The functional – 1019C/G HTR1A polymorphism and mechanisms of fear

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INTRODUCTION

Albeit animal studies showed genetic modulation of fearful behaviour by the serotonin receptor 1a gene (Htr1a), translational approaches towards anxiety disorders are missing. The present study aimed to close this gap by investigating behavioural and neural consequences of HTR1A variation in panic disorder with agoraphobia (PD/AG).

In rodents, disruption of Htr1a has been linked to increased defensive behaviour,1–4 particularly with regard to ambiguous, potential threat indicating stimuli.5,6 In these studies, ambiguous cues have been created, for example, by combining unaffected tactile and olfactory cues with spatial cues that were already cues have been created, for example, by combining unaffected.

The 5-HT1A receptor acts as a presynaptic inhibitory auto- and postsynaptic heteroreceptor mediating serotonin regulation.10 HTR1A rs6295 has been described as a potential mechanism in PD with or without AG.7–9 Thus, variation in HTR1A might be relevant for the etiopathogenesis of PD/AG.10 The G allele of HTR1A rs6295 has been proposed to convey risk for the development of PD/AG.11–14 However, despite strong evidence for the role of HTR1A in fear processing and PD/AG, the mechanisms underlying altered behavioural and neural responses are largely unknown.

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The 5-HT1A receptor acts as a presynaptic inhibitory auto- and postsynaptic heteroreceptor mediating serotonin regulation.10 HTR1A rs6295, in the transcriptional control region of HTR1A (~1019C/G), modulates the expression of 5-HT1A receptors and hence auto-inhibitory feedback on the presynaptic serotonergic neuron. While the G allele increases receptor expression at the presynapse and thereby reduces serotonergic neurotransmission due to enhanced auto-inhibitory feedback, it also reduces the expression of postsynaptic 5-HT1A leading to an overall reduction in
serotonergic neurotransmission, especially in neuronal structures characterized by postsynaptic 5-HT1A heteroreceptors such as frontal cortex, hippocampus and amygdala. Elevated defensive behaviours including escape and avoidance have been demonstrated in Htr1a KO mice and are also important characteristics of patients with PD/AG. Healthy subjects show shortened reaction times during the anticipation of defensive stimuli.

### Table 1. Demographic and clinical characteristics of the fMRI and BAT samples according to rs6295 (−1019C/G HTR1A) genotype

#### Genetic-BAT-sample (N = 245)

| Genotype | CC | CG | GG | Differences (CC vs GG) |
|----------|----|----|----|------------------------|
| N        | 60 | 120| 65 | $\chi^2$/F | P |
| Female (n(%)) | 42 (70.21) | 82 (68.33) | 54 (83.08) | 2.99* | 0.08 |
| Age (years) | 36.38 (10.88) | 35.58 (11.50) | 35.46 (10.23) | 0.24 | 0.63 |
| Clinical characteristics at baseline |
| SIGH-A total | 24.53 (5.04) | 23.71 (5.23) | 24.46 (5.55) | 0.01 | 0.94 |
| PAS total | 26.96 (9.64) | 26.14 (9.96) | 28.23 (9.33) | 0.57 | 0.45 |
| CGI | 5.17 (0.74) | 5.18 (0.72) | 5.29 (0.61) | 1.09 | 0.30 |
| ASI total | 31.15 (9.96) | 30.51 (11.60) | 32.28 (12.61) | 0.31 | 0.58 |
| BDI II total | 16.19 (8.82) | 16.56 (8.24) | 15.87 (9.02) | 0.04 | 0.84 |
| MI7 | 2.07 (0.92) | 1.93 (0.99) | 1.92 (0.99) | 0.69 | 0.41 |

#### Genetic-BAT-treatment-sample (N = 171)

| Genotype | CC | CG | GG | Differences (CC/CG vs GG) |
|----------|----|----|----|--------------------------|
| N        | 43 | 85 | 43 | $\chi^2$/F | P |
| Female (n(%)) | 29 (67.44) | 57 (67.06) | 34 (79.07) | 2.17* | 0.34 |
| Age (years) | 36.28 (11.31) | 36.60 (12.09) | 33.28 (9.40) | 1.31 | 0.27 |
| Clinical characteristics at baseline |
| SIGH-A total | 13.65 (7.89) | 11.78 (7.57) | 12.60 (6.90) | 0.89 | 0.41 |
| PAS total | 13.68 (8.93) | 14.21 (9.52) | 14.18 (8.14) | 0.05 | 0.95 |
| CGI | 3.42 (0.85) | 3.46 (1.11) | 3.40 (1.00) | 0.06 | 0.94 |
| ASI total | 17.23 (10.36) | 15.86 (10.42) | 16.47 (10.04) | 0.26 | 0.77 |
| BDI II total | 8.49 (8.36) | 8.62 (7.99) | 8.44 (8.26) | 0.01 | 0.99 |
| MI7 | 1.97 (0.89) | 1.83 (0.88) | 1.87 (0.89) | 0.35 | 0.70 |

#### Clinical characteristics at post-treatment |
| SIGH-A total | 9.78 (3.99) | 12.38 (6.66) | 13.44 (9.38) | 1.16 | 0.29 |
| PAS total | 14.00 (7.67) | 16.05 (7.40) | 18.67 (11.51) | 1.03 | 0.33 |
| CGI | 1.53 (0.68) | 1.29 (0.49) | 1.47 (0.68) | 2.43 | 0.09 |

#### Genetic-fMRI-treatment-sample (N = 39)

| Genotype | CC | CG | GG | Differences (CC vs GG) |
|----------|----|----|----|------------------------|
| N        | 9  | 21 | 9  | $\chi^2$/F | P |
| Female (n(%)) | 7 (77.78) | 14 (66.67) | 5 (55.56) | 1.00* | 0.62 |
| Age (years) | 30.11 (11.47) | 37.67 (10.01) | 36.11 (7.57) | 1.76 | 0.20 |
| Clinical characteristics at baseline |
| SIGH-A total | 22.89 (4.68) | 23.62 (5.34) | 26.22 (5.93) | 1.75 | 0.20 |
| PAS total | 22.99 (6.72) | 24.19 (9.33) | 31.90 (7.80) | 6.74 | 0.02 |
| CGI | 5.00 (0.71) | 5.38 (0.59) | 5.67 (0.62) | 5.33 | 0.04 |
| ASI total | 30.44 (6.50) | 29.14 (9.08) | 36.00 (11.41) | 1.61 | 0.22 |
| BDI II total | 14.00 (7.63) | 16.05 (7.40) | 18.67 (11.51) | 1.03 | 0.33 |
| MI7 | 1.84 (0.71) | 1.84 (0.93) | 1.65 (1.00) | 0.22 | 0.65 |

#### Clinical characteristics at post-treatment |
| SIGH-A total | 9.78 (3.99) | 12.38 (6.66) | 13.44 (9.38) | 1.16 | 0.29 |
| PAS total | 9.15 (5.28) | 13.57 (8.96) | 18.54 (8.79) | 7.54 | 0.01 |
| CGI | 3.02 (0.97) | 3.62 (1.24) | 3.78 (0.67) | 2.00 | 0.18 |
| ASI total | 13.00 (7.81) | 15.05 (7.41) | 19.11 (11.94) | 1.65 | 0.22 |
| BDI II total | 4.78 (6.40) | 9.67 (6.16) | 9.89 (11.17) | 1.42 | 0.25 |
| MI7 | 1.45 (0.71) | 1.24 (0.38) | 1.20 (0.41) | 0.82 | 0.38 |

Abbreviations: ASI, Anxiety Sensitivity Index; BDI II, Beck Depression Inventory II; CGI, Clinical Global Impressions Scale; PAS, Panic and Agoraphobia Scale; MI7, 7-day version of the Movement Inventory (accompanied); SIGH-A, Hamilton Anxiety Scale. *Pearson’s Chi-square. Means (s.d.) except where noted. Due to missing values, MI7 scores were available in the BAT total sample only in 229 patients (CC: 57, CG: 112, GG: 60) and in the BAT treatment group sample only in 160 patients (CC: 41, CG: 80, GG: 39).
of threat stimuli if carrying the rs6295 GG genotype, probably as a result of sensitized neural circuits predisposing to enhanced processing of fear stimuli. Furthermore, in healthy subjects, a reduced amygdala activity has been observed in HG homoygotes during face processing (face > shapes), which could reflect an inhibition process. However, in PD/AG patients we recently observed distinct defensive behaviours depending upon threat imminence. During a standardized behavioural avoidance test (BAT), acute panic and associated escape behaviour was accompanied by intense autonomic mobilization, previously associated with imminent threat processing. Variation across patients in escape behaviour during the BAT could be partly explained by a hitherto unidentified genetic predisposition regarding the serotonergic system, for example, in HTR1A. In addition to defence mechanisms, PD/AG was linked to aberrant fear conditioning, overgeneralization of fear, and dysfunction of related neural networks. Findings in anxiety disorders paralleled increased fear conditioning found in HTR1A KO mice mediated by hippocampus and amygdala. The neural network implicated in fear conditioning overlaps with brain regions that are affected by 5-HT1A-mediated serotonergic neurotransmission (specifically amygdala, PAG and ACC). However, the effect of genetic variations in HTR1A on the neural correlates of fear conditioning in PD/AG is unknown.

With regard to treatment, cognitive-behavioural therapy (CBT) has proven its efficacy for most mental disorders, and particularly PD. More recently, neurofunctional brain changes related to psychotherapy, particularly CBT, have been investigated. However, despite first evidence indicates that specific genetic polymorphisms may contribute to CBT outcome and changes on the neural and behavioural level, the effect of variation of HTR1A on changes in context of psychotherapeutic interventions are unknown. Considering, however, the converging evidence suggesting a central role of HTR1A for fear processing, it is likely that variation in HTR1A contributes to CBT effects in PD/AG.

In summary, animal studies have demonstrated that reduced Htr1a expression goes along with a bias towards threat stimuli predominantly mediated by hippocampus and amygdala. Variations in HTR1A might be of relevance to PD/AG, as increased defence reactivity and an overgeneralization of conditioned fear is an important mechanism in this disorder. rs6295 GG genotype—going along with reduced serotonergic tone in frontal cortex, amygdala and hippocampus—has been associated with PD/AG. Deviations on the functional level, that is, defence reactivity and fear conditioning and effects of exposure-based CBT, might thus be influenced by rs6295. To test this empirically, we used a multilevel strategy to link HTR1A genotype to behaviour, neurofunctional activation and its changes in the course of cognitive-behavioural therapy, respectively. We hypothesized that the rs6295 GG genotype (a) facilitates escape behaviour during the BAT, (b) goes along with increased fear responses reflected by enhanced amygdala activity towards not fully predictive conditioned stimuli (CS+) and CS − during early acquisition when initial pairings of unconditioned stimulus and CS occur and (c) reduced effects of CBT on neural correlates of fear conditioning and behavioural defence reactivity.

MATERIALS AND METHODS

Participants

All patients with PD/AG investigated in this study participated in the Mechanism of Action in CBT study (see Table 1, Supplementary Figure S1) that has been described in detail earlier. Inclusion criteria were: (a) a current primary diagnosis of PD/AG; (b) a clinical interview score > 18 on the structured interview for the Hamilton anxiety scale (SIGH-A in anxiety and depression); (c) a score > 4 on the clinical global impressions scale; (d) an age of 18–65 years; and (e) the ability and availability to regularly attend treatment sessions. Exclusion criteria were: (a) comorbid DSM-IV-TR psychotic or bipolar I disorder; (b) current alcohol dependence/current abuse or dependence on benzodiazepine and other psychoactive substances; (c) current suicidal intent; (d) borderline personality disorder; (e) concurrent ongoing psychotherapeutic or psychopharmacological treatment for PD/AG or another mental disorder; (f) antidepressant or anxiolytic pharmacotherapy; and (g) physician-verified contraindications of exposure-based CBT (that is, severe cardiovascular, renal or neurological diseases). Additional exclusion criteria were applied to fMRI subjects: cardiac pacemaker, ferromagnetic metal implants, tattoos or permanent make-up with ferromagnetic colours.

Eight treatment centres in Germany participated in the clinical multicentre trial including BAT procedure as part of the baseline diagnostics (Aachen, Berlin-Adlershof, Berlin-Charniere, Bremen, Dresden, Greifswald, Münster, Würzburg). In the study, exposure-based CBT was administered in 12 twice-weekly sessions based on a highly standardized and controlled treatment protocol. The treatment procedure was shown to be highly effective.

In total, n = 369 patients were enrolled in the clinical study. Here, we refer to four different subgroups of this clinical sample to investigate genotype effects on (1) exposure behaviour, (2) on BAT before and (3) after CBT, as well as (4) on the neural correlates of fear conditioning (see Supplementary Figure 1 and ref. 40 for further details).

Exposure sample. For the investigation of genotype effects on exposure behaviour, data of 184 patients were available (CC = 45; CG = 91; GG = 48).

BAT t1 sample. In total, 364 patients performed the BAT. From 306 patients, who entered the BAT box and were not re-randomized from the waiting list group, blood samples were available in 245 patients (CC = 60; CG = 120; GG = 65).

BAT t2 sample. Of the 245 patients from the BAT t1 sample, 171 were randomized to one of two active treatment conditions and also repeated BAT during post-assessment (CC = 43; CG = 85; GG = 43).

fMRI sample. In total, 89 patients took part in the neuroimaging study, because only four (Aachen, Berlin, Dresden and Münster) of the eight treatment centres had fMRI technique assessable. Quality-controlled fMRI data were available before and after CBT from 42 patients. Blood samples for genotyping were obtained from 39 of these 42 patients (CC = 9; CG = 21; GG = 9).

Clinical and demographic data of the BAT and fMRI subcohorts are comparable to the scores of the whole sample (n = 369) of the clinical trial (compare Table 1 with Gister et al. and Straube et al.).

Genotyping of rs6295 (HTR1A −1019C/G)

Genomic DNA was extracted from blood by using a standard de-salting procedure. A 163-bp fragment was amplified by polymerase chain reaction (PCR). The PCR reaction mix included 25 ng of genomic DNA in 2.1 μl Gold Star buffer, 25 mM MgCl2, 2.5 μM of each nucleotide, 10 μM of each forward and reverse primer and 0.5 μl of Taq polymerase. Primer sequences were 5-GGAAGAAGGACCGGTCTCAT-3 and 5-GCCGACTGTTAAGTATAACG-3. After an initial denaturation step for 5 min at 95 °C, 38 cycles of denaturing at 95 °C for 30 s, annealing at 59.5 °C for 40 s and extension at 72 °C for 50 s were performed, followed by a final extension step at 72 °C for 5 min. PCR products were digested with BseGI and visualized on a 5% agarose gel containing ethidium bromide.

The rs6295 genotype groups did not significantly differ in age, gender and clinical characteristics between the different subsamples (see Table 1). Genotypes in the total cohort, the BAT and fMRI subcohort did not deviate from Hardy-Weinberg equilibrium (P > 0.2).

Treatment intervention

For detailed information of the clinical and treatment aspects of the study, please see Gister et al. and Straube et al. Sessions 1–3 consisted of psychoeducation and an individualized behavioural analysis of the patient’s symptoms and coping behaviours. Sessions 4–5 provided the treatment rational for exposure and implemented interoceptive exposure exercises in the therapy room identically for both groups. Sessions 6–8 consisted of standardized in situ exposure exercises (bus, shopping mall and forest), which were implemented after the patient agreed to enter the situation without engaging in safety behaviours and waiting for the anxiety to take its natural course. Session 9 reviewed progress to date and
addressed anticipatory anxiety. Sessions 10–11 again consisted of in situ exposures but now targeted the patients’ two most significant feared situations. Session 12 repeated crucial elements of the manual and instructed patients to continue exposing themselves to feared situations. Since effects of genotype were expected specifically on exposure behaviour, data of the exposure sessions (Sessions 6–8 and 10–11), where patients where specifically motivated to do exposure homework, had been collapsed for respective analyses (see below; and Glöster et al.\textsuperscript{25} for an identical approach).

Behavioural avoidance test (BAT)

BAT procedure is described in detail elsewhere.\textsuperscript{17} Briefly, patients were instructed first to sit in front of an open test chamber (75 x 120 x 190 cm) while defensive reactivity during anticipation of the upcoming exposure was measured (for 10 min). Afterwards, patients were asked to sit in the dark and locked chamber as long as possible (maximum 10 min). Stopping exposure in the test chamber was always possible. Defensive reactivity was measured by self-reports of anxiety on a visual analogue scale, and by observable behaviour (premature escaping behaviour during exposure). Defensive reactivity during anticipation and exposure was analysed as a function of rs6295 \textit{HTR1A} genotype.

fMRI

Parallel versions of a previously validated differential conditioning paradigm were applied during fMRI data acquisition (Figure 1, details in Reinhardt et al.\textsuperscript{26}) before and after CBT (see Kircher et al.\textsuperscript{24} for methodological details). The fMRI brain images were acquired using a 3T Philips Achieva (Muenster and Aachen, Germany), a 3T Siemens Trio (Dresden, Germany) and a 3T General Electric Healthcare (Berlin, Germany) scanner (for acquisition parameters see Kircher et al.\textsuperscript{24}). MR images were analysed using standard procedures of the software Statistical Parametric Mapping (SPM5; www.fil.ion.ucl.ac.uk) implemented in MATLAB 7.1 (the Mathworks, Sherborn, MA, USA).

At the single-subject level, the realignment parameters of each patient were included as regressors into the model to account for movement artefacts. The BOLD response for each event type (\textit{CS+} paired, \textit{CS+} unpaired, \textit{CS−}, unconditioned stimulus) and each phase (familiarization phase (F); early (F1) and late (F2); acquisition phase (A): early (A1) and late (A2); extinction phase: early (E1) and late (E2)) was modelled by the canonical haemodynamic response function used by SPM5 within the framework of the general linear model to analyse brain activation differences related to the onset of the different stimuli.\textsuperscript{27} Parameter estimates (B−) and t-statistic images were calculated for each subject.

Group analyses were performed by entering contrast images into flexible factorial analyses as implemented in SPM5, in which subjects are treated as random variables. The fMRI centre was introduced as a covariate to account for scanner differences. To investigate the influence of rs6295 on neural activity, we compared the genetic subgroups during the processing of \textit{CS+} unpaired and \textit{CS−} in the early acquisition phase of the fear-conditioning paradigm (where the most pronounced effects and the neural plasticity induced by CBT in PD/AG were detected, see Kircher et al.\textsuperscript{24}). Analyses were performed by contrasting the extreme groups of the three genetic subgroups GG (n = 9), CG (21) and CC (n = 9). Due to the small sample size, these analyses should be considered as preliminary. To explore general effect of genotype on the neural processing of not fully conditioned stimuli in the early acquisition phase, the genotype main effect (GG vs CC) independent of time point (t1/t2) and stimulus type (CS+ vs CS−) had been calculated. To test for genotype-specific effects on CBT-related changes, interaction analyses had been performed (GG/CC × t1/t2 × CS+ vs CS−).

The identical cluster threshold of at least 142 voxels at SPM significance level of \( P < 0.005 \) uncorrected (based on a Monte Carlo simulation for correction of multiple testing\textsuperscript{45}), as in previous investigations of this multicentre trial has been applied.\textsuperscript{27,40} For the anatomical localization, functional data were referenced to probabilistic cytoarchitectonic maps\textsuperscript{46} and the AAL toolbox.\textsuperscript{49}

RESULTS

Clinical characteristics

There was no significant effect of genotype on baseline characteristics (t1) and post-treatment characteristics (t2) in the BAT and fMRI samples (see Table 1).

Despite absence of effects on primary clinical outcome variables, we found variation in \textit{HTR1A} (GG vs CC) to be related to differences in exposure behaviour during CBT (interaction effect of \( HTR1A \times \text{CBT session} \): F(1,91) = 3.976, \( P < 0.05 \)), indicating that CC in contrast to GG homozygotes performed more exposure on their own during later exposure sessions of therapy; specifically during the exposure sessions 10 and 11 (CC > GG, t\textsubscript{91} = 2.025, \( P < 0.05 \); linear effect CC > GG > GG: F(1,181) = 4.203; \( P < 0.05 \), see Figure 1). Importantly variation in \textit{HTR1A} is not related (\( P > 0.2 \)) to treatment variants (therapist vs self-guided exposure), which has been previously shown to be related to exposure behaviour\textsuperscript{25} and the neural correlates of conditioning.\textsuperscript{20}

Behavioural avoidance test

\textit{Effect of HTR1A.} Risk genotype was significantly associated with acute flight behaviour before therapy (t1): GG genotype carriers escaped more often during the exposure to the test chamber as compared with CC carriers (\( χ^2 = 5.12, P < 0.05 \); see Figure 2a). Univariate analysis of variance with genotype (GG carriers vs CC carriers) and behaviour (escapers vs non-escapers) as between-subjects variables revealed significant interaction effects between genotype and behaviour on reported anxiety during anticipation period (F(1,121) = 5.42, \( P < 0.05 \)) and exposure period (F(1,121) = 6.40, \( P < 0.05 \)). Post hoc analysis displayed that CC carriers who showed escaping behaviour during the exposure already reported significantly more anticipatory anxiety as compared with non-escaping patients at the anticipation period (behaviour F(1,58) = 8.57, \( P < 0.01 \)) while anticipatory anxiety between escaping and non-escaping G allele homozygotes was comparable (behaviour F(1,63) = 0.11, \( P = 0.75 \); see Figure 2b) suggesting that pronounced self-reported anticipatory anxiety preceded escape behaviour only if carrying the CC gene variant. During exposure, reported anxiety was significantly increased in escaping patients as compared with non-escaping patients in both, C allele (behaviour F(1,58) = 31.03, \( P < 0.001 \)) and G allele homozygotes (behaviour F(1,63) = 12.88, \( P < 0.01 \)). However, escaping CC carriers reported significantly higher anxiety than G allele homozygous escapers (genotype F(1,28) = 6.96, \( P < 0.05 \)) while no significant difference between genotypes was observed
reported anxiety depending on genotype in escaping patients. \(P(1,167) = 10.14\), anticipation period \((F(1,167) = 6.34\), P = 0.01\), post hoc completers: genotype \(F(1,136) = 0.17\), P = 0.68; exposure: genotype \(\times\) behaviour \(F(1,167) = 8.92\), P < 0.01, post hoc escapers: genotype \(F(1,31) = 12.72\), P = 0.001, post hoc completers: genotype \(F(1,136) = 0.97\), P = 0.33) were no longer observable during t2 (anticipation period: genotype \(F(1,167) = 0.13\), P = 0.72; genotype \(\times\) pretreatment behaviour \(F(1,167) = 0.10\), P = 0.75; exposure period: genotype \(F(1,167) = 0.34\), P = 0.56; genotype \(\times\) pretreatment behaviour \(F(1,167) = 0.01\), P = 0.93). In line with the results above, no significant differences between genotype in the frequency of escape behaviour were observed during t2 (CC/CG: N = 5, 3.9%; GG: N = 5, 11.6%; exact Fisher’s P = 0.12).

FMRI results

**Effect of HTR1A.** The main effect of genotype (GG vs CC) for the processing of \(CS^+\text{unpaired}\) and \(CS^-\) during early acquisition phase of the conditioning paradigm baseline (t1) and post-assessment (t2) revealed activity in the bilateral amygdalae, hippocampi as well as distributed regions including predominantly parietal, temporal and cerebellar structures (see Figure 4a; Table 2). Risk genotype carriers (GG; N = 9) in contrast to CC genotype carriers generally demonstrated higher activity in these regions independent of time point or stimulus type. Bar graphs in Figure 4a illustrate the contrast estimates for the activity in the left amygdala. Contrast estimates for all other activation clusters demonstrate a similar pattern of increased activity in GG carriers independent of measurement point.

**Effect of CBT.** The interaction of genotype (GG vs CC), processing of \(CS^+\text{unpaired}\) vs \(CS^-\) during early acquisition phase and baseline (t1) vs post-assessment effects (t2) revealed activation in the bilateral insulae, the middle cingulate cortex and distributed regions of the parietal and occipital lobe (see Figure 4b, Table 2). Bar graphs in Figure 4b illustrate the contrast estimates for the activity in the left insula. Contrast estimates for all other activation clusters show similar patterns. Risk genotype carriers demonstrated relatively stable activity in the illustrated regions independent of time point or stimulus type. By contrast, patients with the protective genotype (CC; N = 9) showed a reduced activation for the \(CS^+\text{unpaired}\) after treatment and an opposite effect for the \(CS^-\).

Exploratory correlation analyses were performed to reveal the association of BAT anxiety ratings, genotype and fMRI activity. While amygdala activity was correlated with numbers of G alleles (left amygdala: \(r = 0.450\), P = 0.004 uncorrected, P = 0.036 corrected for multiple comparisons; right amygdala: \(r = 0.513\), P = 0.001 uncorrected, P = 0.008 corrected for multiple comparisons), no association between amygdala activity and anxiety ratings from anticipation and exposure phase of the BAT task could be observed (for all \(P > 0.20\)). For differential conditioning (\(CS^+\text{unpaired} > CS^-\)), the right insula correlated negatively with anxiety ratings during the anticipation of BAT exposure before treatment \((r = -0.344\), P = 0.032 corrected, P = 0.324 corrected for multiple comparisons). Activation change \((t2−t1)\) for the differential conditioning (\(CS^+\text{unpaired} > CS^-\)) in the right insula was positively correlated with the number of G alleles \((r = 0.404\), P = 0.011 uncorrected, P = 0.099 corrected for multiple comparisons) and negatively correlated with changes in the anxiety reports during BAT exposure after CBT \((t2−t1); r = 0.339, P = 0.035\) uncorrected, P = 0.315 corrected for multiple comparisons).

**DISCUSSION**

The rationale of this study was built upon conclusive evidence from animal research suggesting that lack of Htri1a in hippocampus and amygdala neurons leads to increased fear response to...
defensive response during the BAT as well as neurofunctional changes with regard to differential conditioning activity after 12 sessions of CBT.\textsuperscript{27,35} Despite these differences, both groups demonstrated clinical improvement. However, these might be obtained by different components of CBT as indicated by increased exposure behaviour in CC genotype carriers. Synthesizing this data, we argue that \textit{HTR1A} genotype contributes to predisposing a patient to preferentially utilize different neural pathways of fear (supported by escape behaviour and amygdala activity in GG carriers and subjective anxiety and CBT effects on fear conditioning in C allele carriers). Our data suggest that there are neurogenetic subgroups of PD/AG patients and, depending on genotype, CBT may act upon different pathways of fear. These findings might be useful in the future for informing clinical decisions regarding CBT treatment.

In line with the hypothesis that the GG genotype of \textit{HTR1A} should facilitate flight behaviour, GG homozygotes more often escaped from a small, dark and closed test chamber during a standardized BAT. Extensive animal research suggests that defensive reactivity is dynamically organized as a function of threat proximity\textsuperscript{51,52} resulting in different patterns of defensive reactions, for example, increased autonomic arousal, and related brain circuit activation. In the case of imminent threat, the dorsal periaqueductal grey was shown to mediate the expression of defensive behaviour\textsuperscript{53–55} and is also relevant for fear conditioning in PD/AG\textsuperscript{9}. Electric or chemical stimulation of the PAG in animals induces strong increases in autonomic arousal and flight/flight behaviour, which are the dominant characteristics of defensive responses during acute threat in general, but also during acute panic states and escape behaviour in PD/AG patients.\textsuperscript{17} As 5-HT inhibits PAG-mediated panic and escape behaviour\textsuperscript{56}, decreased serotonergic neurotransmission as a consequence of the \textit{HTR1A} GG genotype might well contribute to increased escape behaviour during the standardized BAT. Interestingly, escape behaviour was preceded by increased anticipatory anxiety in CC but not GG genotype carriers. Moreover, reported anxiety immediately before escape was more pronounced in CC carriers as compared with GG carriers. Although it remains speculative, our results suggest that acute escape in C allele homozygotes might be driven by the motivation to reduce anxious apprehension. In contrast, escape behaviour in GG carriers might be less depending on previous subjective distress. Future research has to clarify whether G allele-associated flight behaviour in humans is indeed associated to a more sensitive PAG as supposed by animal models and how functionality of that brain structure might be affected by anticipatory anxiety.

In line with the BAT data and the assumption that the presence of G alleles goes along with increased fear reactions towards not fully predictive conditioned stimuli, our preliminary neuroimaging data suggest that \textit{HTR1A} GG homozygotes show increased activation of the bilateral amygdalae upon presentation of conditioned stimuli (CS+unpaired and CS−) as an indicator of potential threat (unconditioned stimuli) detection. Evidence for increased activation of the amygdala can also be found in response to viewing emotional stimuli (faces) in patients with panic disorder carrying the rs6295 GG genotype,\textsuperscript{13} whereas in healthy subjects, even reduced amygdala activity has been reported for the processing of faces.\textsuperscript{19} Intriguingly, increased amygdala activation in GG homozygotes in our small fMRI sample was highly stable and remained constant even after successful CBT.

Previously, we have shown that PD/AG patients exhibit altered top-down (prefrontal regions) and bottom-up processing (midbrain regions) of conditioned stimuli compared with healthy individuals.\textsuperscript{9} Further, we also demonstrated that CBT predomi-}

ambiguous stimuli.\textsuperscript{5,6} Thus, genetic variation in human \textit{HTR1A} should also be of relevance for the pathophysiology of PD/AG, as generalization of fear to ambiguous or even safety signals is an important aetiological mechanism for the disorder.\textsuperscript{7,9} In translating evidence from rodent models to humans with PD/AG, we found that \textit{HTR1A} rs6295 risk genotype (GG) carriers display increased threat-related defensive reactivity (escape behaviour) during BAT and increased amygdala activity—measured with fMRI—for both threat as well as safety cues during fear conditioning. Both behavioural styles can be interpreted as increased fear-related flight behaviour in response to ambiguous cues, just as observed in \textit{Htr1a} knockout mice. In contrast, we found the CC allele carriers to be associated with pronounced decreases of
preliminary evidence for the effects of HTR1A on the neural correlates of fear conditioning and related changes in the context of CBT (in the CC group only). Amygdala activity upon CS+ unpaired and CS− presentation in the GG group suggest a dysfunctional differential conditioning or general increased reactivity reflected in a hyper-reactivity to both fear and safety cues in these PD/AG risk genotype carriers, paralleling the reaction towards ambiguous cues in Htr1a KO mice. Although this effect was not affected by CBT, HTR1A CC homozygotes demonstrated effects of CBT on the differential processing of CS+ unpaired and CS− in the early acquisition phase, as indicated by a significant interaction of genotype group (GG vs CC), treatment (t1 vs t2) and stimulus (CS+ unpaired vs CS−). After CBT, only the HTR1A CC group demonstrated reduced activation in response to the CS+ unpaired in a network including the bilateral insulae, the anterior/middle cingulate cortex and more distributed regions of the parietal and occipital lobe. Especially the involvement of the bilateral insulae might indicate successful differential conditioning and a reduced interoceptive attention after CBT in this genotype patient group.

Our findings can only provide a starting point for further investigations on the role of HTR1A in PD/AG and its treatment and should be interpreted in light of some limitations. Especially, the results of the fMRI analyses have to be interpreted with caution because of the small sizes of the genotype subgroups. Due to the small sample size, we cannot exclude that our results either represent false positive effects or that important differences...
might have been missed due to false negative findings. Especially, activation of the parietal lobe has to be interpreted with caution since activation change in this region could also be observed in activation of the parietal lobe has to be interpreted with caution.

On the other hand, our data benefitted from coming from a large and controlled trial and from converging lines of evidence that strengthen our findings. For example, here we had the opportunity to perform correlations between anxiety ratings during BAT and fMRI activity. Such exploratory analyses indicate, for example, that activity predominantly in the right insular cortex is associated with the subjective experience and evaluation of anxiety in context of the BAT, whereas amygdala activity was unrelated to subjective anxiety ratings. Another issue to be kept in mind is that variation in HTR1A, which causes rather subtle molecular changes, is not identical to a corresponding knockout in animals. Therefore, it is even more remarkable that we still observe paralleling defensive behaviour and fear conditioning to ambiguous conditioned stimuli in humans and animals on neural and behavioural level.

Taken together, we demonstrated the effect of HTR1A on mechanisms of fear, reflected in increased threat-related defensive reactivity and dysfunctional differential conditioning processes indicated by amygdala activity for both threat and safety cues in GG homozygotes. On the other hand, in CC genotype carriers, we found increased subjective anxiety as a precursor of escape behaviour during BAT. Furthermore, only the latter group demonstrated neurofunctional changes with regard to differential conditioning activity due to CBT. Our results, therefore, translate evidence from animal studies to humans and suggest a central role for HTR1A in differentiating subgroups of patients with anxiety disorders. Because therapy was effective for all patients investigated with fMRI and BAT (see Table 1), our data could be explained by the fact that distinct components of CBT influence the processing of fear in different ways, as manualized CBT embraced several interventions (such as cognitive strategies, exposure therapy and so on,) with the overall goal of helping as many patients as possible. Longer exposure times in CC homozygote carriers suggest that exposure is the important component of CBT, which might be responsible for the neurofunctional changes within this patient subgroup. If future

### Table 2. fMRI results (coordinates and statistics)

| Contrast/region                  | Cluster extensions/submaxima | x   | y   | z   | t-value | P uncorrected | Cluster size |
|----------------------------------|------------------------------|-----|-----|-----|---------|---------------|--------------|
| **Main effect: GG > CC**         |                              |     |     |     |         |               |              |
| Right Amy/HC                     | Amy (SF, 69.7%; CM, 80.3%), HC (CA, 8.5%) | 18  | −6  | −16 | 4.05    | < 0.001       | 837          |
|                                  | Right putamen                | 30  | −8  | −6  | 3.54    | < 0.001       |              |
|                                  | Right insula                 | 32  | −18 | 20  | 4.01    | < 0.001       | 965          |
| Left SPL                         | Left postcentral gyrus       | −18 | −40 | 72  | 3.69    | < 0.001       |              |
| Right postcentral gyrus          | Right postcentral gyrus      | 32  | −38 | 52  | 3.80    | < 0.001       | 802          |
|                                  | Right precentral gyrus       | 28  | −26 | 68  | 3.53    | < 0.001       |              |
| Right calcarine gyrus            | Right precuneus              | 16  | −58 | 12  | 3.44    | < 0.001       | 695          |
|                                  | Right precuneus              | 18  | −54 | 16  | 3.26    | < 0.001       |              |
| Right thalamus                   | 8                            | −14 | 24  | 3.80 | < 0.001 | 460          |
| Right SPL                        | 24                            | −66 | 52  | 3.20 | < 0.001 | 303          |
|                                  | Right cuneus                 | 18  | −76 | 38  | 3.17    | < 0.001       |              |
| Left HC/Amy                      | Amy (SF, 31.5%), HC (CA, 7.4%; FD, 13.3%) | −14 | −12 | −14 | 3.81    | < 0.001       | 279          |
|                                  | Left HC                      | −28 | −20 | 12  | 3.30    | < 0.001       |              |
| Left SMA                         | BA 6                         | −6  | 10  | 70  | 3.56    | < 0.001       | 223          |
|                                  | Right SMA                    | 2   | 0   | 66  | 2.94    | 0.002         |              |
| Thalamus                         | −20                          | −14 | 8   | 3.17 | 0.001  | 202          |
|                                  | Left insula                  | −34 | −20 | 4   | 3.06    | 0.001         |              |
|                                  | Left cerebellum              | −12 | −68 | −16 | 3.74    | < 0.001       | 143          |
| **Interaction: genotype (CC > GG) x time ((t > 12) x stimulus (CS+ = CS− = CS−))** |                              |     |     |     |         |               |              |
| Left precentral gyrus            | −38                          | −12 | 58  | 4.37 | < 0.001 | 4471         |
|                                  | Right SMA                    | 8   | 6   | 60  | 4.36    | < 0.001       |              |
|                                  | Left precentral gyrus        | −28 | −18 | 72  | 4.18    | < 0.001       |              |
| Right middle occipital gyrus     | 30                            | −74 | 30  | 4.34 | < 0.001 | 2360         |
|                                  | Right postcentral gyrus      | 34  | −32 | 68  | 3.94    | < 0.001       |              |
|                                  | Right precentral gyrus       | 30  | −28 | 74  | 3.94    | < 0.001       |              |
| Right temporal pole              | 54                            | 18  | −16 | 3.69 | < 0.001 | 455          |
|                                  | Right temporal pole          | 60  | 14  | −4  | 3.17    | 0.001         |              |
|                                  | Right insula                 | 46  | 18  | −4  | 2.99    | 0.002         |              |
| Left insula                      | −46                          | 8   | −4  | 3.47 | < 0.001 | 330          |
|                                  | Left temporal pole           | −54 | 10  | −10 | 3.22    | 0.001         |              |
|                                  | Left IFG (pars opercularis)  | −40 | 8   | 8   | 2.93    | 0.002         |              |
| Right MFG                        | 48                            | 48  | 6   | 3.71 | < 0.001 | 149          |
|                                  | Right MFG                    | 40  | 56  | 8   | 3.12    | 0.001         |              |
| Left ACC                         | −10                          | 34  | 26  | 3.18 | 0.001  | 144          |
|                                  | Left superior medial gyrus   | −2  | 32  | 34  | 2.98    | 0.002         |              |
|                                  | Left ACC                     | −6  | 42  | 18  | 2.86    | 0.002         |              |
| Left STG                         | −52                          | −18 | 10  | 3.10 | 0.001  | 142          |

Abbreviations: ACC, anterior cingulate gyrus; Amy, amygdala; CA, cornu ammonis; CM, centromedial group; FD, fascicular dentata; HC, hippocampus; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SF, superficial group; SPL, superior parietal lobe; STG, superior temporal gyrus. Significance level, t-values, uncorrected P-value and the size of the respective cluster (voxels) at $P < 0.05$, corrected (MC), were mentioned. Coordinates are listed in MNI atlas space. Contrasts are named in italic letters. Cluster extensions demarcate activated regions for larger voxel clusters encompassing different brain areas and should be considered approximate. Anatomical regions have been defined by the anatomy toolbox of statistical parametric mapping.46,59
studies are able to identify further components of CBT, a more effective and personalized therapy for the individual patient might ultimately be possible.

CONFLICT OF INTEREST
VA is a member of the advisory boards and/or gave presentations for the following companies: AstraZeneca, Janssen-Organon, Lilly, Lundbeck, Servier, Pfizer and Wyeth. He also received research grants from AstraZeneca, Lundbeck and Servier. He chaired the committee for the Wyeth Research Award Depression and Anxiety. JD received in the past 3 years honoraria by Janssen, Bristol-Myers Squibb, Wyeth, Lundbeck, AstraZeneca and Pfizer and Grant Support by Medice, Lundbeck and AstraZeneca. TK received fees for educational programs from Janssen-Cilag, Eli Lilly, Servier, Lundbeck, Bristol-Myers Squibb, Pfizer and AstraZeneca; travel support/sponsorship for congresses from Servier; speaker’s honoraria from Janssen-Cilag; and research grants from Pfizer and Lundbeck. CRK received fees for educational programs from Esparrma GmbH/Aristo Pharma GmbH, Lilly Deutschland GmbH, Servier Deutschland GmbH and MagVenture GmbH. AR has received research support from PsyNova, and AR and KD have received research grants from AstraZeneca. KD has received honoraria for scientific talks from Pfizer, Lilly and Bristol-Myers Squibb and has been a consultant for Johnson & Johnson. AS received research funding from Lundbeck, and speaker honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Lundbeck, Wyeth and UCB. Educational grants were given by the Stiftverband für die Deutsche Wissenschaft, the Berlin Brandenburgische Akademie der Wissenschaften, the Boehringer Ingelheim Fonds and the Eli Lilly International Foundation. H-UW has served as a general consultant (non-product related) for Pfizer, Organon, Servier and Essex Pharma and has received grant funding for his institution from Sanofi Aventis, Pfizer, Lundbeck, Novartis, Essex Pharma, Servier and Wyeth. The remaining authors declare no conflict of interest.

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REFERENCES
1. Parks CL, Robinson PS, Bilicke E, Shenk T, Toth M. Increased anxiety of mice lacking the serotonin1A receptor. Proc Natl Acad Sci USA 1998; 95: 10734–10739.
2. Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelson M et al. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA 1998; 95: 1446–1447.
3. Heisler LC, Chu HM, Brennan TJ, Danao JA, Baija P, Parsons LH et al. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proc Natl Acad Sci USA 1998: 95: 15049–15054.
4. Gross C, Santarelli L, Brunner D, Zhaung X, Hen R. Altered fear circuits in 5-HT(1A) receptor KO mice. Biol Psychiatry 2000; 48: 1157–1163.
5. Klemmehagen KC, Gordon JA, David DJ, Hen R, Gross CT. Increased fear response to contextual cues in mice lacking the 5-HT1A receptor. Neuropsychopharmacology 2006; 31: 101–111.
6. Tsetsenis T, Ma XH, Lo IL, Beck SG, Gross C. Suppression of conditioning to ambiguous cues by pharmacogenetic inhibition of the dentate gyrus. Nat Neurosci 2007; 10: 896–902.
7. Lissek S, Rabin S, Heller RE, Lukenbaugh D, Geraci M, Pine DS et al. Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. Am J Psychiatry 2010; 167: 47–55.
8. Lueken U, Straube B, Konrad C, Wittchen HU, Stöehr A, Wittmann A et al. Neural substrates of trait response to cognitive-behavioral therapy in panic disorder with agoraphobia. Am J Psychiatry 2013; 170: 1345–1355.
9. Lueken U, Straube B, Reinhardt I, Maslowski NI, Wittchen HU, Stöehr A et al. Altered top-down and bottom-up processing of fear conditioning in panic disorder with agoraphobia. Psychol Med 2014; 44: 381–394.
10. Akimova E, Lanzenberger R, Kasper S. The serotonin-1A receptor in anxiety disorders. Biol Psychiatry 2009; 66: 627–635.
11. Rothe C, Gutknecht L, Freitag C, Tauber R, Mosnner R, Franke P et al. Association of a functional 1019C>G 5-S-HT1A receptor gene polymorphism with panic disorder with agoraphobia. Int J Neuropsychopharmacol 2004; 7: 189–192.
12. Le Francois B, Czesak M, Steubl D, Albert PR. Transcriptional regulation at a HTR1A gene polymorphism associated with major illness. Neuropsychopharmacology 2008; 35: 977–985.
13. Domshikie K, Braun M, Ohrmann P, Sulsow T, Kugel H, Bauer J et al. Association of the functional –1019C/G 5-HT1A polymorphism with prefrontal cortex and amygdala activation measured with 3 T fMRI in panic disorder. Int J Neuropsychopharmacol 2006; 9: 349–355.
14. Huang YY, Battistuzzi C, Quendo MA, Harkavy-Friedman J, Greerhill L, Zalsman G et al. Human 5-HT1A receptor C(–1019)G polymorphism and psychopathology. Int J Neuropsychopharmacol 2004; 7: 441–451.
15. Lemonde S, Turecki G, Bakish D, Du L, Hrinda PD, Bowden CD et al. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci 2003; 23: 8788–8799.
16. Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L et al. Serotonin1A receptor acts during development to establish normal anxiety-like behavior in the adult. Nature 2002; 416: 396–400.
17. Richter J, Hamm AO, Pané-Farré CA, Gerlach AL, Gloster AT, Wittchen HU et al. Dynamics of depressive reactivity in patients with panic disorder and agoraphobia: implications for the etiology of panic disorder. Biol Psychiatry 2012; 72: 512–520.
18. Schmitz A, Kirsch P, Reuter M, Alexander N, Kozyra E, Kuepper Y et al. The 5-HT1A C(-1019)G polymorphism, personality and electrodifferential reactivity in a reward/punishment paradigm. Int J Neuropsychopharmacol 2009; 12: 383–392.
19. Fakra E, Hyde LW, Gorka A, Fisher PM, Munoz KE, Kimak M et al. Effects of HTR1A C(−1019)G polymorphism on amygdala reactivity and trait anxiety. Arch Gen Psychiatry 2009; 66: 33–40.
20. Lang PJ, Bradley MM. Emotion and the motivational brain. Biol Psychol 2010; 84: 437–450.
21. Bouton ME, Mineka S, Barlow DH. A modern learning theory perspective on the etiology of panic disorder. Psychol Review 2001; 108: 4–32.
22. Rachman S. The conditioning theory of fear-acquisition: a critical examination. Behav Res Ther 1977; 15: 375–387.
23. Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C et al. Classical fear conditioning in the anxious disorders: a meta-analysis. Behav Res Ther 2005; 43: 1391–1424.
24. Damsa C, Kosel M, Moussally J. Current status of brain imaging in anxiety disorders. Curr Opin Psychiatry 2009; 22: 96–110.
25. de Carvalho MR, Dias GP, Cosci F, de-Melo-Neto VL, Bevilacqua MC, Gardino PF et al. Current findings of fMRI in panic disorder: contributions for the fear neuropsychiatric and CBT effects. Expert Rev Neurother 2010; 10: 291–303.
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26 Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder. revised. Am J Psychiatry 2000; 157: 493–505.

27 Kircher T, Arot V, Jansen A, Pyka M, Reinhartd I, Kellermann T et al. Effect of cognitive-behavioral therapy on neural correlates of fear conditioning in panic disorder. Biol Psychiatry 2013; 73: 93–101.

28 Buchel C, Dolan RJ, Armony JL, Friston KJ. Amygdala-hippocampal involvement in human aversive face conditioning revealed through event-related functional magnetic resonance imaging. J Neurosci 1999; 19: 10869–10876.

29 Sehmeyer C, Schoning S, Zwitserlood P, Pfieferder B, Kircher T, Arot V et al. Human fear conditioning and extinction in neuroimaging: a systematic review. PLoS One 2009; 4: e8865.

30 Reinhartd I, Jansen A, Kellermann T, Schuppen A, Kohn N, Gerlach AL et al. Neural correlates of aversive conditioning: development of a functional imaging paradigm for the investigation of anxiety disorders. Eur Arch Psychiatry Clin Neurosci 2010; 260: 443–453.

31 Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP et al. Serotonin 5-HT1A receptor binding in people with panic disorder: positron emission tomography study. Br J Psychiatry 2008; 193: 229–234.

32 Neumeister A, Bain E, Nugent AC, Carson RE, Bonne O, Luckenbaugh DA et al. Reduced serotonin type 1A receptor binding in panic disorder. J Neurosci 2004; 24: 589–591.

33 McHugh RK, Smits JA, Otto MW. Empirically supported treatments for panic dis- order. Consult Clin Psychol 2010; 78: 443–453.

34 Sanchez-Meca J, Rosa-Alcazar AI, Marin-Martinez F, Gomez-Conesa A. Psycholo- gical treatment of panic disorder with or without agoraphobia: a meta-analysis. Clin Psychol Rev 2010; 30: 37–50.

35 Gloster AT, Wittchen HU, Einsle F, Lang T, Helbig-Lang S, Fydrich T et al. Psychological treatment for panic disorder with agoraphobia: a randomized con- trolled trial to examine the role of therapist-guided exposure in situ. J Consult Clin Psychol 2011; 79: 406–420.

36 Prasko J, Horacek J, Zalesky R, Kopecek M, Novak T, Paskova B et al. The change of regional brain metabolism (18FDG PET) in panic disorder during the treatment with cognitive behavioral therapy or antidepressants. Neuro Endocrinol Lett 2004; 25: 340–348.

37 Sakai Y, Kumanoh H, Nishikawa M, Sakano Y, Kaya H, Imabayashi E et al. Changes in cerebral glucose utilization in patients with panic disorder treated with cognitive-behavioral therapy. Neuroimage 2006; 33: 218–226.

38 Kumari V, Peters ER, Fannon D, Antonova E, Premkumar P, Anilkumar AP et al. Dorsolateral prefrontal cortex activity predicts responsiveness to cognitive-behavioral therapy in schizophrenia. Biol Psychiatry 2009; 66: 594–602.

39 Fu CH, Williams SC, Cleare AJ, Scott J, Mitterschiffthaler MT, Walsh ND et al. Neural responses to sad facial expressions in major depression following cognitive behavioral therapy. Biol Psychiatry 2007; 61: 1002–1008.

40 Reif A, Richter J, Straube B, Hoffer M, Lueken U, Gloster AT et al. MAOA and mechanisms of panic disorder revisited: from bench to molecular psychotherapy. Mol Psychiatry 2014; 19: 122–128.

41 Lonsdorf TB, Kalisch R. A review on experimental and clinical genetic associations studies on fear conditioning, extinction and cognitive-behavioral treatment. Tranl Psychiatry 2011; 1: e41.

42 Lester KJ, Eley TC. Therapypigenetics: using genetic markers to predict response to psychological treatment for mood and anxiety disorders. Biol Mood Anxiety Disorder 2013; 3: 1–4.

43 Yang Y, Kircher T, Straube B. The neural correlates of cognitive behavioral therapy: Recent progress in the investigation of patients with panic disorder. Behav Res Ther 2014; 62: 88–96.

44 Lueken U, Straube B, Wittchen HU, Konrad C, Strohle A, Wittmann A et al. Therapypigenetics: anterior cingulate cortex-amygdala coupling is associated with 5-HTTLPR and treatment response in panic disorder with agoraphobia. J Neural Transm 2014.

45 Gloster AT, Wittchen HU, Einsle F, Hoffer M, Lang T, Helbig-Lang S et al. Mechanism of action in CBT (MAG): methods of a multi-center randomized controlled trial in 369 patients with panic disorder and agoraphobia. Eur Arch Psychiatry Clin Neurosci 2009; 259: 155–166.

46 Straube B, Lueken U, Jansen A, Konrad C, Gloster AT, Gerlach AL et al. Neural correlates of procedural variants in cognitive-behavioral therapy: a randomized, controlled multicenter FMRI study. Psychother Psychosom 2014; 83: 222–233.

47 Slotnick SD, Moo LR, Segal JB, Hart J. Distinct prefrontal cortex activity associ- ated with item memory and source memory for visual shapes. Brain Res Cogn Brain Res 2003; 17: 75–82.

48 Eickhoff SB, Stephan KE, Mohlberg H, Greffkes C, Fink GR, Amunts K et al. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. Neuroimage 2005; 25: 1325–1335.

49 Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 2002; 15: 273–289.

50 Straube B, Lueken U, Jansen A, Konrad C, Gloster AT, Gerlach AL et al. Neural correlates of procedural variants in cognitive behavioral therapy: a randomized, controlled multicentre fMRI study. Psychother Psychosom 2013; 82: 222–233.

51 Timberlake W. Behavior systems and reinforcement: an integrative approach. J Exp Anal Behav 1993; 60: 105–128.

52 McNaughton N, Corr PJ. A two-dimensional neuropsychology of defense: fear/ anxiety and defensive distance. Neurosci Biobehav Rev 2004; 28: 305–306.

53 Del-Ben CM, Graeff FG. Panic disorder: is the PAG involved? Eur Arch Psychiatry Clin Neurosci 2004; 254: 226.

54 Mobbs D, Marchant JL, Hassabi D, Seymour B, Tan G, Gray M et al. From threat to fear: the neural organization of defensive fear systems in humans. J Neurosci 2009; 29: 12236–12243.

55 Lueken U, Hillbort K, Stolyar V, Maslowski NI, Wittchen H-U. Neural substrates of defensive reactivity in two subtypes of specific phobia. Soc Cogn Affect Neurosci 2014; 9: 1668–1675.

56 Graeff FG, Zangrossi H Jr. The dual role of serotonin in defense and the mode of action of antidepressants on generalized anxiety and panic disorders. Cent Nerv Syst Agents Med Chem 2010; 10: 207–217.

57 Craig AD. How do you feel—now? The anterior insula and human awareness. Nat Rev Neurosci 2009; 10: 59–70.

58 Paulus MP, Stein MB. An insular view of anxiety. Biol Psychiatry 2006; 60: 383–387.

59 Amunts K, Kedo O, Kindler M, Pieperhoff P, Mohlberg H, Shah NJ et al. Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. Anat Embryol (Berl) 2005; 210: 343–352.

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