 8 min long. During resting-state measurements, participants saw a black screen and were instructed to relax and keep their eyes open without falling asleep. Using a detailed debriefing, we confirmed that none of the participants fell asleep during the resting-state measurement. Two hundred forty functional images were acquired per run, using a spin-echo EPI sequence with the following acquisition parameters: TR = 2000 ms, TE = 28 ms, flip angle = 77°, FOV = 192 × 192 mm², matrix size = 64 × 64, 34 transversal slices, voxel size = 3.5 × 3.5 × 3.75 mm³. High-resolution anatomical images were acquired for each participant using an MP-RAGE sequence with the following acquisition parameters: TR = 1900 ms, TE = 2.52 ms, flip angle = 9°, FOV = 256 × 256 mm², 176 sagittal slices, voxel size = 1 × 1 × 1 mm³.

**Image Processing**

SPM8 (Institute of Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm) was used for preprocessing as well as later voxel-wise statistics, whereas FSL MELODIC (FMRIB, University of Oxford, UK; www.fmrib.ox.ac.uk/fsl/medlic2/index.html) (Beckmann and Smith 2004) was used for ICA. For preprocessing, the first 5 volumes of each functional time series were discarded, so that the brain could reach a stable magnetized state, preventing artifacts from transient signal changes at the beginning of the functional run. To correct for movement artifacts, functional images were realigned using a least-squares approach and a 6-parameter rigid body spatial transformation. A two-pass procedure was applied to register functional images to the mean image after the first realignment. Through visual inspection, we verified that none of the participants exceeded the predefined movement limits of 3 mm, or 3°. Subsequently, within-subject registration was performed between functional and anatomical images, using the functional images as a reference image. Co-registered anatomical images were segmented, using tissue probability maps of the International Consortium for Brain Mapping template (ICBM; http://www.loni.ucla.edu/ICBM/ICBM_TissueProb.html), aligned with an atlas space, corrected for inhomogeneities, and classified into gray matter, white matter, and cerebrospinal fluid. Using affine transformations, these data were then registered to MNI space. Finally, functional images were re-sampled to 2-mm³ resolution using sinc interpolation, and spatially smoothed using an 8-mm FWHM Gaussian kernel to account for intersubject variability.

**Probabilistic ICA and Automatic Extraction of RSN**

ICA was applied using the “single-session ICA” MELODIC algorithm implemented in FSL. The probabilistic ICA (pICA) that is applied in MELODIC enables the assignment of significance values (P values) to spatial maps (Beckmann and Smith 2004). Functional data were divided into a set of spatially independent maps, each providing internally consistent temporal dynamics characterized by a specific time course (McKeown et al. 1998). The advantage of pICA is that it provides z-scores (i.e., intensity values), which in turn provide a measure of the contribution of a time course of a specific component to the measured signal within a voxel. The resulting spatial maps can be seen as the result of a multiple regression model, and therefore, pICA allows the user to segregate functional networks and to create a voxel-wise map of quantitative measures of resting-state functional connectivity. Note that, for convenience and better legibility, we prefer to use the term activity as a synonym for the term resting-state functional connectivity throughout the whole manuscript.

For each participant separately, all functional images of a run were concatenated at time zero to create a single 4D image, which was subsequently analyzed using the “single-session ICA” model implemented in MELODIC (Beckmann and Smith 2004). As part of the pICA, a high-pass filter (>0.009 Hz) was applied to remove low-frequency drifts, and a low-pass filter (<0.18 Hz) was applied to remove cardiac- and breathing-related artifacts. The software was set to output 40 components, as this number of components represents one sixth of the number of functional volumes per run. During pICA, components were estimated using the Laplace approximation of the Bayesian model evaluation. Subsequently, the algorithm implemented in MELODIC allowed separation of activation (e.g., resting-state maps) and artifact-related (e.g., physiological noise) components, in the absence of a specifically defined time series model (Beckmann and Smith 2005). Thus, artifact-related components were removed and all further analysis focused only on nonartifact components.

For the identification of RSN, we employed a fully automated method originally proposed by Greicius et al. (2004); however, the same methodology has been used also by other researchers (Greicius et al. 2007, 2008; Supelkar et al. 2010; Minga et al. 2012, 2013). This method provides automatic identification of the most consistent RSN, and it is based on an assessment of the similarity of the individual components and predefined RSN templates (Greicius et al. 2004, 2007). These templates included the following RSN: DMN, salience, executive control, 2 frontoparietal networks (left- and right-lateralized), sensorimotor, cerebellum, auditory, and 3 visual networks. All templates were derived from the study by Smith et al. (2009), except for the template of the salience network, which was derived from the study by Seeley et al. (2007). All RSN templates were binarized, so that they could be used as mask images. To avoid confusion, it should be noted that these binarized mask images were only used for the process of component selection. For all further random effects, RFX analyses, the original data from the ICA was used. Using MATLAB, we then employed an automated algorithm to select the component reflecting the respective RSN best. In the course of this selection procedure, each component was paired with each template. The algorithm employed here follows the method developed by Greicius et al. (2004). We always took the average z-score of all voxels within the template minus the average z-score of all voxels outside the template. For each participant, the component with the greatest difference (i.e., goodness of fit) was selected as reflecting the participant’s respective RSN. The most important advantages of this methodology are 1) that it allows for automatic selection, without any visual inspection of the component that reflects a specific RSN best, and 2) that P values are assigned to each voxel within the entire brain, allowing for calculation of voxel-wise statistics.

**Random Effects Group Analyses**

Recent studies demonstrated that in-scanner head motion might be a confounding factor for between-group differences in resting-state and connectivity analyses (Power et al. 2012; Satterthwaite et al. 2012; Van Dijk et al. 2012). Therefore, we used the 6 motion parameters estimated during realignment to calculate the frame-wise displacement (FD), a standard metric for quantifying mean motion per scan. For the calculation of the FD, we employed the exact same formula given in the original publication by Power et al. (2012). The applied procedure resulted in 1 FD value for each scan, for each participant. After calculating all FD values for each participant, we averaged FD across the group and computed mean FD values for each group, between 2 experimental groups using two-sample t-tests. Given the potentially nonparametric nature of the FD values, we also compared the group means using the Mann–Whitney test for nonparametric data. For both tests, the significance level was adjusted using Bonferroni corrections, to account for the high number of multiple comparisons (i.e., 234 separate tests). Additionally, we averaged the FD values across all scans for each participant and computed the group means for these average values. These average FD values were also compared between the 2 experimental groups using the two-sample t-test and the Mann–Whitney test for nonparametric data.

To compare the different RSN between the 2 groups, we used SPM8. The first step for RFX analyses was to pool the components, derived from the ICA, representing the respective RSN for all subjects into a second-level analysis at an uncorrected voxel-level threshold of $P < 0.001$. The resulting statistical maps were used as an inclusive mask to limit all further comparisons to those voxels which are, based on the total cohort of all participants, significantly involved in the respective RSN. A comparable approach for masking subsequent between-group comparisons has already been employed in previous studies (Greicius et al. 2004; Supelkar et al. 2010). Thus, for each RSN, the specific contrast (e.g., M1OA-L > M1OA-H) was masked inclusively with the contrast defining the RSN in the total cohort of all participants, i.e., (M1OA-L + M1OA-H > 0). This should ensure that, for each RSN, we only report group differences for areas actually falling within the
Results

Demographic Data
We first checked whether the 2 experimental groups differed significantly concerning demographic factors, such as age and IQ. The groups were well matched, and no significant differences were found, neither for age \((P = 0.056, t_{(52)} = 1.96)\), nor for IQ \((P = 0.841, t_{(52)} = -0.2)\). Furthermore, no significant difference was found when comparing the mean AQ score between the 2 experimental groups \((P = 0.313, t_{(52)} = -1.02)\). The results of the \(\chi^2\) test revealed that also gender did not differ significantly between the 2 experimental groups, \(X^2 (1, N = 54) = 2.42, P = 0.121\).

fMRI Results
When comparing mean FD values for each scan between the 2 experimental groups, no significant differences were found using two-sample \(t\)-tests \((P > 0.05)\) or Mann–Whitney tests \((P > 0.05)\). Due to the high number of multiple comparisons \((i.e., 234 \text{ separate tests})\), the significance level for these tests had to be adjusted to \(P < 0.0002\), using Bonferroni correction. We also compared the group means after averaging FD values across all scans for each participant. No significant difference was found with the two-sample \(t\)-test \((P = 0.461, t_{(52)} = 0.745)\) or the Mann–Whitney test \((P = 0.383, Z(52) = -0.872)\). Thus, the results indicate that in-scanner head motion was not significantly different between the 2 experimental groups, and we cautiously conclude that this factor did not significantly affect the between-group differences presented here.

It should be noted that we were able to detect a component that was consistent with \(i.e.,\) resembled the RSN templates provided in the literature \((\text{Seeley et al. 2007; Smith et al. 2009})\) for all participants. Using two-sample \(t\)-tests, we examined the goodness of fit scores, which did not differ significantly between the 2 experimental groups for any of the RSN \((P > 0.05)\). Given the potentially nonparametric nature of the data, we also compared the group means using the Mann–Whitney test \((P > 0.05)\), corroborating the results of the two-sample \(t\)-tests. This indicated that RSN were equally easy to identify in both experimental groups. Thus, on the level of the goodness of fit scores \((\text{representing a quantitative evaluation of the overlap between the RSN templates and the selected components})\), no group differences were detected. Aside from the goodness of fit scores, group comparisons revealed significantly different activity within 5 of the 11 RSN. A detailed overview of the results can be found in Table 1. For all group analyses reported here, gender and age were included as covariates, to remove variance related to these factors and thus obtain results that are not confounded by age or gender of participants. In the following paragraphs, we describe the results in more detail.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of fMRI results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAOA-L &gt; MAOA-H</td>
</tr>
<tr>
<td>Auditory</td>
<td>–</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Anterior lobe of cerebellum (RH)</td>
</tr>
<tr>
<td>DMN</td>
<td>SFG (BA 8)/IPL (BA 40, RH)/MTG (BA 21, LH)</td>
</tr>
<tr>
<td>Executive control</td>
<td>–</td>
</tr>
<tr>
<td>Right frontoparietal</td>
<td>MFG (BA 6, LH)</td>
</tr>
<tr>
<td>Left frontoparietal</td>
<td>–</td>
</tr>
<tr>
<td>Salience</td>
<td>Dorsal ACC (BA 32)/MFG (BA 10, LH)</td>
</tr>
<tr>
<td>Sensorimotor</td>
<td>–</td>
</tr>
<tr>
<td>Visual (medial)</td>
<td>–</td>
</tr>
<tr>
<td>Visual (occipital)</td>
<td>–</td>
</tr>
<tr>
<td>Visual (lateral)</td>
<td>–</td>
</tr>
</tbody>
</table>

The table summarizes all results for the 11 RSN and the 2 contrasts of interest. For both contrasts, increased activity is displayed at a cluster-level threshold of \(P < 0.05\) (FDR corrected).

Default Mode Network
First, the contrast \((\text{MAOA-L} > \text{MAOA-H})\) was examined. When compared with the MAOA-H group, the MAOA-L group exhibited increased activity within several parts of the DMN: in the frontal part, namely in the bilateral superior frontal gyrus \((\text{SFG})\) at Brodmann area \((\text{BA})\) 8, in the parietal part, around the right inferior parietal lobe \((\text{IPL})\) at \(\text{BA} 40\), and in the temporal part, in the left middle temporal gyrus \((\text{MTG, BA 21})\). Activity within the SFG covered parts of the right and the left SFG, as depicted in Figure 1A. The reverse contrast \((\text{MAOA-H} > \text{MAOA-L})\) revealed that the posterior cingulate was the only region showing reduced activity for the MAOA-L group \((\text{see Fig. 1B})\). All results for these 2 contrasts, including MINI coordinates, \(t\)-statistics, and \(P\) values for peak voxels of all activated clusters, are summarized in Table 2 and illustrated in Figure 1.

Executive Control Network
The contrast \((\text{MAOA-L} > \text{MAOA-H})\) did not yield any significant results for the executive control RSN. However, for the reverse contrast \((\text{MAOA-H} > \text{MAOA-L})\), reduced activity was found in participants with MAOA-L at 2 right frontal sites. For this contrast, all results, including MINI coordinates, \(t\)-statistics, and \(P\) values for peak voxels of all activated clusters, are summarized in Table 3 and can be seen in Figure 2A. Reduced activity in the right middle frontal gyrus \((\text{MFG})\), at \(\text{BA 6}\), was located close to the presupplementary motor area \((\text{pre-SMA})\). Furthermore, reduced activity was observed in right \(\text{MFG at BA 9}\), close to the dorsolateral prefrontal cortex \((\text{DLPFC})\).

Right Frontoparietal Network
The contrast \((\text{MAOA-L} > \text{MAOA-H})\) for the right frontoparietal RSN did not reveal any significant results. For the reverse contrast \((\text{MAOA-H} > \text{MAOA-L})\), reduced activity for participants with MAOA-L was found in the right \(\text{MFG at BA 6}\). Comparable with the executive control RSN, reduced activity was found close to the pre-SMA. All results for this contrast are summarized in Table 3 and are shown in Figure 2B.

Salience Network
The contrast \((\text{MAOA-L} > \text{MAOA-H})\) did not reveal significant results for the salience RSN. But the contrast \((\text{MAOA-H} > \text{MAOA-L})\) revealed reduced activity for participants with MAOA-L.
within the dorsal ACC (dACC) at BA 32, and in the left MFG at BA 10. Activity within dACC was slightly more extended toward the left hemisphere. All results for the salience RSN, including MNI coordinates, t-statistics, and P values for peak voxels of all activated clusters, are summarized in Table 3 and can be seen in Figure 3A.

Table 2
Overview of group differences in DMN

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>t-Statistic</th>
<th>P value</th>
<th>No. of voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN: MAOA-H &gt; MAOA-L</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>M posterior cingulate cortex</td>
<td>31</td>
<td>4</td>
<td>-46</td>
<td>56</td>
<td>4.72</td>
<td>&lt;0.001</td>
<td>530</td>
</tr>
<tr>
<td>DMN: MAOA-L &gt; MAOA-H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R and L SFG</td>
<td>8</td>
<td>-20</td>
<td>44</td>
<td>56</td>
<td>4.46</td>
<td>&lt;0.001</td>
<td>1140</td>
</tr>
<tr>
<td>L middle temporal gyrus</td>
<td>21</td>
<td>-52</td>
<td>10</td>
<td>-34</td>
<td>4.57</td>
<td>&lt;0.001</td>
<td>286</td>
</tr>
<tr>
<td>R inferior parietal lobe</td>
<td>40</td>
<td>44</td>
<td>-56</td>
<td>32</td>
<td>3.59</td>
<td>&lt;0.001</td>
<td>313</td>
</tr>
</tbody>
</table>

All x, y, and z values represent coordinates according to the MNI coordinate system (ICBM 152). Statistical values correspond to the t-statistics and P values of the peak voxel within each anatomical region. For both contrasts, increased activity is displayed at a cluster-level threshold of P < 0.05 (FDR corrected). BA, Brodmann area; DMN, default mode network; L, left hemisphere; M, medial part of the brain; MAOA-H, high-activity allele group; MAOA-L, low-activity allele group; R, right hemisphere; SFG, superior frontal gyrus.

Cerebellar Network
The contrast (MAOA-L > MAOA-H) revealed that the MAOA-L group exhibited increased activity within the anterior lobe (lobule VI) of the right cerebellum, when compared with the MAOA-H group. A detailed summary of this cerebellar activation is provided in Table 3, and the cluster is visualized in Figure 3B. The reverse contrast (MAOA-H > MAOA-L) did not yield any significant activation.

Discussion
In the present study, fMRI was used to determine whether differences in RSN were influenced by MAOA genotype. In this
exploratory study, we employed a rather novel approach, by using RSN as a particularly suitable tool to gain insights into how the MAOA genotype might influence brain activity already during rest, independent from any task and cognitive or emotional load. Using templates provided by previous studies (Seeley et al. 2007; Smith et al. 2009), 11 common RSN were examined in a large sample of healthy participants. For distinct RSN, we found genotype-related functional activity differences. Nevertheless, it should be noted that the sample examined here was psychiatrically healthy, showing no behavioral

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>r-Statistic</th>
<th>P value</th>
<th>No. of voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum RSN: MAOA-L &gt; MAOA-H</td>
<td>26</td>
<td>−46</td>
<td>−42</td>
<td>3.89</td>
<td>&lt;0.001</td>
<td>321</td>
<td></td>
</tr>
<tr>
<td>Executive control RSN: MAOA-H &gt; MAOA-L</td>
<td>9</td>
<td>44</td>
<td>34</td>
<td>40</td>
<td>4.66</td>
<td>&lt;0.001</td>
<td>97</td>
</tr>
<tr>
<td>R middle frontal gyrus/dorsolateral prefrontal cortex</td>
<td>6</td>
<td>26</td>
<td>6</td>
<td>68</td>
<td>4.26</td>
<td>0.002</td>
<td>81</td>
</tr>
<tr>
<td>R middle frontal gyrus/presupplementary motor area</td>
<td>6</td>
<td>28</td>
<td>2</td>
<td>62</td>
<td>3.55</td>
<td>0.001</td>
<td>256</td>
</tr>
<tr>
<td>Right frontoparietal RSN: MAOA-H &gt; MAOA-L</td>
<td>10</td>
<td>−32</td>
<td>52</td>
<td>14</td>
<td>3.27</td>
<td>&lt;0.001</td>
<td>310</td>
</tr>
<tr>
<td>M-dorsal anterior cingulate cortex</td>
<td>32</td>
<td>−14</td>
<td>22</td>
<td>32</td>
<td>3.73</td>
<td>&lt;0.001</td>
<td>486</td>
</tr>
<tr>
<td>L middle frontal gyrus</td>
<td>6</td>
<td>28</td>
<td>2</td>
<td>62</td>
<td>3.55</td>
<td>0.001</td>
<td>256</td>
</tr>
</tbody>
</table>

All x, y, and z values represent coordinates according to the MNI coordinate system (ICBM 152). Statistical values correspond to the t-statistics and P values of the peak voxel within each anatomical region. For all contrasts, increased activity is displayed at a cluster-level threshold of P < 0.05 (FDR corrected). BA, Brodmann area; L, left hemisphere; M, medial part of the brain; MAOA-H, high-activity allele group; MAOA-L, low-activity allele group; RH, right hemisphere; RSN = resting-state network.
abnormalities. Thus, the differences in RSN described here must be interpreted cautiously and most likely do not indicate increased risk of aggressive behavioral outcome, but rather represent a neural mechanism that might interact with several other (environmental) factors to contribute to increased risk of aggressive behavior (Caspi et al. 2002; Meyer-Lindenberg et al. 2006). Thus, in agreement with previous studies, we would like to point out that in order to evoke abnormally aggressive behavioral phenotypes, MAOA-L has to be present in combination with early environmental exposure to abusive or aggressive treatment (Caspi et al. 2002; Kim-Cohen et al. 2006; Ducci et al. 2008). Accordingly, the behavioral results of the present study revealed that the continuous scale measurement of aggression (AQ) did not differ significantly between the genotype groups. Furthermore, there were no significant correlations (all P values > 0.05; all correlation coefficients between \( r = -0.13 \) and \( r = 0.17 \)) between RSN activity and the mean AQ scores. This might be attributed to the fact that we compared 2 groups of healthy participants. We suggest that significant differences or correlations with the AQ scores might be found when comparing healthy participants with psychiatric patients suffering from a disease associated with pathological aggression (e.g., borderline personality disorder). However, the present results clearly demonstrate that differences in RSN of healthy participants can be influenced by MAOA.

**RSN with Increased Activity for MAOA-L**

Our RSN study revealed that the participants in the MAOA-L group, when compared with the MAOA-H group, exhibited increased activity within several parts of the DMN. However, due to the exploratory nature of the present study and the limited knowledge about potential interactions between the MAOA genotype and RSN, it is rather difficult to interpret these differences within the DMN in relation to the MAOA genotype. One might speculate that, for those participants with MAOA-L, increased activity already during rest could also lead to differential brain activity during cognitive and/or emotional tasks. During tasks assessing arousal, affect, and/or inhibitory control, it was already shown that decreased brain activity was linked to MAOA-L (Fan et al. 2003; Meyer-Lindenberg et al. 2006; Passamonti et al. 2006). Thus, our results add to previous findings by providing the first evidence using RSN, showing that the DMN is hyperactive in participants with MAOA-L, during rest and in the absence of any aggression-related experimental challenge.

**Figure 3.** Voxel-wise difference maps for cerebellum and salience RSN. Activity is displayed at a cluster-level threshold of \( P < 0.05 \) (FDR corrected) and projected on the MNI template brain (ICBM 152). Panel (A) shows, within the salience RSN, the dorsal anterior cingulate cortex (dACC) and the anterior frontal cortex at BA 10. For both areas, activity was reduced for the MAOA-L group. Panel (B) shows a cerebellar cluster at the anterior lobe of the cerebellum, showing increased activity for the MAOA-L group. BA, Brodmann area; FDR, false discovery rate; LH, left hemisphere; MAOA-H, high-activity allele group; MAOA-L, low-activity allele group; RH, right hemisphere; RSN, resting-state network.
and hence under low environmental influence. Overall, brain activity in people with MAOA-L seems to be overly decreased during cognitive and emotional tasks and overly increased (at least in the DMN) during rest. A rather important implication here is that differences in task-related activity might thus be reduced in people with MAOA-L because their brain is already more active at baseline (i.e., in default mode areas), diminishing differences between task and baseline conditions. The results and the proposed explanation presented here might thus be important for designing future studies and also for the interpretation of previous fMRI studies showing task-related differences between MAOA-L and MAOA-H.

The MAOA-L group also showed increased activity within the anterior lobe of the cerebellum. Previous findings indicate that the anterior lobe of the cerebellum is primarily responsible for sensorimotor tasks (Grodd et al. 2001; Stoodley and Schmahmann 2009; Stoodley et al. 2012). Future studies have to investigate specifically whether altered activity during rest in brain areas related to sensorimotor processing (e.g., within the sensorimotor RSN) might be accompanied by behavioral changes such as disrupted impulse control.

Overall, the following preliminary conclusion for participants with MAOA-L can be drawn from the present results: carriers of the MAOA-L genotype exhibit increased brain activity in several default mode areas of the brain. Such a widespread hyperactivity within the DMN implies that already during rest, the brains of MAOA-L carriers function differently when compared with those of MAOA-H carriers. Future studies should specifically investigate whether such differential activity patterns during rest are also related to increased aggression, in healthy participants and psychiatric patients. Note that all conclusions presented here should be interpreted cautiously because they are derived from results obtained in a nonclinical, healthy population of participants. Moreover, the present study is highly exploratory, being the first study investigating this topic and therefore all results and conclusions need to be replicated—preferably also in clinical populations—for consolidation. Also, it has to be acknowledged that the limbic system is not covered by any of the common RSN. Nevertheless, areas like the amygdala have been shown to play an important role in emotion regulation and aggression (Schneider et al. 1995; Habel et al. 2004; Buckholtz and Meyer-Lindenberg 2008). Future studies may evaluate whether the hyperactivity in the DMN of the MAOA-L group might be accompanied by hyperactive amygdala functioning.

**RSN with Reduced Activity for the MAOA-L Group**

As expected, we found differences in the executive control RSN. Here, the MAOA-L group exhibited reduced activity within 2 right frontal areas, which have been previously related to inhibitory control and emotion regulation (Raine et al. 1994, 1998; Grafman et al. 1996; Pietrini et al. 2000; Soderstrom et al. 2002; Yang and Raine 2009; Coccaro et al. 2011). The right frontal region at BA 6 was also found to be less active within the frontoparietal network, indicating an important role for this region. We suggest that the finding of less frontal activity during rest for MAOA-L confirms previous results (Meyer-Lindenberg et al. 2006; Passamonti et al. 2006), which demonstrated decreased prefrontal activity in MAOA-L during emotionally arousing tasks. Both, the previous and the present results, indicate that the frontal cortex might be a primary area of interest for genetic differences related to MAOA. Future research will clarify whether the pattern of reduced frontal activity for MAOA-L and increased frontal activity for MAOA-H persists in both task and resting-state paradigms. However, as noted earlier, it should be kept in mind that so far, there are no RSN explicitly covering the limbic system, although it has been shown to play an important role in explaining aggressive behavior and emotion regulation (Schneider et al. 1995; Habel et al. 2004; Buckholtz and Meyer-Lindenberg 2008).

The present findings for the salience network indicate that people with MAOA-L have already less dACC activity during rest. Comparable with the frontal areas, the dACC is thought to be responsible for attentional and executive control, as well as conflict and error monitoring, and thus acting as a central regulatory structure (Paus et al. 1997; Menon et al. 2001; Sturm and Willnes 2001; Kerns et al. 2004; Mottaghy et al. 2006; Clemens et al. 2011, 2013). Activation differences within this structure during rest could be related to the interaction of the MAOA genotype and aggression. Such an interpretation would be supported by 1) previous neuroimaging findings showing that the dACC is specifically responsible for regulating and controlling motivational impulses (Banks et al. 2007; Pezawas et al. 2005), and 2) clinical studies showing that disrupted ACC activity is linked to increased aggressive behavior, as well as impaired inhibitory control and emotion regulation (Volvaka 1995; Grafman et al. 1996; Danckert et al. 2000; Hornak et al. 2003). Whereas differences in dACC and frontal regions have been previously related to MAOA genotype only during task demands, we provide here neurofunctional evidence that these regions also differ during rest. Moreover, the present results fit well with previous findings: Reduced or disrupted dACC activity was previously associated with higher likelihood of aggressive behavior and impaired inhibitory control (Danckert et al. 2000; Hornak et al. 2003; Yang and Raine 2009). We show here that reduced dACC activity during rest was found for the MAOA-L genotype, which was previously related to increased likelihood of aggressive behavior. Taking into account the present and previous findings, we cautiously hypothesize that less dACC activity results in an increased likelihood of aggressive behavior, and vice versa. Corroborating this hypothesis, previous findings in pathologies associated with increased aggressive behavior (e.g., borderline personality disorder, psychopathy) revealed decreased functional activity and recued anatomical volume in dACC (Kiehl et al. 2001; van Elst et al. 2003; Birbaumer et al. 2005; Whittle et al. 2006; Enzi et al. 2013). A potential explanation for the link between the dACC and aggression could be that reduced dACC functioning leads to reduced action, conflict, and error monitoring, and thus to increased aggressive behavior. Another potential explanation for the relationship between reduced dACC functioning and increased aggression is that less dACC activation results in less inhibition and thus overly increased activity of subcortical areas, which in turn leads to less inhibition of aggressive tendencies and thus more aggressive behavior (Raine et al. 1998; Coccaro et al. 2007; Yang and Raine 2009).

Moreover, the MAOA-H group showed increased activity within one part of the DMN, namely the posterior cingulate. For both the posterior and the anterior cingulate regions, it should be kept in mind that these regions have the highest density of serotonin receptors in the human cortex (Varnas et al. 2004). The fact that MAOA specifically interacts with sero-
tonin and dopamine might partly explain these differences in cingulate brain regions.

Conclusions
We conclude that investigating RSN in relation to MAOA provides useful information on how differences in brain activity—even during rest—are influenced by MAOA genotype. Our work is exploratory and encouraging to increase further research to fully elucidate the interactions between the MAOA genotype and brain activity. Especially, research on RSN should be intensified, considering the relatively convenient manner in which resting-state data can be acquired. Based on the present results we conclude that the MAOA genotype, which has been repeatedly associated with aggression, influences brain activity already during rest. The impact of MAOA influence is observable in brain areas playing an important role in emotion regulation and pathological aggression, and future studies should clarify whether behavioral abnormalities might be partly explained by MAOA-related differences in brain activity during rest. These differences include increased DMN activity and decreased activity within areas related to executive control for the MAOA-L group. Carriers of the MAOA-H genotype apparently exhibit increased activity in the posterior cingulate part of the DMN. Once more, we must emphasize the fact that the data presented here are derived from healthy participants with no behavioral phenotype, and that an interaction between MAOA-L and aggression is highly dependent upon early environmental exposure to abusive or aggressive behavior. Therefore, all conclusions of this exploratory study need to be interpreted cautiously and warrant further confirmation. Future studies combining analyses of MAOA and RSN in psychiatric populations could help to further elucidate the link between the resting brain and the MAOA genotype.

Funding
This work was supported by the Interdisciplinary Center for Clinical Research within the Faculty of Medicine at the RWTH Aachen University (IZKF Aachen) (grant number N4-4), and by the State of North Rhine-Westphalia (NRW, Germany) and the European Union through the ‘NRW Ziel2 Program’ as a part of the European Fund for Regional Development. The funding sources had no role in study design, in collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Notes
The authors express their gratitude to Andre Schueppen, from the Brain Imaging Facility of the Interdisciplinary Centre for Clinical Research at the RWTH Aachen University, and to the radiographers Cordula Kemper and Maria Peters, for their technical support and assistance with data acquisition. Furthermore, we thank 2 reviewers for their helpful comments and constructive criticism on this manuscript. Conflict of Interest: None declared.

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