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Cortico-Limbic Connectivity in *MAOA*-L Carriers is Vulnerable to Acute Tryptophan Depletion

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Abstract: *Introduction:* A gene–environment interaction between expression genotypes of the monoamine oxidase A (MAOA) and adverse childhood experience increases the risk of antisocial behavior. However, the neural underpinnings of this interaction remain uninvestigated. A cortico-limbic circuit involving the prefrontal cortex (PFC) and the amygdala is central to the suppression of aggressive impulses and is modulated by serotonin (5-HT). *MAOA* genotypes may modulate the vulnerability of this circuit and increase the risk for emotion regulation deficits after specific life events. Acute tryptophan depletion (ATD) challenges 5-HT regulation and may identify vulnerable neuronal circuits, contributing to the gene–environment interaction. *Methods:* Functional magnetic resonance imaging measured the resting-state state activity in 64 healthy males in a double-blind, placebo-controlled study. Cortical maps of amygdala correlation identified the impact of ATD and its interaction with low- (*MAOA*-L) and high-expression variants (*MAOA*-H) of *MAOA* on cortico-limbic connectivity. *Results:* Across all Regions of Interest (ROIs) exhibiting an ATD effect on cortico-limbic connectivity, *MAOA*-L carriers were more susceptible to ATD than *MAOA*-H carriers. In particular, the *MAOA*-L group exhibited a larger reduction of amygdala connectivity with the right prefrontal cortex and a larger increase of amygdala connectivity with the insula and dorsal PCC. *Conclusion: MAOA*-L carriers were more susceptable to a

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central 5-HT challenge in cortico-limbic networks. Such vulnerability of the cortical serotonergic system may contribute to the emergence of antisocial behavior after systemic challenges, observed as gene-environment interaction. *Hum Brain Mapp 38:1622–1635, 2017.*

Key words: amygdala; serotonin; MAOA; aggression; resting state fMRI

INTRODUCTION

Behavioral and personality traits can emerge on the grounds of gene–environment interactions [South and Krueger, 2008]. In particular, antisocial behaviors may result from childhood maltreatment, specifically in carriers of the low expressing variant of the monoamine oxidase A gene [*MAOA*-L; Caspi et al., 2002; meta-analysis in Byrd and Manuck, 2014]. Antisocial behavior is related to dysfunctions in cortico-limbic inhibition [Buckholtz et al., 2008]. In particular, amygdala responses to aversive stimuli are modulated by the prefrontal cortex [PFC, Frodl et al., 2009; Lemogne et al., 2011], which in turn receives dense serotonergic projections [Challis and Berton, 2015; Peyron et al., 1998]. *MAOA*-L carriers may be more sensitive to disturbances of serotonin (5-HT) metabolism, which may affect connectivity between PFC and limbic structures. One approach to investigate the neural processes of altered 5-HT availability in *MAOA*-L carriers is acute tryptophan depletion (ATD). This method decreases the availability of the essential 5-HT precursor tryptophan [Delgado et al., 1990, 1991] and may test for serotonergic vulnerability of the cortico-limbic network in different *MAOA* genotypes.

Abbreviations

5-HT	serotonin;
ACC	anterior cingulate cortex;
ANOVA	analysis of variance;
ATD	acute tryptophan depletion;
BAL	balanced;
CN	caudate nucleus;
HCl	hydroxychloride;
IFG	inferior frontal gyrus;
KCl	potassiumchloride;
LN	lentiform nucleus;
MAOA	monoaminoxidase A;
MAOA-L	low expressing allele of the MAOA gene;
MAOA-H	high expressing allele of the MAOA gene;
$MgCl_2$	magnesium dichloride;
PCC	posterior cingulate cortex;
PCR	polymerase chain reaction;
PFC	prefrontal cortex;
PG	precentral gyrus;
ROI	region of interest;
RS	resting state;
SFG	superior frontal gyrus;
STG	superior temporal gyrus

Regulation of the amygdala seems highly relevant for behavioral disorders. The amygdala is hyperactive in *MAOA*-L carriers during the recall of negative events [Meyer-Lindenberg et al., 2006] and anger control [Denson et al., 2014]. In particular, during aggressive behavior, amygdala regulation is pivotal for emotion processing [Klasen et al., 2013; Mathiak and Weber, 2006]. Conceivably, vulnerable amygdala regulation may contribute to dysfunctional emotion regulation, thereby increasing the risk for antisocial behavior in *MAOA*-L carriers. Clinical effects of 5-HT enhancing drugs on aggressive behavior suggest an involvement of the serotonergic system [Siever, 2008].

The PFC regulates amygdala responses to emotional stimuli [Lemogne et al., 2011]. This cortico-limbic network is associated with impulse control and reduced activation in the ACC during a Go/NoGo task, emerging in *MAOA*-L carriers only [Buckholtz and Meyer-Lindenberg, 2008; Meyer-Lindenberg et al., 2006]. Indeed, impaired cortico-limbic regulation is supposed to contribute to antisocial behavior in *MAOA*-L carriers [Chester et al., 2015; Davidson et al., 2000]. Therefore, a potential neural mechanism of a higher vulnerability in *MAOA*-L carriers may originate from impaired regulation in the outlined network.

ATD is an established method to experimentally challenge the central nervous 5-HT system [Delgado et al., 1990; Zimmermann et al., 2012]. This procedure reduces central 5-HT availability [Nishizawa et al., 1997; Shoaf et al., 1998] and allows investigations on the effects of reduced central 5-HT on the cortico-limbic circuitry. Lowering brain 5-HT synthesis yielded a bias to aversive emotional stimuli [Klaassen et al., 2002; Passamonti et al., 2012], and increased state aggression [Cleare and Bond, 1995; Pihl et al., 1995]. These factors probably contribute to developing aggressive behavior. Further, ATD affected the activity of the amygdala and the PFC [Bremner et al., 1997; Morris et al., 1999; Smith et al., 1999a; Neumeister et al., 2004; Lemogne et al., 2011]. In particular, emotionally salient stimuli yielded higher responses in the PFC as a result of impaired evaluation after ATD [Roiser et al., 2009b]. Thus, PFC regulation of emotion processing at the amygdala seems to depend on 5-HT availability.

On the neural level, ATD reduced connectivity of the amygdala with the PFC and perturbed emotion regulation to aggressive social stimuli [Crockett et al., 2009; Hindi Attar et al., 2012; Passamonti et al., 2012; Roiser et al., 2009a; Robinson et al., 2012]. Passamonti et al. [2012] showed that ATD reduced PFC-amygdala connectivity specifically for angry faces, supporting neurobiological models that postulate facilitation of the suppression of negative affect via the PFC-amygdala circuit [Davidson et al., 2000; Miczek et al.,

2007; Siever, 2008]. Such negative affect bias is a specific feature of Borderline and impulsive aggressive personality disorders in which serotonergic neurotransmission is disturbed [Lee et al., 2012; Leyton et al., 2001]. Moreover, activation of brain areas related to aggression and impulsivity (i.e., such as the orbitofrontal cortex) were correlated with ATD-related depletion magnitude and trait-impulsivity [Helmbold et al., 2015]. In summary, genetic and pharmacological alterations of 5-HT levels affect neural correlates of emotion regulation and impulse control. Therefore, ATD may test the vulnerability to dysregulation of the cortico-limbic network in *MAOA*-L carriers.

The regulation networks for emotions have been widely investigated [see meta-analysis in Kohn et al., 2014]. Task-dependent paradigms focus on differential aspects of emotion regulation but may miss fundamental network changes. Therefore, one beneficial strategy to assess network activity is resting state [RS; Roy et al., 2009]. RS-fMRI measurements identify neural networks independently from specific task demands or emotion-eliciting stimuli [Beckmann et al., 2005]. During RS, functionally connected networks show synchronized activity even in the absence of a task and can be identified [Damoiseaux et al., 2006] and dysfunctional emotion regulation networks can be detected [e.g., in depression; Anand et al., 2009]. Therefore, RS-fMRI may investigate the vulnerability of the cortico-limbic network in *MAOA* genotypes to ATD.

We investigated whether the cortico-limbic circuit is more vulnerable to changes of brain 5-HT in *MAOA*-L than in *MAOA*-H carriers. Reducing central 5-HT availability by ATD should reveal neural mechanisms of this vulnerability. Therefore, our study aimed to identify the influence of ATD on functional connectivity in *MAOA*-L and -H carriers during resting state fMRI. First, we aimed to test whether the finding by Passamonti et al. [2012], that ATD reduces amygdala connectivity with the PFC to aggressive faces, extends to connectivity during stimulus-independent resting state. Second, diminished central 5-HT availability may affect *MAOA*-L stronger than *MAOA*-H carriers. In particular, we hypothesized an interaction between the *MAOA* genotype and ATD on cortico-limbic connectivity, which may contribute to a higher vulnerability in *MAOA*-L carriers.

METHODS

Participants

Sixty-four male volunteers (25.0 ± 3.8 years, all German mother tongue, and of Caucasian ethnicity) participated in the present study. In a standardized assessment, all volunteers reported normal vision, normal hearing, no contraindications against MRI investigations, and no history of neurological or psychiatric disorders. The subjects were drug-screened, right-handed as assessed with the Edinburgh Handedness Inventory [Oldfield, 1971], and had normal intelligence according to the multiple-choice-word test [Lehrl, 2005]. The experiment was conducted in accordance with the Code of Ethics of the World Medical Association [Declaration of Helsinki, 2008], and the study protocol was approved by the local ethics committee. The subjects were financially compensated for their participation.

Genotyping

Prior to the fMRI scanning, all participants underwent a 9 mL venous blood sampling. Genomic DNA was isolated from peripheral lymphocytes with a routine salting-out procedure. For the determination of the *MAOA* genotype, standard polymerase chain reaction (PCR) amplification was performed in a 25 μL volume containing 80 ng of genomic DNA, unit of recombinant *Taq* polymerase (Invitrogen, Darmstadt, Germany), PCR buffer (10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, pH 8.3), 200 mM deoxynucleotides, and 20 pmol of each primer. *MAOA* primer sequences were obtained from Sabol et al. [1998]. The PCR was run on an MJ PTC200 Temperature Cycler (Biozym, Hessisch-Oldendorf, Germany), and each of the 35 cycles consisted of a 95°C denaturation step for 45 s, a 62°C annealing step for 30 s, and, finally, a 72°C elongation step for 90 s. PCR products were run on an automated sequencing system (AB3130, Applied Biosystems, Darmstadt, Germany), and the electropherograms were analyzed with gene mapping software. According to Sabol et al. [1998], 3 and 5 repeats were classified as representing a low expression (*MAOA*-L) and 3.5 and 4 repeats as representing a high *MAOA* expression (*MAOA*-H).

Serotonergic Challenge

We studied effects of ATD in a cross-over, double-blind design with blocked-random group allocation. Two conditions were compared: (1) body weight-adapted ATD according to the Moja-De scheme [Demisch et al., 2002; Moja et al., 1988]; and (2) an L-Tryptophan–balanced control drink (BAL). For ATD, a tryptophan-free amino acid beverage was applied: for each 10 kg of body weight, 0.084 g L-Isoleucine, 0.132 g L-Leucine, 0.12 g L-Lysine-HCL, 0.05 g L-Methionine, 0.132 g L-Phenylalanine, 0.06 g L-Threonine, and 0.096 g L-Valine were introduced. In the BAL condition, an extra amino acid mixture was provided, which included an additional 0.7 g of tryptophan per 10 kg body weight (BAL). The latter composition acts as a control and does not impact 5-HT synthesis in the brain [Biskup et al., 2012].

As a manipulation check, blood serum was collected immediately before beverage administration and 3 h later, that is, before the start of the fMRI resting state measurement. With an ANOVA, we tested effects of the within-subject factors "Condition" (ATD and BAL) and "Time" (Before beverage administration and before resting state) as well as the between-subject factors "Genotype" (*MAOA*-H and *MAOA*-L) and "Order" (first session ATD and BAL) on tryptophan levels.

Mood Assessment

ATD effects on mood across healthy men are inconsistent [Evers et al., 2006; Young and Leyton, 2002]. Therefore, we assessed mood using the Positive And Negative Affect Scale [PANAS; Watson et al., 1988] before beverage administration and before resting state in both conditions. In the ATD and BAL condition, differences between before beverage administration and before resting state fMRI were calculated separately for positive and negative affect, corresponding to baseline-corrected effects on mood. Positive and negative affect changes were analyzed using repeated measures ANOVAs with the within-subject factor "Condition" (ATD and BAL) and the between-subject-factor "Genotype" (*MAOA*-H and *MAOA*-L) in SPSS 21 (IBM Corp., Armonk, New York).

Task and Procedure

Participants were instructed to adhere to a tryptophan-free diet from 8 pm the evening before each measurement. In the morning, the participants arrived at 8 am at the study site. At 8:30 am, an amino acid beverage (ATD or BAL) was administered blind to the experimenter and the participants. At 11:30 am, a known time point associated with a robust decline of TRP influx in the brain [Dingerkus et al., 2012], a resting state fMRI session was recorded for 8 min with eyes open (white fixation cross on a black background). The participants were instructed to relax, to think of nothing in particular, and to stay awake. The measurement was followed by a high resolution anatomical T1 scan. Individual measurements were separated 14.82 days on average (minimum 7 days, maximum 99 days). To assess the effect size of tryptophan depletion, we estimated Cohen's d for the effect of ATD on tryptophan depletion (compared with BAL).

Brain Imaging

Magnetic resonance imaging was conducted on a 3 Tesla Siemens Trio scanner (Siemens Medical, Erlangen, Germany). For fMRI, echo-planar imaging (EPI) acquired 34 transversal slices for a total of 240 volumes (TE = 28 ms, TR = 2,000 ms, flip angle = 77°, voxel size = 3 × 3 mm with 64 × 64 matrix, 3 mm slice thickness, 0.75 mm gap). After the RS measurements, a high resolution, whole brain anatomical image was acquired (MPRAGE, T1-weighted, TE = 2.52 ms; TI = 900 ms; TR = 1,900 ms; flip angle = 9°; FOV 256 × 256 mm^2; 1-mm isotropic voxels; 176 sagittal slices).

fMRI Preprocessing

Resting state measurements were analyzed with *BrainVoyager QX 2.8* (Brain Innovation, Maastricht, The Netherlands). To avoid T1 saturation effects, the first five images of each session were discarded. Preprocessing of functional MR images included slice scan time correction, 3D motion correction, spatial smoothing (6 mm FWHM), and high-pass filtering including linear trend removal. Additionally, rigid-body motion parameters as well as cerebrospinal fluid and white matter time courses were regressed out to reduce physiological noise [Hutton et al., 2011]. Functional images were co-registered individually to the anatomical images and transformed into Talairach space [Talairach and Szikla, 1980].

Seed-Based Functional Connectivity Analysis

Amygdala maps were imported from the Anatomy Toolbox [Eickhoff et al., 2005] in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) to BrainVoyager software (V 2.8) and transformed into Talairach space. Functional connectivity using the bilateral amygdala seed was calculated for each voxel of the whole brain. The *t*-tests determined the effect of ATD on amygdala connectivity after Fisher's z-transformation. Further *t*-tests explored the effects of *MAOA* expression variants and the differences between left and right amygdala connectivity. The resulting maps for the amygdala connectivity were thresholded at a voxelwise $P < 0.001$ and corrected for multiple comparisons using Monte–Carlo simulation at $P < 0.05$, resulting in a cluster threshold of $k > 12$ voxels.

ROIs were functionally defined, based on the main effect of ATD on amygdala connectivity, and labeled according to the location of their peak voxels (Table I). At these peak voxels, connectivity values were extracted for both conditions to investigate the influence of *MAOA* expression variants and to control for carry-over effects. ROI analyses were conducted in SPSS 21 (IBM Corp., Armok, New York). Peak voxels of ROIs that exhibited increased amygdala connectivity (positive connectivity change) after the ATD challenge and those of ROIs with reduced amygdala connectivity (negative connectivity change) were averaged separately. We tested for effects of *MAOA* expression on these average scores in an ANOVA with the within-subject factor "Direction-ATD-effect" (positive connectivity change and negative connectivity change) and the between-subject factors "Genotype" (*MAOA*-H and *MAOA*-L) and "Order" (first session ATD and BAL). Post hoc *t*-tests determined directionality of the effects. Further exploratory paired *t*-tests compared *MAOA* genotype effects on connectivity change of the amygdala, after the ATD challenge to each ROI. We estimated Cohen's d to evaluate the effect sizes of *MAOA* genotype effects on amygdala connectivity.

RESULTS

Genotype Results

In line with previously reported distributions of *MAOA* allele expression [Sabol et al., 1998], 21 out of 64 participants (32.8%) were classified as *MAOA*-L (20 × 3 repeats; 1 × 5 repeats) and 43 (67.2%) as *MAOA*-H carriers (2 × 3.5 repeats; 41 × 4 repeats).

TABLE I. Clusters of ATD effect on amygdala connectivity

Anatomical regions	Brodmann areas	Peak voxel *x y z*	Cluster size (mm^3)	T_{peak}
Right PFC				
Middle frontal gyrus R	6, 9, 32	20 31 39	6067	−5.39
Cingulate gyrus R				
Inferior frontal gyrus R	9			
Medial frontal gyrus R	6, 8, 9, 10			
Precentral gyrus R	6, 9			
Superior frontal gyrus R	6, 8, 9, 10			
Left PFC				
Middle frontal gyrus L	6, 8, 9, 46	−43 13 30	1705	−4.22
Inferior frontal gyrus L	9, 46			
Precentral gyrus L	9			
Superior frontal gyrus L	8			
Right IFG				
Inferior frontal gyrus R	11, 47	35 28 −16	1743	−5.14
Middle frontal gyrus	11, 47			
Superior frontal gyrus R	11			
Left SFG				
Superior frontal gyrus L	8, 9, 10	−22 13 39	1750	−4.13
Cingulate gyrus L	32			
Medial frontal gyrus L	6, 8, 9, 32			
Middle frontal gyrus L	6, 8, 9			
Left PG				
Precentral gyrus L	6, 9	−46 4 36	592	−3.94
Inferior frontal gyrus L	6, 9			
Middle frontal gyrus L	6, 8, 9			
Right CN				
Caudate nucleus R		11 −2 24	1756	−5.16
Cingulate gyrus R	24			
Right LN				
Cingulate gyrus R	25	8 1 −9	757	−4.45
Caudate R				
Lentiform nucleus R				
Subcallosal gyrus R	25			
Left CN				
Caudate nucleus L		−16 10 9	722	−3.85
Claustrum L				
Lentiform nucleus L				
Right insula				
Claustrum R		32, 17 15	1650	4.82
Insula R				
Lentiform nucleus R				
Superior temporal gyrus R				
Thalamus R				
Right ventral PCC				
Posterior cingulate cortex R	29,30	14 −56 9	1132	4.43
Lingual gyrus R	18, 19			
Parahippocampal gyrus R				
Left ventral PCC				
Posterior cingulate L R	23, 29, 30	−4 −35 18	3468	4.71
Caudate nucleus R				
Cingulate gyrus L R	23, 31			
Parahippocampal gyrus R	30			
Thalamus				
Left dorsal PCC				
Cingulate gyrus L	31	−13 −26 33	744	5.85
Caudate nucleus L				
Posterior cingulate L	23			

TABLE I. (continued).

Anatomical regions	Brodmann areas	Peak voxel *x y z*	Cluster size (mm^3)	T_{peak}
Left precuneus				
Precuneus L	7, 31	−19 −59 27	593	4.10
Cingulate gyrus L	31			
Left lingual gyrus				
Declive		11 −80 −15	1376	4.84
Inferior occipital gyrus R				
Lingual gyrus R				
Right STG				
Caudate nucleus R		35 −41 6	726	5.28
Middle temporal gyrus R	22			
Parahippocampal gyrus R	19			
Hippocampus R				
Superior temporal gyrus R	22, 41			

Abbreviations for cluster labels: PFC, prefrontal cortex; CN, caudate nucleus; IFG, inferior frontal gyrus; SFG, superior frontal gyrus; LN, lentiform nucleus; PG, precentral gyrus; PCC, posterior cingulate cortex; STG, superior temporal gyrus.

Serotonin Challenge and Mood

Five participants were excluded from the analysis due to drop-outs or technical problems. Of the remaining sample, 28 participants received the BAL condition and 31 participants the ATD condition first. We found significant main effects for the factors "Condition" ($F(1,57) = 1014.3$, $P < 0.0001$) and "Time" ($F(1,57) = 700.8$, $P < 0.0001$) as well as a significant "Condition*Time" interaction ($F(1,57) = 1107.4$, $P < 0.0001$). Post-hoc t-tests showed a significant increase of tryptophan levels, comparing before beverage administration and before resting state in the BAL condition (before beverage: 65.58 ± 1.39 mmol/L; before resting state: 446.63 ± 11.96 mmol/L; $t(59) = -32.6$, $P < 0.0001$; Cohen's $d = 5.83$) and significantly reduced tryptophan serum levels comparing before beverage administration and before resting state in the ATD condition (before beverage: 65.9 ± 1.65 mmol/L; before resting state: 22.51 ± 0.99 mmol/L; $t(59) = -30.3$, $P < 0.0001$; Cohen's $d = -4.15$). According to our hypotheses, we focused on the change of tryptophan levels for the "Condition*Time" interaction between before beverage administration and before resting state in the ATD and BAL conditions. The *t*-test confirmed significant reduction of tryptophan levels in the ATD condition compared with the BAL condition before resting state (ATD: −40.08 ± 6.36 mmol/L, BAL: 356.14 ± 16.56 mmol/L; $t(60) = -22.4$, $P < 0.0001$; Cohen's $d = 4.35$). Neither the factors "Genotype" or "Order" nor any further interaction yielded significance (all $F < 3.2$).

Due to incomplete PANAS forms, seven participants who received ATD first and seven participants who received BAL first were excluded from the behavioral analysis. In line with previous studies [Booij et al., 2005; Roiser et al., 2009b], we found no main effect for "Condition" or "Genotype," or any interaction on mood (all $P > 0.5$).

Seed-Based Functional Connectivity Analysis

After the ATD challenge, amygdala connectivity was reduced to the bilateral prefrontal cortex (right/left PFC), the bilateral caudate nucleus (right/left CN), the right inferior frontal gyrus (right IFG), the left superior frontal gyrus (left SFG), the left precentral gyrus (left PG), and the right lentiform nucleus (right LN). Furthermore, *t*-tests revealed increased amygdala connectivity to the right insula, the bilateral ventral and left dorsal posterior cingulate cortex (right/left ventral and left dorsal PCC), the right superior temporal gyrus (right STG), the right lingual gyrus, and the left precuneus after ATD challenge (Table I, Fig. 1). No differences emerged between the *MAOA* genotypes or between left and right amygdala connectivity at the same statistical threshold.

Functional connectivity changes were extracted and averaged from the peaks of the seven positive (higher connectivity after ATD) and the eight negative ROIs (lower connectivity after ATD). In addition to the trivial main effect "Direction-ATD-effect" ($F(1,60) = 88.0$; $P < 0.0001$), the interaction "Direction-ATD-effect*Genotype" ($F(1,60) = 12.7$; $P < 0.001$) yielded significance. There was no significant effect of "Genotype" or "Order" or any further interaction on connectivity change (all $F < 0.6$, $P > 0.4$). Post hoc *t*-tests revealed a higher negative ($t(62) = -2.8$, $P < 0.014$, after Bonferroni correction; $d = -2.269$; Fig. 2) as well as a higher positive connectivity change ($t(62) = 2.8$, $P < 0.014$, after Bonferroni correction; $d = 1.928$; Fig. 2) in the *MAOA*-L group compared with the *MAOA*-H group.

Further, an exploratory analysis considered the connectivity values in all ROIs separately. The paired *t*-tests yielded significant differences in the right PFC, the right insula, and the left dorsal PCC (right PFC: $t(61.85) = -2.1$, $P = 0.037$ after Greenhouse–Geisser correction, $d = -0.15$; right insula: $t(62) = 2.7$; $P = 0.009$, $d = 0.72$; left dorsal PCC: $t(62) = 3.3$, $P = 0.002$, $d = 0.93$; Fig. 2 and Table II).

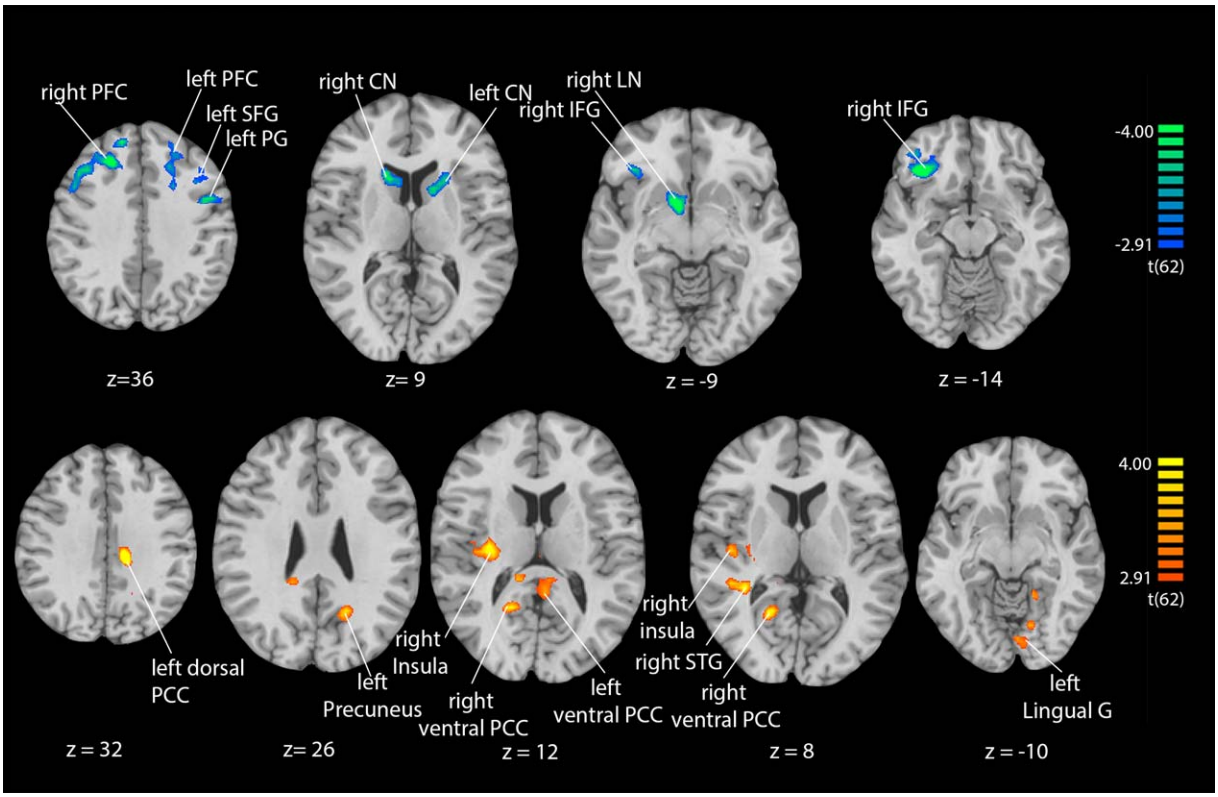

Figure 1.

ATD effects on amygdala connectivity. Functional connectivity with the amygdala was reduced in prefrontal and striatal structures (cold colors; upper row) and increased to right insula, right STG, and the PCC (warm color; lower row). For abbreviations see Table I. [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

The present study investigated the effects of ATD and its interaction with *MAOA* expression alleles on amygdala connectivity. After the ATD challenge, amygdala connectivity was reduced to frontal areas, whereas connectivity to the insula and the posterior cingulate cortex was increased. On average, these ATD effects were more than twice as strong in the *MAOA*-L as in the *MAOA*-H carriers. Thus, the ATD challenge unmasked a vulnerability of cortico-limbic connectivity in *MAOA*-L carriers to reduced 5-HT availability. In this genotype, the instable PFC-amygdala connectivity may confer a risk for impaired emotion regulation, which is associated with antisocial behaviors.

Impact of ATD and BAL on Tryptophan Levels

Tryptophan levels in blood serum decreased to about a third after ATD. Such a reduction of tryptophan confers to diminished central 5-HT availability [Sánchez et al., 2015]. In the BAL condition, serum tryptophan after BAL increased to about the 6-fold level of before beverage intake, similarly to a previous study by Dingerkus et al. [2002]. However, such marked increase in the serum did not affect 5-HT levels in the brain [Biskup et al., 2012].

Behavioral Effects of ATD and MAOA

The ATD challenge investigates the role of 5-HT on mood in healthy subjects [Evers et al., 2010] and in patients with psychiatric disorders [Booij et al., 2003; Golightly et al., 2001; Zimmermann et al., 2012]. Apparently, mood decreased in healthy men after ATD [Young and Leyton, 2002]; although, recent research suggests only small, if any, effects of the ATD challenge on the mood in healthy men [Young, 2013]. Our study confirmed no ATD effect on mood; positive and negative affect neither differed between ATD and BAL conditions nor did an interaction with the genotype emerge. In previous research, mood effects emerged primarily in patient groups and their relatives [Benkelfat et al., 1994]. A different 5-HT-related gene—the 5-HT transporter polymorphism (5-HTTLPR)—yielded an effect in women only [Neumeister

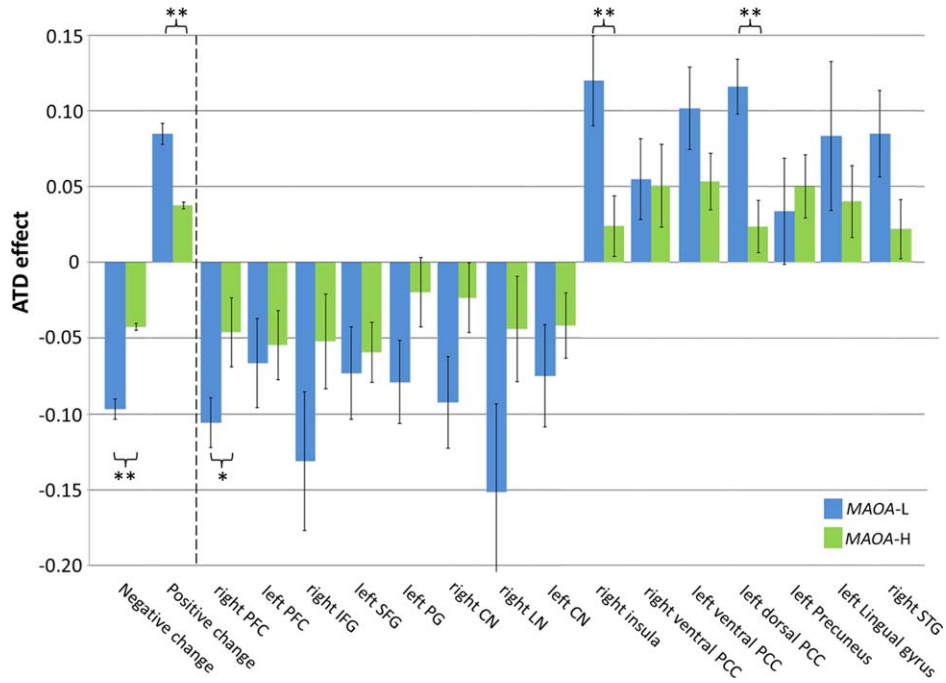

Figure 2.

ATD effect on amygdala connectivity in *MAOA*-L and -H carriers. Risk allele carriers (*MAOA*-L) were more affected by ATD across ROIs (left-most bars: "Negative change" in ROIs with reduced connectivity after ATD and "Positive change" in the other ROIs) and for the single ROIs yielding significance in right PFC, right insula, and left dorsal PCC. (Changes of correlation coefficients are shown after Fisher's *z*-transformation with 95%-confidence interval as error bars; *MAOA*-L: blue; *MAOA*-H: green; ** = $P < 0.01$; * = $P < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

et al., 2002]. Thus, mood effects of ATD may depend on sex and genes regulating 5-HT. We observed no such interaction for expression variants of the *MAOA* gene in healthy males.

Altered central nervous 5-HT neurotransmission may contribute to the emergence of antisocial behavior. In a previous ATD challenge study, healthy men responded with more aggression to repeated provocation as 5-HT levels decreased [Bjork et al., 1999; Young and Leyton, 2002]. Subsequent investigations replicated an increase of aggression after an ATD challenge [Bjork et al., 2000; Moeller et al., 1996]. Thus, the ATD challenge provides an interesting model to investigate the neural correlates involved in aggressive behavior. Conceivably, our participants have had an increased potential for aggressive behavior after the ATD challenge as well. Our study, however, chose not to trigger or test aggressive behavior, to obtain unbiased RS-fMRI measurements.

Neural Effects of ATD

Consistent with our hypothesis, cortico-limbic connectivity of the resting-state networks was lower after the ATD challenge. In particular, the PFC exhibited reduced functional connectivity with the amygdala after the ATD challenge. Conceivably, 5-HT mediates the communication between the PFC and the amygdala [Davidson et al., 2000; Siever, 2008]. Our study supports this idea and shows that functional connectivity between the PFC and the amygdala depends on central 5-HT. In a study on impulsive behavior, the central 5-HT availability influenced the cortico-amygdala connectivity as well as the inhibitory control [Passamonti et al., 2006]. A few further studies in this field confirmed that reduced central 5-HT was associated with decreased right prefrontal activity and increased impulsive behavior [Lamar et al., 2009; Rubia et al., 2005]. Although functional implications remain speculative, we hypothesize that lower PFC-amygdala connectivity after the ATD challenge may reflect the neural basis of impaired impulse control and reduced emotion regulation as observed in behavioral studies.

Attempts were made to specify the roles of the regions of the PFC in emotion regulation. In our study, we found reduced IFG-amygdala connectivity during resting state after the ATD challenge. The IFG achieved top–down-regulation of negative emotional responses [Ochsner et al., 2004] and impulse control [Coccaro et al., 2007; Smith et al., 1999b]. This frontal region seems to be a core area for emotion regulation, in particular for negative emotions and impulse control [Kohn et al., 2014]. Studies in patients

TABLE II. ATD effects in *MAOA*-H and -L carriers

Average ATD effect on amygdala connectivity (±SD)	*MAOA*-L ($N = 21$)	*MAOA*-H ($N = 43$)
Right PFC	−0.11 ± 0.08*	−0.05 ± 0.15
Left PFC	−0.07 ± 0.13	−0.05 ± 0.15
Right IFG	−0.13 ± 0.21	−0.05 ± 0.20
Left SFG	−0.07 ± 0.14	−0.06 ± 0.13
Left PG	−0.08 ± 0.12	−0.02 ± 0.15
Right CN	−0.09 ± 0.14	−0.02 ± 0.15
Right LN	−0.15 ± 0.27	−0.04 ± 0.23
Left CN	−0.07 ± 0.15	−0.04 ± 0.14
Right insula	0.12 ± 0.14*	0.02 ± 0.13
Right ventral PCC	0.05 ± 0.12	0.05 ± 0.18
Left ventral PCC	0.10 ± 0.13	0.05 ± 0.12
Left dorsal PCC	0.11 ± 0.08*	0.02 ± 0.11
Left precuneus	0.03 ± 0.16	0.05 ± 0.14
Left lingual gyrus	0.08 ± 0.23	0.04 ± 0.16
Right STG	0.02 ± 0.13	0.08 ± 0.13

SD, Standard deviation; *: significant group difference, see text; abbreviations: see Table I.

with borderline personality disorder reported an association between reduced IFG activation, and aggressive and impulsive behavior during a Go/NoGo task in contrast to controls without an emotion regulation disorder [Jacob et al., 2013]. Our study may provide a new perspective for the influence of 5-HT on the IFG in cortico-limbic regulation. The IFG's function during emotion regulation critically depends on 5-HT levels [Rubia et al., 2005]. Indeed, after the ATD challenge, IFG activation was reduced and aggression levels were increased [Allen et al., 2006; Rubia et al., 2005]. Conceivably, IFG-amygdala connectivity may reflect an additional mechanism for emotion regulation and impulse inhibition modulated by central 5-HT.

Insula–Emotion Processing and Aggression

In contrast to frontal regions, amygdala connectivity with the insula was increased after the ATD challenge. The insula has abundant reciprocal connections to the amygdala [Nieuwenhuys, 2012] and is central to emotion processing [for a review, see Klasen et al., 2014]. Enhanced activation in the insula emerged particularly after provocation and is associated with aggressive retaliation [Krämer et al., 2007], and damage to the insula may reduce impulsive behavior [Naqvi et al., 2007]. Therefore, the insula may be considered as a modulating factor for aggressive and impulsive behavior. Indeed, insula activation was reduced after ATD challenge during the decision phase in Taylor's aggression paradigm [Krämer et al., 2011], which may be in line with a tighter coupling of limbic responses, as disclosed by our connectivity analysis.

The insula is related to the detection of, and the reaction to aggressive stimuli. On the one hand, during the Taylor aggression paradigm, insula activation increased after aggressive reactions to the opponent [Dambacher et al., 2015]. On the other hand, the insula is part of the salience network [Seeley et al., 2007] and contributes to the allocation of attention to salient environmental stimuli [Menon and Uddin, 2010] by integrating information for higher cognitive processing [Damasio et al., 2000]. Indeed, the insula is consistently involved in the processing and regulation of emotions, in particular of negative affect [Ochsner et al., 2004; Sambataro et al., 2006]. Hypothetically, an alteration of the insula–amygdala connectivity may reflect modulated integration and cognitive regulation of negative affective information derived from the environment.

Posterior Cingulate Cortex Connectivity after ATD

The PCC is the core hub of the default mode network [DMN; Raichle et al., 2001], and the DMN activity in the PCC and precuneus was influenced by the ATD challenge [Kunisato et al., 2011]. In our study, the amygdala connectivity with the PCC increased after the ATD challenge. This is in line with the lowered DMN activity under reduced 5-HT metabolism [Scharinger et al., 2014] and tighter coupling to limbic activity. Indeed in patients with depression, the DMN activity during resting state was found to extend further into the limbic system [Sheline et al., 2009], and was restored to normal levels after increasing 5-HT availability [Cullen et al., 2016].

The DMN and the PCC are involved in emotion processing, regulation, and introspection [Klasen et al., 2011; Maddock et al., 2003; Otto et al., 2014; Singer, 2006; Teasdale, 1999]. Similar to the insula, the PCC is involved in processing and control of salient negative emotional stimuli [Vogt, 2005], and increased activation is associated to situations with immediately harmful outcome for specific opponents [Helmbold et al., 2015; White et al., 2014]. On the molecular level, local and autoinhibitory 5-HT1A binding inversely modulated the posterior cingulate cortex [Hahn et al., 2012], which may render this region particularly sensitive to changes in 5-HT availability. Conceivably, the disturbed PCC-amygdala network may reflect impaired processing of negative affect after the ATD challenge. Therefore, the increased amygdala connectivity with the PCC and the insula may show that the attention bias to negative and aggressive emotions processed in the amygdala depends on 5-HT availability.

Vulnerability in *MAOA*-L Carriers

In the current study, *MAOA*-L carriers showed much stronger ATD effects as compared with *MAOA*-H carriers, presumably reflecting a higher vulnerability of their serotonergic system. A similar interaction between a nutritional and a genetic factor was reported previously by for phenylketonuria—first described by Følling [1934]. In this disorder, the consumption of the amino acid phenylalanine led

to severe impairments of brain development in carriers of a dysfunctional phenylketon-hydroxylase gene [Pietz et al., 1999]. The gene–nutritional or –environmental interactions are not limited to somatic phenomena but extends to behavior and its neural correlates as well [for a review, see Burt, 2008].

In our study, the PFC, the insula, and the PCC were more susceptible to the ATD challenge in *MAOA*-L than in *MAOA*-H carriers. We discussed the functional relevance of these regions for emotion regulation above. Hypothetically, these regions may contribute to poor emotion regulation in *MAOA*-L carriers after adverse events. *MAOA*-L carriers are more likely to engage in delinquent behaviors [Beaver et al., 2014; Brunner et al., 1993; Cases et al., 1995; Guo et al., 2008] and constitute a higher proportion of violent offenders among incarcerated felons [Stetler et al., 2014]. Though several studies replicated the association between antisocial behavior and carriers of the *MAOA*-L gene, a direct link has not been reported consistently [for a review, see Vassos et al., 2014]. These contradictory reports need to be seen in the light of neglecting environmental influences. Caspi et al. [2002] first reported that the interaction of *MAOA*-L and childhood maltreatment contributed significantly to the emergence of antisocial behavior in males. This gene–environment interaction was repeatedly replicated in larger samples for the male sex only [see meta-analysis in Byrd and Manuck, 2014]; although, some skepticism emerged as well [for a comprehensive review, see Ficks and Waldman, 2014]. Low expression of *MAOA* may lead to impaired inhibitory control of impulsive behaviors after developmental stress. However, the neural mechanisms underlying the vulnerability in *MAOA*-L carriers are unknown. Our results may reflect alterations of the serotonergic system to the outlined cortico-limbic network that become apparent after external stress. Therefore, the outlined cortico-limbic network may represent the neural basis of 5-HT contributing to the risk of developing impulsive-aggressive behavior in *MAOA*-L carriers when exposed to adverse environmental challenges [Caspi et al., 2002; Kim-Cohen et al., 2006]. Further monoaminergic neurotransmitters such as adrenaline may contribute to this risk as well, as they are also affected by *MAOA* expression. These neurotransmitters are out of the scope of this study and need to be addressed in further research.

In previous studies, ATD and low expression of *MAOA* affected 5-HT availability in opposite directions but both factors were associated with impulsive aggressive behavior [Caspi et al., 2002; Chester et al. 2015; Manuck et al., 2006]. Although a comprehensive explanation for this phenomenon is still lacking, adaptation during development may reverse the effect of high 5-HT. Thus, the same cortico-limbic network may contribute to higher impulsivity after the ATD challenge and after aversive child hood experiences in *MAOA*-L carriers. Mouse models [see Uchida et al., 2005, 2007] may help to disentangle short- and long-term effects of altered 5-HT levels within specific neural circuits.

LIMITATIONS

Notably, the gene–environment interaction usually refers to an adverse environmental condition during development, and the current study employs the ATD challenge as an acute model. In a mouse model, chronic tryptophan depletion (1 month) induced lower anxiety and higher social dominance behavior [Uchida et al., 2005, 2007]. In a similar vein, increased impulsivity in humans may arise from reduced anxiety, and an increased stress response after reduced central 5-HT during development. Furthermore, both—*MAOA*-L and ATD—challenged the regulatory resources of the cortico-limbic system in our study. Therefore, we suggest that the synergistic effects of both conditions may affect neural correlates, promoting impulsive behavior.

Contributions of distinct neurotransmitter systems to the development of antisocial behavior are rather unclear so far. *MAOA* degrades not only 5-HT but other monoamines such as norepinephrine and dopamine as well. These confounds cannot be dismissed but we argue that the observed effects arise due to effects on 5-HT, since *MAOA* influenced the 5-HT levels most strongly compared with norepinephrine and dopamine [Nair et al., 1993]. Conceivably, the observed neural effects were most likely mediated by effects on 5-HT availability rather than norepinephrine or dopamine.

The statistical power to detect medium-sized effects ($d = 0.5$) in the fMRI experiment was limited by the size of the *MAOA*-L group [*MAOA*-L $d = 0.58$; *MAOA*-H $d = 0.89$; calculated in GPower; Faul et al., 2007, 2009]. Therefore, the detection of other group differences may have failed in the current study. Nevertheless, statistics showed stable effects of *MAOA* genotype on amygdala connectivity after the ATD challenge.

In addition to 5-HT, ATD affects the kynurenine system as well. Kynurenine is a metabolite of tryptophan and thus reduced after ATD. In a controversially discussed article, Van Donkelaar et al. [2011] speculated that memory impairments after ATD may also be due to reduced kynurenine. In our study, we investigated neural correlates of emotion regulation. Emotion regulation and impulse control has been consistently associated with 5-HT. We argue that lowered 5-HT synthesis was the main factor for altered cortico-limbic connectivity.

CONCLUSION

The cortico-limbic connectivity was affected by the ATD challenge during resting state. *MAOA*-L carriers were more vulnerable to the depletion effects and are a known risk group for impulsive antisocial behavior. Altered cortico-limbic connectivity is a promising candidate for the neural mechanism enhancing vulnerability to external stressors in *MAOA*-L carriers and may contribute to the emergence of antisocial behaviors.

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