Allelic variation in *CRHR1* predisposes to panic disorder by biasing towards fear

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Abstract

Corticotropin releasing hormone (CRH) is a major regulator of the hypothalamic-pituitary-adrenal (HPA) axis. Binding to its receptor CRHR1 triggers the downstream release of cortisol, a hormone needed for regulation of stress responses. Biochemical, behavioral and genetic studies revealed *CRHR1* as a possible candidate gene for mood and anxiety disorders. Here, we aimed to evaluate *CRHR1* as a candidate molecule in panic disorder (PD). Allelic variation throughout the *CRHR1* gene was captured by 9 selected single nucleotide

polymorphisms (SNPs); these were genotyped in 531 matched case/control pairs (discovery sample (n=239); replication sample (n=292)). Four SNPs were found to be associated with PD, in at least one sub-sample. The minor alleles of rs17689918 and rs17689966 were found to significantly increase risk for PD in females of the discovery, the replication and the combined sample, both withstanding correction for multiple testing ($p_{rs17689918}=1.3*10^{-4}$; $p_{rs17689966}=0.042$). Expressional analysis demonstrated that both minor alleles of rs17689918 and rs17689966 significantly decreased *CRHR1* mRNA in the forebrain and amygdala. Bioinformatical analysis revealed a high proportion of differential neuro-relevant transcription factor binding possibly underlying expression changes. When investigating the neural correlates underlying this association, risk allele carriers of rs17689918 and rs17689966 showed aberrant differential conditioning and safety signal processing arguing for predominant generalization of fear and hence anxious apprehension. Furthermore, the minor risk (A) allele of rs17689918 led to less flight behavior during fear provoking situations, but rather increased anxious apprehension and went along with increased anxiety sensitivity. Thus, reduced CRHR1 expression driven by *CRHR1* risk allele leads to a phenotype characterized by a fear bias and hence sustained fear. These results strengthen the role of *CRHR1* in PD and clarify the mechanisms by which genetic variation in *CRHR1* is linked to this disorder.

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis with its primary effector cortisol plays a central role in the response to stress and disorders relating to it, such as affective and anxiety disorders. The neurobiological and neurofunctional mechanisms that may lead to these disorders are however elusive. The present study therefore aimed to link genetic variation in the HPA system to the clinical phenotype by utilizing a multi-level approach in which genetic variants were linked to molecular, physiological and behavioral data relating to panic disorder (PD).

Disruptions of internal homeostasis or exposure to external threat activate the HPA axis, triggered by the secretion of corticotropin releasing hormone (CRH). Binding to the CRH receptor (CRHR), it stimulates the release of corticotrophin (ACTH) in the pituitary gland and thus leads to increased levels of cortisol (CORT; [1, 2]). Two different forms of CRHR (CRHR1

and CRHR2) exist, which differ in expression pattern [3]. CRHR1 function has been examined in detail in mice where the *Crhr1* gene has been knocked out (*Crhr1* -/-). In global *Crhr1* -/- mice, stress-induced ACTH and CORT levels were markedly blunted, anxiety was decreased and exploratory as well as locomotor behavior was increased [4]. To dissect the role of Crhr1 in specific brain regions, conditional knock-out mice were generated where the gene was only deleted in brain regions expressing CaMKIIα, i.e. the forebrain and the limbic system. In contrast to global knockout mice, these animals were hypersensitive to stress [5] but had normal spatial memory and hippocampal morphology following chronic social defeat stress [6], arguing that these negative effects of stress are mediated by forebrain Crhr1. Ablation of *Crhr1* in the hippocampus prevented the down-regulation of the glucocorticoid receptor after stress and did not display by stress-induced sequelae in adulthood following early life stress [7]. These data suggest an important role of CRHR1 in short- and long-term adaptive responses to stress, probably by attenuating the impact of circulating ACTH and CORT, while CRHR1 directly exerts negative effects on learning and hippocampal morphology as a response to stress. Further adding to this, it was shown that limbic CRHR1 enhances the consolidation of fear memories [8].

There is ample evidence suggesting that the CRH/CRHR system is crucially involved in mood and anxiety disorders [2, 9-12]. Accordingly, targeted association studies were conducted to test whether *CRHR1* polymorphisms – mainly rs7209436, rs110402, and rs242924, forming a haplotype where the TAT combination conveys disease – influence the risk for stress-related disorders like post-traumatic-stress disorder (PTSD; [13, 14]) and depression [15-20]. *CRHR1* accordingly is associated with the stress-sensitive personality trait Neuroticism [21] and depression-related measures such as the Beck Depression Inventory (BDI). Most noteworthy, there is consistent evidence that genetic variation interacts with early adverse life events to increase risk for depression [15-17, 22-25]. In the context of PD, linkage and case/control studies as well as dimensional association studies provided a heterogeneous picture [26-29] regarding an involvement of *CRHR1*. Notably, the initial association of this gene with PD [29] was followed up recently, providing evidence that the *CRHR1* minor rs878886 allele leads to fear-acquisition deficits using a fear condition protocol in healthy volunteers [30]. Furthermore, a neuroimaging study revealed that the processing of emotional stimuli crucially depends on *CRHR1* genotype [31]. Thus, one can assume that *CRHR1* genotype predisposes towards PD, however conclusive evidence and the precise mechanisms are still elusive. The combination

of *CRHR1* genetics with neurofunctional and behavioral data can provide deeper insight in the etiology of PD. To address these issues, we aimed to further clarify the contribution of presumably functional *CRHR1* polymorphisms to PD. To do so, a multilevel approach was applied, by (1) genotyping nine *CRHR1* tag SNPs and determining their corresponding haplotypes in two independent, large and well controlled case-control studies and (2) assess the effect of significantly associated SNPs on mRNA expression in human post-mortem brain and, (3) examining the effect of these SNPs on brain activation correlates of fear conditioning in patients with PD, (4) testing the association of risk SNPs on psychophysiological parameters such as heart rate and symptom ratings during a behavioral avoidance test (BAT), (5) studying the effect of significant SNPs on basal scores of dimensional anxiety traits and test for possible gene × environment effects and finally (6) compute possible SNP function using various bio-informatical tools.

Materials and Methods

Samples

SNPs examined in this study were genotyped in PD patients matched by sex to an equal number of healthy controls. Details on case/control pairs of the discovery and the replication samples are specified below. Cases and controls were unrelated and of Caucasian origin. Patients with schizoaffective or other psychotic disorders, comorbid substance abuse disorders, mental retardation, neurological or neurodegenerative disorders were excluded. Only patients and volunteers who gave written informed consent were enrolled in the study, which complied with the Declaration of Helsinki and was approved by the respective local Ethic Committee. Genomic DNA of all individuals included in the case-control study was extracted from venous blood by a routine desalting method.

The discovery sample was described previously [32] and consisted of 239 PD patients recruited in Germany (female=143, male=96; mean age 37.6±11.1). The diagnosis of PD and presence of comorbid agoraphobia (PD/AG; 68.6%; n=75) was ascertained by experienced psychiatrists on the basis of medical records and structured clinical interviews (Composite International Diagnostic Interview, CIDI) according to the criteria of DSM-III-R or DSM-IV, respectively [33-35]. The sex-matched control group comprised 239 blood donors (female=143,

male=96; mean age 36.18±11.78) of German descent, who were not controlled for the absence of mental disorders due to anonymity requirements.

The replication patient sample (n=292; female=216, male=76; mean age 36.0±10.8) was obtained from the national research initive "*Panic-Net*" founded by the Federal Ministry of Education and Research [36]; details on the sample as well as the study workflow were previously published [32, 37]. The diagnosis of PD/AG (co-morbid AG was an inclusion criterion, so all patients suffered thereof) was established by a structured clinical interview (CIDI) according to DSM-IV criteria [35]. Control subjects (n=292; female=216, male=76; mean age 28.8±7.38) were matched and drawn from a larger number of screened healthy controls (n=581; female=408, male=173; mean age 25.4±6.4). Absence of mental axis 1 disorders was established by experienced psychologists on the basis of a structured clinical interview (Mini International Neuropsychiatric Interview, MINI) according to the criteria of DSM-IV [35]. For both patient and control groups, agoraphobic traits and anxiety sensitivity were evaluated by German versions of the Agoraphobic Cognitions Questionnaire (ACQ, [38]) and Anxiety Sensitivity Index (ASI, [39]).

SNPs which remained significant after Bonferroni correction were analyzed for gene × environment effects in a second independent replication sample (SHIP-PD sample) for which lifetime data on traumatic life events were available. The sample comprised 160 individuals reporting panic attacks (female=103, male=57; mean age 51.4±12.0; of those, 74 met diagnostic criteria of PD with or without agoraphobia [female=54, male=20; mean age 50.9±11.1]) and their sex-matched healthy controls (n=160; female=101, male=59; mean age 51.4±12.0). All participants were drawn from a follow-up examination of 6,267 participants of the epidemiological Study of Health in Pomerania (SHiP), reported in detail elsewhere [40, 41]. Criteria of a panic attack or confirmed PD were assessed using a standardized computer-assisted personal version of the Munich Composite International Diagnostic Interview (M-CIDI;[42]). Lifetime diagnoses were assigned according to diagnostic hierarchy rules and exclusion criteria as stated in the DSM-IV-TR. Further details on sampling, assessment procedures and quality assurance are reported in detail elsewhere [40].

For expression analysis, a sample (MRC-sample) of human post-mortem tissue was obtained from the Medical Research Council (MRC) Sudden Death Brain and Tissue Bank, Edinburgh [43]. DNA and RNA of 76 deceased individuals, aged between 16 and 74 (n=76;

female=18, male=58; mean age 48.6±12.8) were isolated from the three brain regions amygdala, forebrain and midbrain, with the MELT™ Total Nucleic Acid Isolation System (Applied Biosystem, AM Foster City, 1983) and stored at -80°C until use.

Genotyping and mRNA Quantification

CRHR1 spans 51 kb on chromosome 17q21 and is composed of 13 exons. In order to capture allelic variation in the *CRHR1* gene with a minimal genotyping effort, 9 tag SNPs were derived from HapMap CEU data [44] using the *Tagger* function as implemented in Haploview V4.2 [45] with default settings. The 9 tag SNPs represent the allelic variation of a 71.2 kb region spanning the *CRHR1* gene as well as the 5´ and 3´ flanking regions. SNP genotyping was performed using Sequenom's MassArray® system according to the instructions supplied by the manufacturer. All PCR reactions were done with the iPlex® chemistry following the MassArray® iPlex® standard operation procedure. Primer sequences can be found in Supplementary Table 1.

All polymorphisms that were found to be associated with PD were genotyped in the MRC sample, using custom Taqman SNP genotyping 5' exonuclease assays from Applied Biosystems (Darmstadt, Germany; rs17689918: Cat. # 4351379 and rs17689966: Cat. #4351379). Amplification and allelic discrimination was performed in duplicates on a Bio-Rad CFX384 real-time PCR cycler, with 10µl reaction volumes, as recommended by the manufacturer. Discrepancies or drop outs were resolved by repeated genotyping.

For quantification of *CRHR1* expression, total RNA from three different regions (forebrain, midbrain and amygdala) of human post-mortem brains was reversely transcribed by applying the iScript™ cDNA synthesis kit (Bio-Rad, München, Germany) to 1000 ng RNA of each sample. Quantification was performed in triplicates on a Bio-Rad CFX384 real-time PCR detection system, using the iQ™ SYBR green supermix (Bio-Rad) and QuantiTect primer (QT00059122) from Qiagen in a reaction volume of 10 µl. PCR conditions were 5 min at 95°C, 40 cycles of 10 s at 95°C, 30 s at 60°C, followed by a melting curve analysis with a gradient from 65°C to 95°C of 0.5°C per 5s.

Statistical Analysis: Single- and Multi-marker Associations

Prior to statistical analysis, SNPs had to pass several quality control criteria. The minimal call rate threshold in the combined sample was set to 85%; rs12938031 yielded a call rate below the threshold and was thus excluded from further analysis. Deviations from Hardy-Weinberg equilibrium (HWE) were considered to be indicative for the presence of genotyping errors; all SNPs met a HWE p-value threshold above 0.05. Also only polymorphic variants with a minor allele frequency (MAF) above 1% were examined further.

Statistical analysis of genotype data was performed with PLINK V1.07 [46] and HaploView V4.1 [45]. Single marker associations were calculated by comparison of allele and genotype counts in 1 and 2-degree-of-freedom χ^2 tests. For multi-marker association tests, haplotype blocks were defined according to the solid spine method; inferred haplotype counts in groups were compared with 1-degree-of-freedom χ^2 tests. Single-marker and haplotype analysis were adjusted for multiple testing using the conservative Bonferroni correction. In this analysis setting, we achieve a power of 36% in the discovery sample, 42% in the replication sample and 65% in the combined sample to detect SNP and haplotype associations, respectively, conveying a relative risk of 1.5 to develop PD assuming a co-dominant model and a MAF of 0.05 [47].

Statistical Analysis: Trait psychometry relevant for Panic Disorder: ASI, ACQ and Gene x Environment

Associations of minor allele dosages with dimensional anxiety traits were analyzed with linear regressions modeling the ACQ and the ASI sum scores. Gene x Environment interaction analyses were performed by testing for significant interactions between the Childhood Trauma Questionnaire (CTQ, [48]) dimensions sub-scores, the CTQ sum score, or the number of critical life events experienced five years prior to the onset of panic attacks and rs17689918 (AA/AG vs. GG) or rs17689966 (GG/GA vs. AA) in a conventional logistic regression model using the occurrence of panic attack or PD, respectively, as a categorical outcome.

Statistical Analysis: CRHR1 expression analysis in human post-mortem tissue

Normalized relative *CRHR1* expression in the respective brain section was calculated with mean efficiencies derived from LinReg [49] and the normalization factors obtained from the three most stable of six investigated housekeeping genes, glyceraldehyde-3-phosphate

dehydrogenase *(GAPDH),* TATA box binding protein *(TBP)* and succinate dehydrogenase complex, subunit A, flavoprotein *(SDHA),* generated by geNorm [50]. To examine the influence of *CRHR1* polymorphisms on gene expression, we carried out ANOVAs on normalized mean expression values in a genotypic model. Allele-specific changes of gene expression were examined with linear regressions. Throughout the manuscript, p-values<0.05 indicate significant associations.

Functional MRI

The task design and analyses has been described in detail elsewhere [51, 52]. Briefly, we employed a differential fear conditioning task that consisted of three phases (familiarisation (F); acquisition (A) and extinction (E)), each subdivided into an early and a late phase. In the acquisition phase, the US (white noise) was pseudo-randomly paired with one of the CS's (coloured spheres). MR images were analyzed using Statistical Parametric Mapping (SPM5; www.fil.ion.ucl.ac.uk) implemented in MATLAB 7.1 (Mathworks Inc., Sherborn, MA; see [51]). At first level, the BOLD response for each event type and phase was modeled by the canonical hemodynamic response function within the framework of the general linear model to analyze brain activation differences related to the onset of the different stimuli. Parameter estimates (ß-) and t-statistic images were calculated. At the second level, group analyses were performed separate for rs17689918 and rs17689966 by entering contrast images into a flexible factorial analysis, in which subjects are treated as random variables. Potential differences between males and females were considered by including the factor sex in the analyses. See supplementary table 2 for sample characteristics. An fMRI centre variable was introduced as a covariate in order to account for scanner differences between sites. *Contrasts of interest:* We first tested for effects of genotype on *differential conditioning* processes in the early acquisition phase, since in this phase strongest effects for patients in contrast to healthy control subjects have been observed [51]. In a second step we tested for genotype effects on *safety signal processing* by focussing on activation differences during the processing of the CS- in contrast to resting baseline (fixation cross). For both analyses data were first analyzed across the whole group, and then in the female group alone to check for sex specific effects. A Monte Carlo simulation was conducted to establish an appropriate voxel contiguity threshold [53]. Thus, for all analyses voxels with a significance level of $p < 0.005$ uncorrected belonging to clusters with at least 142 voxels are reported

(see also [51, 52, 54, 55]). Whole-brain analysis was carried out first. Post-hoc region-of-interest (ROI) analyses were performed for the amygdala to ensure that activity of the amygdala was not excluded due to the large cluster threshold.

Behavioral Avoidance Test (BAT)

267 patients of the replication sample were recruited to take part in the BAT. 120 patients were excluded for further analysis (27 avoided exposure to the test chamber and 93 patients reported no or minimal anxiety during the exposure period indicating that the test was not relevant for these patients) resulting in a BAT-sample of 147 patients. The BAT procedure is described in detail elsewhere [56]. Briefly, during an anticipation period patients were instructed to sit in front of an opened test chamber (120 x 120 x 190 cm) for ten minutes. Afterwards, patients were asked to sit in the dark and locked chamber as long as possible (exposure period; max. ten minutes). Stopping exposure in the test chamber was always possible. Finally, a recovery period outside the test chamber was conducted. Defensive reactivity was indexed by self-reports of anxiety on a visual analog scale (VAS; 1-10), by intensity of autonomic arousal as measured by the heart rate, and by observable fear behavior (premature escaping behavior during exposure). Defensive reactivity during anticipation and exposure was analyzed as a function of rs17689918 (AA/AG vs. GG) and rs17689966 genotype (AA vs. AG/GG; genotype available for 133 patients). See supplementary table 3 for sample characteristics. Due to missing values and the exclusion of patients taking beta-blockers (N=10), heart rate was available only in 122 patients (anticipation), 121 (exposure), and 119 (recovery), respectively.

To test the effect of SNP genotypes on behavior response type (Escapers vs. Completers), we applied $\chi 2$-Tests or, if necessary, Fisher's exact tests, respectively. To test the effect of genotype on subjective report and heart rate during anticipation, exposure and recovery periods, we separately applied model analysis of variance including genotype as between-subject factor.

Results

Single- and Multi-marker Associations

Single- and multi-marker-associations were first calculated using the discovery sample (Supplementary Table 4 and 5), next using an independent replication sample (Supplementary Table 6 and 7) to confirm the results and finally with the combined (discovery plus replication) sample to obtain maximal power (Table 1 and 2).

Of the eight single markers that passed the stringent inclusion criteria, rs7209436, rs12936181, rs17689918 and rs17689966 were significantly associated with PD. The major risk allele (C) of rs7209436 was not associated with disease in one of the individual samples, yet it became nominally significant ($p_{nominal}$=0.038) in females in the combined dataset due to the increased power. In contrast, the major allele (T) of rs12936181 conveyed genetic risk exclusively in males; this was true for the discovery and the combined (p_{nomial}=0.020; p_{nomial}=0.036), but not the replication sample. The strongest association was found for rs17689918, whose minor allele (A) was significantly enriched in cases as compared to unaffected individuals. This allelic association, first discovered in the female subset of the discovery sample ($p_{nominal}$=0.016), was confirmed in the total replication sample ($p_{corrected}$=0.028) as well as its female subset ($p_{corrected}$=0.003). In the combined discovery plus replication sample as well as its female subsample, corrected p-values of 0.005 and 0.00013, respectively, were achieved. Moreover, genotypic associations for this SNP were found in the replication ($p_{nominal}$=0.009) and in the combined sample with a corrected p-value of 0.015. Finally, the minor allele (G) of rs17689966 increased the risk for PD marginally in the female subset of the discovery sample ($p_{marginal}$=0.053), but significantly in the replication ($p_{nominal}$=0.044) and the combined ($p_{corrected}$=0.042) sample. In the genotypic test, both the female subsets ($p_{nominal}$=0.009; $p_{nominal}$=0.007) and the total replication and combined samples ($p_{nominal}$=0.027; $p_{nominal}$=0.034) yielded significant results. For the remaining four SNPs (rs7225082, rs4458044, rs3785877 and rs4792825), no significant associations were detected (Table 1, supplementary Table 4 and 6). Figure 1 provides an overview on the association findings in the various subsamples.

The examined SNPs were in considerable linkage disequilibrium (LD) allowing the definition of two haplotype blocks (see Figure 1; Table 2, supplementary Table 5 and 7). In block 1, consisting of rs7209436, rs4458044, rs12936181, rs3785877 and rs17689918 (three of which conveyed risk in the single-marker analysis), two haplotypes showed significant differences between patients and controls. The CGTGA haplotype, containing all three risk

alleles of rs7209436, rs12936181 and rs17689918, was significantly enriched in the replication ($p_{nominal}$=0.009; $p_{corrected}$=0.050) and combined ($p_{nominal}$=0.006) case samples as well as their respective female subsets and remained significant after Bonferroni correction ($p_{corrected}$=0.011; $p_{corrected}$=0.002). In contrast, the CGCGG haplotype, comprising the rs7209436 risk allele as well as the both protective alleles of rs12936181 and rs17689918, was found to have a protective effect in males in the discovery and the combined sample ($p_{nominal}$=0.017; $p_{nominal}$=0.035). However, in the second block, comprising rs17689966 and rs4792825, the GA and AA haplotypes were significantly associated with PD in the female replication ($p_{nominal}$=0.017) and the combined ($p_{corrected}$=0.008) subsamples as well as the total combined sample ($p_{nominal}$=0.010). Accordingly, the AA haplotype of block 2 including the protective rs17689966 A-allele significantly reduced the risk towards PD in females of the replication ($p_{marginal}$=0.050) and the combined subsample ($p_{nominal}$=0.007; $p_{corrected}$=0.056).

An association of rs17689918 and rs17689966 in the small PD subset of SHIP-PD (n=74) could not be detected (data not shown).

Expression Analysis of rs17689918 and rs17689966

Significant allele- and genotype-specific differences of rs17689918 and rs17689966 alleles on mRNA expression levels were found in the forebrain (rs17689918: p_{ANOVA}=0.009; $p_{Regression}$=0.002 and rs17689966: p_{ANOVA}=0.014; $p_{Regression}$=0.003) and the amygdale (rs17689918: p_{ANOVA}=0.010; $p_{Regression}$=0.003 and rs17689966: p_{ANOVA}=0.044; $p_{Regression}$=0.012), where the respective minor (risk) alleles reduce the mean expression of *CRHR1*. In midbrain, no significant effect of genotype on expression was detected (see Table 3A and B).

Functional MRI

Significant allele- and genotype-specific differences of rs17689918 and rs17689966 alleles on differential conditioning and safety learning were found. For differential conditioning ($CS+_{unpaired}$ > CS-), activation in bilateral frontal cortices was reduced in risk allele carriers (A allele for rs17689918 and G allele for rs17689966; see Figure 2; supplementary Table 8). This effect was specific for females in rs17689966. For safety signal processing, (CS- > baseline [fixation cross]) activation of the amygdala was increased in risk allele carriers of rs17689918 and rs17689966. For rs17689918, this effect was detectable only in females on a liberal significance threshold (see Figure 2; and supplementary Table 9).

Together, these data provide support for aberrant differential conditioning and safety signal processing in risk allele carriers of rs17689918 and rs17689966.

Behavioral Avoidance Test (BAT)

PD/AG patients carrying at least one risk allele of rs17689918 showed less frequent panic associated escape behavior as compared to non-risk allele homozygotes (see figure 3A). The association failed marginally to reach significance level in the entire patient sample (χ^2=3.09; p=0.08) but was significant in the female subgroup (χ^2=3.78; p=0.05). The reduced behavioral tendency to flight from the acute exposure in rs17689918 risk allele carriers was accompanied by a limited heart response during the challenge (genotype F(1,117)=5.03, p=0.027; see figure 3B) in both escaping and non-escaping patients (genotype × behavioral group F(1,117)=1.16; p=0.284). No significant associations were found in heart rate during anticipation and recovery periods, suggesting a specific effect during threat challenge. Importantly, the subjective distress during the task was comparable between genotype groups (see figure 3C). The observed dissociations between physiological/behavioral and subjective responses depending on genotype were supported by following results: in the non-risk genotype patient group, subjective anxiety ratings during exposure were strongly correlated with initial heart rate increase from last minute of anticipation to first minute of exposure (r=0.627, p<0.001) and overall exposure heart rate (r=0.378, p=0.001), suggesting synchrony between physiological and verbal responses; in contrast, heart rate responses did not predict subjective ratings in risk allele carriers (initial heart rate increase: r=0.215, p=0.134; overall heart rate: r=0.239; p=0.091). No significant differences in indicators of defensive reactivity between rs17689966 genotypes on escape behavior, heart rate and reported anxiety were observed (data not shown).

Panic-relevant psychometric traits: ASI, ACQ and Gene x Environment

To validate whether risk and protective alleles influenced dimensional measures of anxiety associated with PD and agoraphobia, we analyzed rs17689918 and rs17689966 (Table 1, supplementary Table 4 and 6) for their effects on the ASI and the ACQ in the replication sample (these data were not available in the discovery sample). In line with results from categorical outcomes, rs17689918 led to an increased ASI sum score by 2.17 (p=0.033) points per A (risk) allele in the combined sample (i.e. cases and controls) and by 2.42 points per A (risk) allele (p=0.040) in the combined female subset. A significant effect of

rs17689918 alleles on the ACQ sum score was only found in the female control subset: each A allele lead to an average reduction of 0.075 points. Furthermore, the minor G allele of rs17689966 was found to decrease the ACQ sum score in the female subset of the control sample (-0.079 points per G allele; p=0.002), but also in the total control sample (-0.064 points per G allele; P=0.002). There was no impact of rs17689966 on ASI sum scores (see Supplementary Table 10 and 11). Finally, rs17689918 and rs17689966 did not display significant gene × environment interaction on any phenotype in the SHIP-PD sample (data not shown).

Bioinformatical Assessment of SNP function

Due to the high consensus of categorical, dimensional, psychophysiological and emotion processing data, both intronic polymorphisms rs17689918 and rs17689966 seem to have a putative functional role on CRHR1 gene function. To identify their possible influence on splice junctions, we analyzed rs17689918 and rs17689966 with the Human Splicing Finder software (HSF) version 2.4.1 [57] and found that the minor risk allele replaces two binding sites (BS) for the Intronic Splicing Enhancers SF2/ASF and SRp55 by a new BS for SC35. Furthermore, we found for both SNPs together, 91 perfect proxies (r^2=D´=1), which also affect neighboring genes C17orf69, IMP5 and MAPT due to extended linkage disequilibrium. Functional prediction revealed a high proportion of differential neuro-relevant transcription factor binding (n=29; MatInspector version 2.1; [58]) and implied allele-specific changes in transcript splicing (n=21). Also, polymorphisms with deleterious effects on the coding sequence (n=6; Patrocles database [59]) were found in adjacent genes (overview in Figure 4; detailed information on supplementary Table 12).

Discussion

In the present study, we applied a multilevel approach to characterize the role of *CRHR1* in panic disorder and thereby demonstrate that alleles in *CRHR1* 1) increase the risk towards PD; 2) go along with reduced expression of the gene product in forebrain and amygdala, resulting in 3) reduced frontal cortical activation upon fear conditioning as well as increased amgydala activation as a response to safety signal presentation while 4) reducing defensive reactivity and 5) increasing anxiety sensitivity. This comprehensive body of

evidence provides evidence on how this component of the HPA axis might contribute to PD and confirms a previously reported finding that allelic variation in the *CRHR1* gene is associated with PD [29].

For replication of the *CRHR1* locus we analyzed eight tagSNPs in two independent case/control samples, and of those, four were found to be associated with PD. rs7209436, which tags rs110402 and rs242924, represents the frequently investigated TAT-haplotype known to predispose to depression [15, 16, 22, 23], but displayed only suggestive association signals with female PD patients. In line with this, rs7209436 was not associated with PD in two further independent studies [28, 29]. Although smaller sample size and mixed-sex analysis may explain this, a comparably small sample revealed a strong association with depression [15]. Therefore, this variant may specifically influence the risk towards depression but not anxiety disorders. Another nominally associated SNP, rs12936181, was restricted to males. In the context of PD, no results of rs12936181 have been published yet; however, its proxy rs4792887 showed strong associations with PTSD in adults after hurricane exposure [14] and affected depression intensity in male suicide attempters [20, 60]. Thus, rs12936181 and/or polymorphisms in high LD may predispose to stress-related disorders in general. For those SNPs that revealed the strongest associations with PD in our current study - rs17689918 and rs17689966 – there is already indirect evidence for effects in anxiety disorders: On the one hand, rs17689918 is in LD with two nominally associated SNPs (rs1396862 and rs1876831) as well as rs878886, the top finding of the Keck study on PD [29]. On the other hand, rs17689966 predisposes to PTSD [14]. Quantitative real-time PCR demonstrated that rs17689918 and rs17689966 reduced *CRHR1* expression in amygdala and forebrain, regions known to regulate anxiety. Furthermore, a recent study to detect functional variation in the human genome [61] classified both SNPs as expression quantitative trait loci (eQTLs); moreover, CRHR1 expression was impaired by rs17689918 at the genome-wide significance level ($p < 5*10^{-8}$). However, despite our data argue for reduced CRHR1 expression to lead to PD, extended linkage disequilibrium at this locus suggests that also neighboring risk genes of psychiatric disorders (C17ORF69, IMP5 and MAPT) may predispose to PD as well. However, this hypothesis needs independent replication to support its validity.

The multi-level approach utilized in the present study allows to investigate the neural mechanisms leading from the molecular changes to disease phenotype. Recently, we found

evidence that the two central clinical symptom complexes of PD, namely acute panic and chronic anxious apprehension, reflect different stages of defensive reactivity as supposed by animal models ([56]; see also review [62]). In this view acute panic and associated flight behavior can be conceptualized as defensive behaviors during imminent threat (phasic fear). In contrast, anxious apprehension can be associated with defensive behaviors during the exposure to *less specific and less predictable* threats (sustained fear/anxiety). Animal models that are differentiating phasic and sustained fear [63] suggest that corticotropin-releasing factor CRF might be involved only in the latter, mainly by acting on the bed nucleus of the stria terminalis (BNST), the core brain region in mediating sustained fear (for reviews see [63-65]). There is evidence that CRH-dependent BNST activation during anxiety states is mediated by CRHR1 activation. For example, in rats, administration of a Crhr1-specific antagonist blocked defensive behaviors during laboratory models of sustained, but not phasic fear [65]. Moreover, *Crh* gene over-expression within the BNST increased the sustained fear response towards a previously conditioned fear cue [66]. *Crh* over-expression was followed by a decreased CRHR1 receptor density within the BNST suggesting that "behavioral effects may be mediated by enhanced CRH receptor signaling or compensatory changes in CRF receptor density within these structures" [66]. Importantly, CRH over-expression was impairing anxiety responses towards threat cues if administered prior to conditioning, and also, limbic CRHR1 was shown to be required for the enhancement of fear memory consolidation [8]. Together these data suggest an important role for CRHR1 in the formation and generalization of fear.

From rodent studies it can therefore be inferred that genetic variation in *CRHR1* might rather affect defensive stages mediating chronic anxious apprehension than defensive stages during acute threat such as panic or escape. Indeed, we found evidence that the PD risk variant rs17689918 diminished defensive behaviors during acute threat during a behavioral avoidance test, as indicated by reduced flight behavior and according autonomic arousal. Importantly, risk allele carriers reported comparable high subjective distress during the test, which, interestingly, was not linked to autonomic mobilization demonstrating the physiological preparation for behavioral responses during acute threat: While patients carrying the non-risk allele variant showed large correlations between reported anxiety and heart rate response during BAT exposure, risk-allele carriers did not. In line with previous results that demonstrated reduced threat related defensive reactivity in those PD/AG patients reporting pervasive agoraphobic apprehension and avoidance, but also broad

dysphoria and general distress [67], the rs16689918 risk allele was associated with pronounced depressive symptoms (BDI-II), general distress (BSI) and agoraphobic avoidance (MI) in the BAT sample (see supplemental table 3). Therefore rs16689918 might increase the risk for PD by facilitating the development of anxious apprehension. In general, reduced CRHR1 expression – as a consequence of rs17689918 and rs17689966 risk alleles – may lead to sustained fear especially in the presence of ambiguous cues.

In line with these assumptions, fMRI findings indicate that risk allele carriers of rs17689918 and rs17689966 demonstrate reduced differential conditioning in predominantly prefrontal cortices (where CRHR1 expression is reduced in risk allele carriers) and increased activation upon safety signal presentation in regions relevant for fear processing such as the amygdala (again, CRHR1 expression is lower here in risk allele carriers), well fitting to the hypothesis of fear generalization (due to amgydala hyper-reactivity in response to non-threatening stimuli and impaired differential conditioning) in risk allele carriers. These data indicate that different top down and bottom-up processes during fear conditioning [55] are affected by genetically driven CRHR1 expression differences in patients with PD/AG. Especially the generalization of fear and dysfunctional safety signal processing has been considered to be a relevant pathological mechanisms of PD/AG [68] and has been shown to be associated to treatment response [54]. Altogether, these data indicate that variation in *CHRH1* genotypes and subsequent expressional changes might contribute to the development of sustained fear due to impaired safety signaling processing and increased amygdala reactivity towards neural stimuli. The fear network in risk allele carriers thus seems to be biased towards fearful processing of neutral or ambiguous stimuli.

In summary, the present study provided compelling evidence for a role of the *CRHR1* gene in the pathogenesis of panic disorder. Allelic variation of the functional *CRHR1* polymorphisms rs17689918 and rs17689966 has been found to predispose to PD, as reflected by emotion processing, psychophysiological parameters and psychometric traits. Our data indicate that CRHR1 has its role in PD in dysfunctional processing of safety cues and goes along with decreased rates of active avoidance, thus resembling states of sustained fear.

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Supporting information cited in this article is available online.

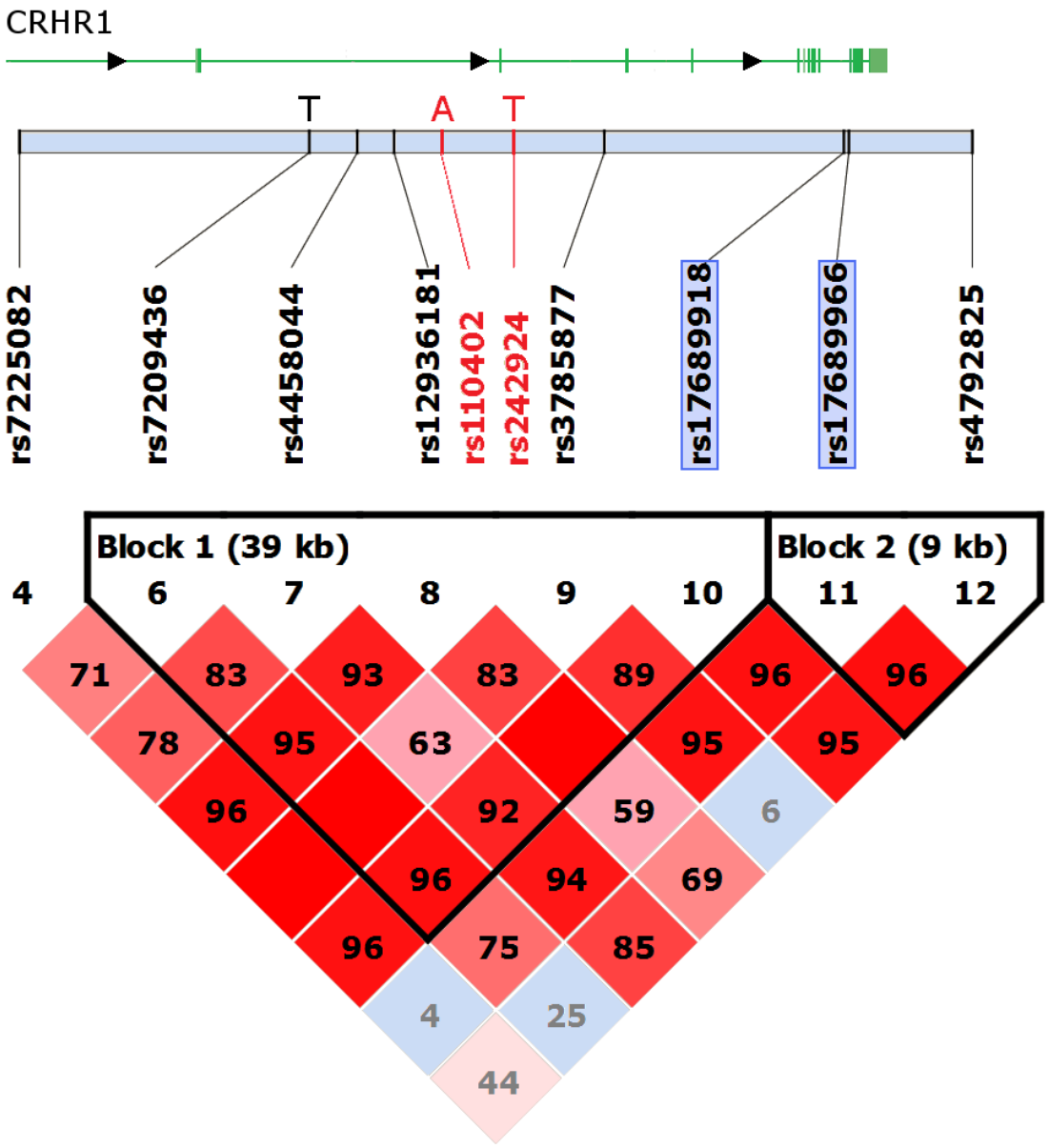

Figure 1: Linkage disequilibrium (LD) plot of *CRHR1*. LD is displayed as D' Haplotype blocks were defined with the solid spine method. In red, SNPs (rs110402 and rs242924) of the TAT haplotype [29] which were not genotyped are indicated. SNPs that are framed and highlighted in blue were found to be significantly associated with panic disorder.

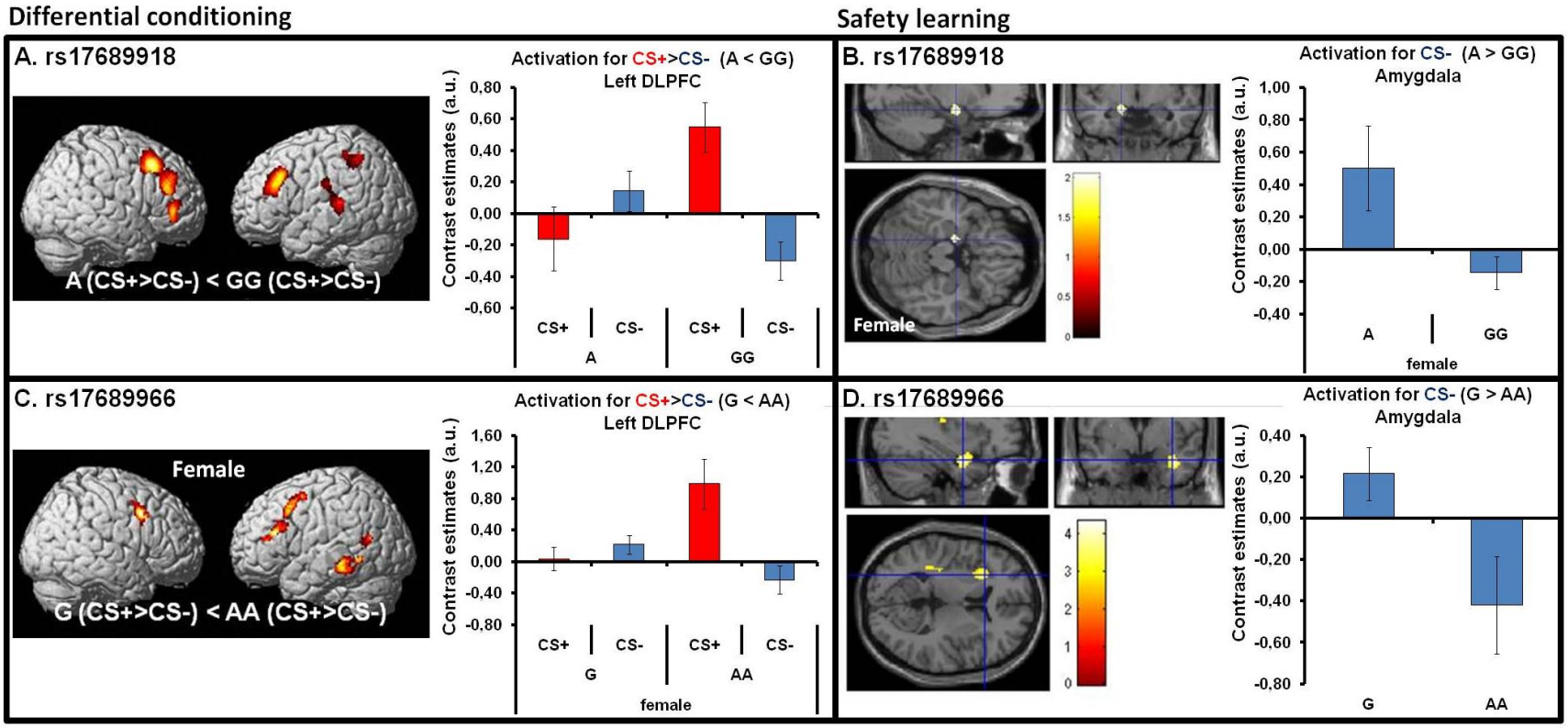

Figure 2: Effect of rs17689918 (top, A, B) and rs17689966 (bottom, C, D) on fMRI results for differential conditioning (left) and safety learning (right). Analyses have been performed either for females only (B, C) or for males and females together (A, D). Bar graphs illustrate the contrast estimates (extracted eigenvariates) for the respective cluster, for males and females together (A, D) or females alone (B, C). For exact locations and statistics please see Supplementary Table 8 and 9. Risk allele carrier (A for rs17689918 and G for rs17689966) demonstrated significant reduced differential conditioning responses (CS+ [red] >CS- [blue]; surface view, left) in predominantly left and bilateral frontal cortices than patients without risk alleles (GG for rs17689918 and AA for rs17689966). By contrast, risk allele carrier (A for rs17689918 and G for rs17689966) in contrast to patients without risk alleles (GG for rs17689918 and AA for rs17689966) demonstrated significant increased activation in the amygdalae for the processing of the safety cues (CS- [blue]; slices view, right).

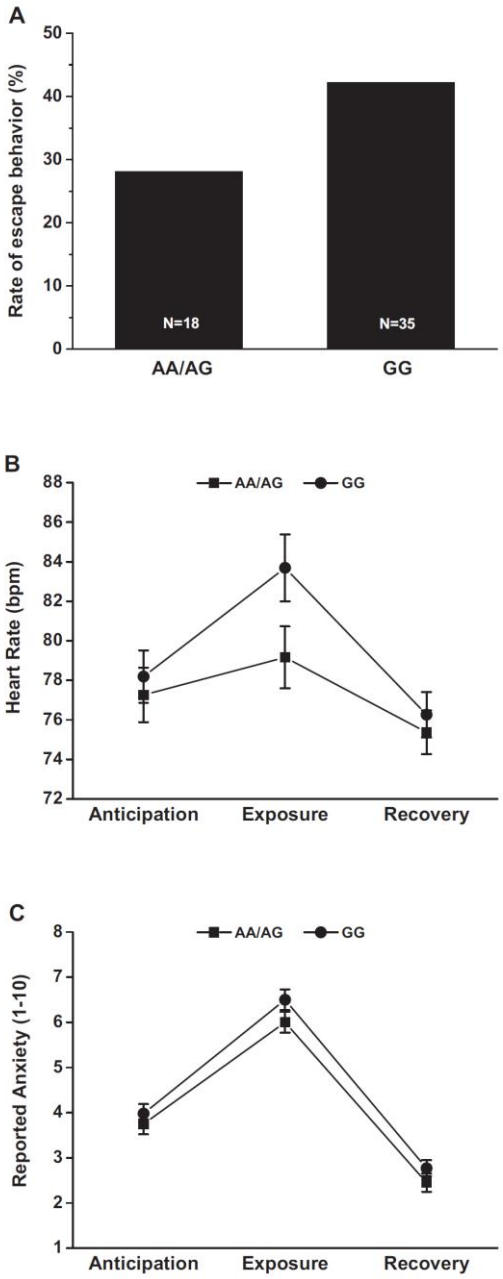
A
Rate of escape behavior (%)
50
40
30
20
10
0
N=18
N=35
AA/AG
GG
B
AA/AG
GG
Heart Rate (bpm)
88
86
84
82
80
78
76
74
72
Anticipation
Exposure
Recovery
C
AA/AG
GG
Reported Anxiety (1-10)
8
7
6
5
4
3
2
1
Anticipation
Exposure
Recovery

Figure 3: Frequency of escape behavior (panel A) and means and SE of heart rate response (panel B) and reported anxiety (panel C) during anticipation, exposure and recovery periods of the BAT depending on rs17689918 genotype.

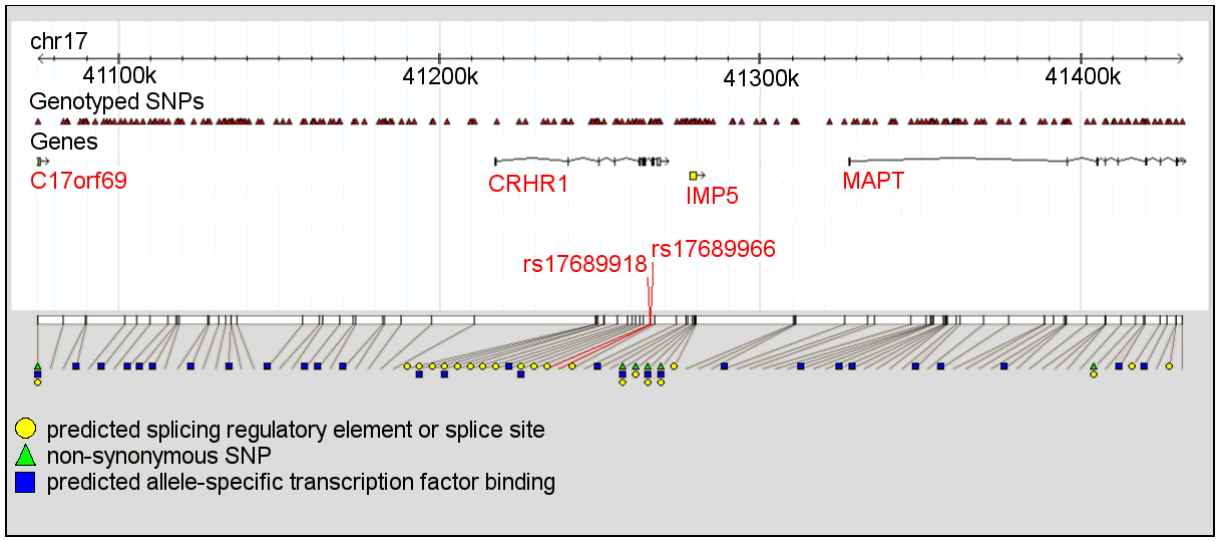

Figure 4: Allele-specific functional predictions of rs17689918, rs17689966 and their 91 perfect proxies (D'= r^2=1) are shown. Predicted allele-specific transcription factor binding is only displayed for neuro-relevant transcription factors. Perfect proxies are found in a large genomic range that includes genes adjacent to *CRHR1*.

Table 1: Association results for examined SNPs along with their minor/major alleles (converted to the coding strand), genotype and allele counts for cases and controls, the nominal and the Bonferroni-corrected p-values for the Combined Sample, as well as the Combined Female and Male Subsamples. Bold indicates p<0.05 in at least one of the samples.

		Combined Sample (N=531)				Combined Female Sample (N=359)				Combined Male Sample (N=172)			
		Controls	Cases			Controls	Cases			Controls	Cases		
	Alleles	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni
SNP	(d/D)	n(d/D)	n(d/D)	P-value	P-value	n(d/D)	n(d/D)	P-value	P-value	n(d/D)	n(d/D)	P-value	P-value
CRHR1; Chromosome 17													
rs7225082	G/T	72/238/165	65/240/195	0.329	1	52/161/112	44/158/133	0.310	1	20/77/53	21/82/62	0.918	1
rs7225082	G/T	382/568	370/630	0.145	1	265/385	246/424	0.131	1	117/183	124/206	0.713	1
rs7209436	T/C	92/230/153	84/244/179	0.412	1	59/166/100	47/165/128	0.107	0.858	33/64/53	37/79/51	0.628	1
rs7209436	**T/C**	**414/536**	**412/602**	**0.186**	**1**	**284/366**	**259/421**	**0.038**	**0.301**	**130/170**	**153/181**	**0.531**	**1**
rs4458044	C/G	40/168/301	33/179/269	0.363	1	27/116/201	17/116/189	0.383	1	13/52/100	16/63/80	0.176	1
rs4458044	C/G	248/770	245/717	0.569	1	170/518	150/494	0.545	1	78/252	95/223	0.073	0.582
rs12936181	C/T	10/104/393	6/85/387	0.349	1	7/67/268	4/64/253	--	--	3/37/125	2/21/134	--	--
rs12936181	**C/T**	**124/890**	**97/859**	**0.143**	**1**	**81/603**	**72/570**	**0.721**	**1**	**43/287**	**25/289**	**0.036**	**0.291**
rs3785877	A/G	0/46/462	1/31/439	--	--	0/31/313	1/20/295	--	--	0/15/149	0/11/144	--	--
rs3785877	A/G	46/970	33/909	0.250	1	31/657	22/610	0.343	1	15/313	11/299	0.513	1
rs17689918	**A/G**	**12/138/321**	**21/197/293**	**0.002**	**0.015**	**5/92/226**	**18/138/187**	**0.054**	**0.433**	**7/46/95**	**3/59/106**	**--**	**--**
rs17689918	**A/G**	**162/780**	**239/783**	**0.001**	**0.005**	**102/544**	**174/512**	**$1.6*10^{-5}$**	**$1.3*10^{-4}$**	**60/236**	**65/271**	**0.771**	**1**
rs17689966	**G/A**	**89/250/164**	**92/276/124**	**0.034**	**0.271**	**54/174/114**	**67/189/75**	**0.007**	**0.057**	**35/76/50**	**25/87/49**	**0.298**	**1**
rs17689966	**G/A**	**428/578**	**460/524**	**0.059**	**0.474**	**282/402**	**323/339**	**0.005**	**0.042**	**146/176**	**137/185**	**0.475**	**1**
rs4792825	G/A	11/125/374	8/109/373	0.557	1	8/82/254	5/73/253	0.617	1	3/43/120	3/36/120	--	--
rs4792825	G/A	147/873	125/855	0.280	1	98/590	83/579	0.358	1	49/283	42/276	0.569	1

Table 2: Association results for haplotypes examined in the Combined Sample, as well as the Combined Female and Subsamples along with frequencies in cases and controls, the nominal and the Bonferroni-corrected p-values. Bold indicates p<0.05 in at least one sample.

Block 1:													
					Combined Sample (N=531)			**Combined Female Sample (N=359)**			**Combined Male Sample (N=172)**		
rs7209436	**rs4458044**	**rs12936181**	**rs3785877**	**rs17689918**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
C	**G**	**T**	**G**	**A**	**0.226/0.176**	**0.006**	**0.050**	**0.247/0.166**	**$2.4*10^{-4}$**	**0.002**	**0.178/0.197**	**0.541**	**1**
C	G	T	A	G	0.033/0.044	0.219	1	0.034/0.044	0.340	1	0.031/0.043	0.411	1
C	**G**	**C**	**G**	**G**	**0.098/0.120**	**0.108**	**0.862**	**0.110/0.118**	**0.671**	**1**	**0.078/0.128**	**0.035**	**0.283**
T	C	T	G	G	0.223/0.228	0.797	1	0.201/0.231	0.174	1	0.267/0.220	0.162	1
C	C	T	G	G	0.024/0.013	0.064	0.514	0.029/0.015	0.078	0.620	0.014/0.008	0.467	1
T	G	T	G	G	0.200/0.207	0.677	1	0.195/0.210	0.485	1	0.210/0.201	0.787	1
C	G	T	G	G	0.197/0.212	0.417	1	0.185/0.216	0.149	1	0.223/0.203	0.544	1
Block 2:													
rs17689966	**rs4792825**				**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
G	G				0.128/0.139	0.470	1	0.126/0.136	0.583	1	0.130/0.143	0.643	1
G	**A**				**0.340/0.285**	**0.010**	0.077	**0.360/0.275**	**0.001**	**0.008**	**0.300/0.308**	**0.831**	**1**
A	**A**				**0.533/0.577**	**0.055**	0.439	**0.514/0.589**	**0.007**	**0.056**	**0.570/0.549**	**0.604**	**1**

Table 3: *CRHR1* variants and expression: Allele-specific mRNA expression changes of rs17689918 (**A**) and rs17689966 (**B**) in the forebrain, amygdala and midbrain were examined with linear regression. For comparison of genotype-specific expression levels single factor analyses of variances (ANOVAs) were carried out.

A

	Forebrain		Amygdala		Midbrain	
rs176889918	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**
ANOVA	**-**	**0.009**	**-**	**0.010**	**-**	0.641
lin. Regression	-0.713	**0.002**	-1.149	**0.003**	-1.528	0.343

B

	Forebrain		Amygdala		Midbrain	
rs176889966	**Beta**	**p-value**	**Beta**	**p-value**	Beta	**p-value**
ANOVA	**-**	**0.014**	**-**	**0.044**	-	0.785
lin. Regression	-0.516	**0.003**	-0.776	**0.012**	-0.522	0.640

Supplementary Table 1: Primer sequences in 5'-3' direction used for Sequenom's MassArray® system.

SNP ID	Primary PCR Primer 1	Primary PCR Primer 2	Extend Primer
rs7225082	ACGTTGGATGATCGATCTCCACCTCTCTTC	ACGTTGGATGTGCAATTCACAGTGGAGCAG	caCTCCCCCTCAGCTCAGC
rs12938031	ACGTTGGATGGGCATCTGCTGAGATATGAC	ACGTTGGATGATTCCAAGTGCGTCAAGCTC	gaggGGCTGGGACTGCAGTGACG
rs7209436	ACGTTGGATGCTAGCTCATCGTGGATCCTG	ACGTTGGATGAGTAGGTGTTTTTGAGCCCC	GTCCCACAACATGGGGTCTTACAG
rs4458044	ACGTTGGATGTCTGTGAGAGCCAAACAGAG	ACGTTGGATGAGAGCTGGCAGTGGGAACGA	CCTGAGTCCCATCCATT
rs12936181	ACGTTGGATGTTTTCTCATCCACCCACAGG	ACGTTGGATGAGTCAAGAGGGCTGAGAAAG	gGACCAGCCAGAGTAAAACTAG
rs3785877	ACGTTGGATGTAATGGACACGAGGTGACTG	ACGTTGGATGAACATTCACAGAGACTCCGC	CGAGGTGACTGCGGGCCAGG
rs17689918	ACGTTGGATGAAGGTTGTTCAGGCTGTGAC	ACGTTGGATGACCACACCTGTCACCCAGT	gaatATTCAGGCTGAGATTGC
rs17689966	ACGTTGGATGTGTCCTGGCCAAGCACTGTC	ACGTTGGATGTAGAAGGCCACAGAGGAAAG	ctTCCCTCCCCATGCCATC
rs4792825	ACGTTGGATGTCAGTCTAATGCTTTGAAGG	ACGTTGGATGTACACTCATCTCACCCACAC	taagGCTTTGAAGGATACTTTACAA

Supplementary Table 2: Demographic and clinical characteristics of the fMRI-samples

	rs17689918						**rs17689966**					
	Risk *(A; n=19)**		**No-Risk *(G/G; n=29)***		**Statistics**		**Risk *(G; n=30)****		**No-Risk *(A/A; n=16)***		**Statistics**	
	Mean	*SD*	*Mean*	*SD*	*Chi/F*	*P-Value*	*Mean*	*SD*	*Mean*	*SD*	*Chi/F*	*P-Value*
Demographic characteristics												
Gender (female. n [%])	16	84%	17	59%	3.50	***0.061***	22	73%	10	63%	0.58	0.447
Education	2/10/7		2/14/13		0.40	0.818	2/16/12		1/7/8		0.43	0.805
Age	36.20	9.94	38.55	10.77	0.58	0.449	36.06	11.12	38.12	8.05	0.43	0.518
Neuropsychological characteristics												
DS Forward	7.58	2.17	7.76	2.12	0.08	0.777	7.73	2.27	7.50	1.97	0.12	0.730
DS Backward	6.74	2.00	7.21	2.02	0.63	0.433	6.83	2.12	7.44	1.67	0.97	0.329
DS total	14.26	3.72	14.97	3.38	0.46	0.502	14.53	3.68	14.94	3.38	0.13	0.717
TMT A *(sec.)*	27.71	6.64	25.31	9.07	0.99	0.326	26.68	8.58	24.19	7.73	0.94	0.338
TMT B *(sec.)*	59.68	20.16	55.48	16.56	0.62	0.434	58.59	17.61	51.13	17.60	1.87	0.178
Clinical characteristics												
HAMA	25.37	5.97	23.17	4.53	2.09	0.155	25.80	5.56	21.13	3.16	9.59	**0.003**
PAS	26.51	7.00	26.90	10.13	0.02	0.886	26.54	8.53	26.47	9.79	0.00	0.981
No. pan. att.	1.68	0.90	1.83	0.77	0.35	0.559	1.83	0.68	1.63	1.07	0.65	0.424
CGI	5.47	0.61	5.41	0.68	0.10	0.758	5.40	0.62	5.44	0.63	0.04	0.847
ASI	32.00	10.36	30.34	9.43	0.33	0.570	31.53	10.33	27.69	8.14	1.66	0.204
ACQ	2.01	0.34	2.21	0.64	1.52	0.224	2.17	0.55	2.17	0.57	0.00	0.981
BDI II	21.32	8.79	13.76	6.76	11.30	**0.002**	18.67	8.62	11.88	4.43	8.66	**0.005**
MI gen. *accompanied*	2.19	0.71	2.09	0.74	0.17	0.687	2.30	0.65	1.86	0.81	3.91	***0.055***
MI gen. *alone*	2.83	0.89	2.73	0.80	0.16	0.687	2.80	0.86	2.72	0.86	0.07	0.793

*differences in sample size due to the fact that tree patients for rs17689966 (n=46) and one for rs17689918 (n=48) of a total of 49 datasets could not be specified in the genetic analysis.

Supplementary Table 3: Demographic and clinical characteristics of the BAT-samples

	rs17689918						**rs17689966**					
	Risk *(AA/AG; n=64)*		**No-Risk *(GG; n=83)***		**Statistics**		**Risk *(AG/GG; n=102)***		**No-Risk *(AA; n=31)***		**Statistics**	
	Mean	*SD*	*Mean*	*SD*	*Chi/F*	*P-Value*	*Mean*	*SD*	*Mean*	*SD*	*Chi/F*	*P-Value*
Demographic characteristics												
Gender (female. n [%])	54	84%	63	76%	1.59	0.206	83	81%	22	71%	1.55	0.213
Education	7/29/28		11/33/39		0.51	0.777	10/45/47		5/9/17		2.56	0.279
Age	34.55	10.55	34.52	11.39	0.01	0.977	34.72	11.32	34.00	11.00	0.09	0.757
Clinical characteristics												
HAMA	25.45	6.16	23.99	4.99	2.54	0.113	25.01	5.81	23.09	4.32	2.88	0.092
PAS	29.77	9.50	28.02	9.89	1.17	0.281	28.53	9.69	27.64	9.54	0.20	0.656
No. pan. att.	3.34	2.53	2.75	2.43	2.10	0.149	2.95	2.51	2.84	2.57	0.05	0.829
CGI	5.28	0.63	5.24	0.73	0.13	0.724	5.25	0.67	5.19	0.70	0.14	0.710
ASI	32.03	11.56	32.30	11.46	0.02	0.887	32.66	11.81	29.36	9.59	2.01	0.159
ACQ	2.12	0.53	2.24	0.61	1.52	0.220	2.17	0.56	2.24	0.60	0.35	0.556
BDI II	19.55	8.14	15.41	8.55	8.81	**0.004**	17.75	9.25	14.68	5.62	3.06	**0.083**
BSI	1.28	0.52	1.05	0.53	6.88	**0.010**	1.18	0.57	0.96	0.35	3.98	**0.048**
MI gen. *accompanied*	2.39	0.75	2.32	0.75	0.23	0.633	2.41	0.75	2.16	0.77	2.34	0.129
MI gen. *alone*	3.24	0.76	2.99	0.83	2.96	**0.088**	3.09	0.78	2.95	0.85	0.75	0.388

Note: Due to missing values reduced samples were available according MI7t accompanied (rs17689918: 59 AA/AG and 77 GG; rs17689966: 92 AG/GG and 30 AA), MI7t alone (rs17689918: 59 AA/AG and 79 GG; rs17689966: 94 AG/GG and 30 AA), MI gen. accompanied + alone (rs17689918: 57 AA/AG and 74 GG; rs17689966: 89 AG/GG and 29 AA).

Supplementary Table 4: Association results for examined SNPs along with their minor/major alleles (converted to the coding strand), genotype and allele counts for cases and controls, the nominal and the Bonferroni-corrected p-values for the Discovery Sample, as well as the Discovery Female and Male Subsamples. Bold indicates p<0.05 in at least one of the samples.

		Discovery Subsample (N=239)				Discovery Female Subsample (N=143)				Discovery Male Subsample (N=96)			
		Controls	Cases			Controls	Cases			Controls	Cases		
	Alleles	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni	n(dd/dD/DD)	n(dd/dD/DD)	Nominal	Bonferroni
SNP	(d/D)	n(d/D)	n(d/D)	P-value	P-value	n(d/D)	n(d/D)	P-value	P-value	n(d/D)	n(d/D)	P-value	P-value
rs7225082	G/T	34/111/78	28/108/94	0.367	1	19/71/46	17/63/58	0.376	1	15/40/32	11/45/36	0.605	1
rs7225082	G/T	179/267	164/296	0.164	1	109/163	97/179	0.234	1	70/104	67/117	0.458	1
rs7209436	T/C	44/107/71	42/109/80	0.809	1	25/71/39	23/64/51	0.365	1	19/36/32	19/45/29	0.622	1
rs7209436	T/C	195/249	193/269	0.514	1	121/149	110/166	0.241	1	74/100	83/103	0.689	1
rs4458044	C/G	20/74/139	17/72/116	0.757	1	13/47/79	9/40/69	0.882	1	7/27/60	8/32/47	0.406	1
rs4458044	C/G	114/352	106/304	0.636	1	73/205	58/178	0.663	1	41/147	48/126	0.202	1
rs12936181	C/T	5/49/178	3/32/169	--	--	4/25/109	2/23/93	--	--	1/24/69	1/9/76	--	--
rs12936181	**C/T**	**59/405**	**38/370**	**0.111**	**0.887**	**33/243**	**27/209**	**0.857**	**1**	**26/162**	**11/161**	**0.020**	**0.163**
rs3785877	A/G	0/19/214	0/14/182	--	--	0/12/127	0/7/106	--	--	0/7/87	0/7/76	--	--
rs3785877	A/G	19/447	14/378	0.701	1	12/266	7/219	0.475	1	7/181	7/159	0.812	1
rs17689918	A/G	6/53/126	10/83/134	0.164	1	1/33/76	8/51/76	--	--	5/20/50	2/32/58	--	--
rs17689918	**A/G**	**65/305**	**103/351**	**0.070**	**0.557**	**35/185**	**67/203**	**0.016**	**0.126**	**30/120**	**36/148**	**0.921**	**1**
rs17689966	G/A	38/116/69	46/120/59	0.449	1	17/75/40	31/71/32	0.079	0.635	21/41/29	15/49/27	0.410	1
rs17689966	G/A	192/254	212/238	0.222	1	109/155	133/135	0.053	0.428	83/99	79/103	0.673	1
rs4792825	G/A	3/61/169	2/46/155	--	--	2/36/101	1/28/90	--	--	1/25/68	1/18/65	--	--
rs4792825	G/A	67/399	50/356	0.373	1	40/238	30/208	0.555	1	27/161	20/148	0.494	1

Supplementary Table 5: Association of haplotypes in the Discovery Sample, as well as the Discovery Female and Male Subsamples with frequencies in cases and controls, nominal p-value, the nominal and the Bonferroni-corrected p-values. Bold indicates p<0.05 in at least one sample.

Block 1:

					Discovery Subsample (N=239)			Discovery Female Subsample (N=143)			Discovery Male Subsample (N=96)		
rs7209436	**rs4458044**	**rs12936181**	**rs3785877**	**rs17689918**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
C	G	T	G	A	0.212/0.180	0.229	1	0.236/0.171	0.071	0.565	0.180/0.192	0.775	1
C	G	T	A	G	0.035/0.038	0.808	1	0.032/0.040	0.628	1	0.043/0.037	0.800	1
C	**G**	**C**	**G**	**G**	**0.089/0.124**	**0.098**	**0.781**	**0.110/0.115**	**0.837**	**1**	**0.063/0.139**	**0.017**	**0.136**
T	C	T	G	G	0.225/0.224	0.994	1	0.219/0.242	0.543	1	0.231/0.199	0.459	1
C	C	T	G	G	0.022/0.015	0.434	1	0.023/0.019	0.744	1	0.026/0.013	0.353	1
T	G	T	G	G	0.215/0.207	0.776	1	0.200/0.212	0.729	1	0.232/0.196	0.398	1
C	G	T	G	G	0.202/0.212	0.727	1	0.181/0.201	0.579	1	0.224/0.223	0.986	1

Block 2:

rs17689966	**rs4792825**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
G	G	0.123/0.140	0.476	1	0.123/0.136	0.674	1	0.124/0.146	0.543	1
G	**A**	**0.346/0.284**	**0.056**	**0.449**	**0.368/0.271**	**0.022**	**0.173**	**0.315/0.303**	**0.820**	**1**
A	A	0.531/0.576	0.195	1	0.509/0.593	0.062	0.498	0.562/0.551	0.836	1

Supplementary Table 6: Association results for examined SNPs along with their minor/major alleles (converted to the coding strand), genotype and allele counts for cases and controls, the nominal and the Bonferroni-corrected p-values for the Replication Sample, as well as Replication Female and Male Subsamples. Bold indicates p<0.05 in at least one of the samples.

		Replication Sample (N=292)				Replication Female Sample (N=216)				Replication Male Sample (N=76)			
		Controls	Cases			Controls	Cases			Controls	Cases		
SNP	Alleles (d/D)	n(dd/dD/DD) n(d/D)	n(dd/dD/DD) n(d/D)	Nominal P-value	Bonferroni P-value	n(dd/dD/DD) n(d/D)	n(dd/dD/DD) n(d/D)	Nominal P-value	Bonferroni P-value	n(dd/dD/DD) n(d/D)	n(dd/dD/DD) n(d/D)	Nominal P-value	Bonferroni P-value
rs7225082	G/T	38/127/87	37/132/101	0.766	1	33/90/66	27/95/75	0.564	1	5/37/21	10/37/26	0.479	1
rs7225082	G/T	203/301	206/334	0.481	1	156/222	149/245	0.327	1	47/79	57/89	0.769	1
rs7209436	T/C	48/123/82	42/135/99	0.459	1	34/95/61	24/101/77	0.183	1	14/28/21	18/34/22	0.895	1
rs7209436	T/C	219/287	219/333	0.234	1	163/217	149/255	0.086	0.685	56/70	70/78	0.637	1
rs4458044	C/G	20/94/162	16/107/153	0.463	1	14/69/122	8/76/120	0.370	1	6/25/40	8/31/33	0.451	1
rs4458044	C/G	134/418	139/413	0.727	1	97/313	92/316	0.707	1	37/105	47/97	0.222	1
rs12936181	C/T	5/55/215	3/53/218	--	--	3/42/159	2/41/160	--	--	2/13/56	1/12/56	--	--
rs12936181	C/T	65/485	59/489	0.582	1	48/360	45/361	0.760	1	17/125	14/128	0.568	1
rs3785877	A/G	0/27/248	1/17/257	--	--	0/19/186	1/13/189	--	--	0/8/62	0/4/68	--	--
rs3785877	A/G	27/523	19/531	0.228	1	19/391	15/391	0.502	1	8/132	4/140	0.219	1
rs17689918	**A/G**	**6/85/195**	**11/114/159**	**0.009**	**0.075**	**4/59/150**	**10/87/111**	**--**	**--**	**2/26/45**	**1/27/48**	**--**	**--**
rs17689918	**A/G**	**97/475**	**136/432**	**0.003**	**0.028**	**67/359**	**107/309**	**0.0003**	**0.003**	**30/116**	**29/123**	**0.750**	**1**
rs17689966	**G/A**	**51/134/95**	**46/156/65**	**0.027**	**0.214**	**37/99/74**	**36/118/43**	**0.009**	**0.070**	**14/35/21**	**10/38/22**	**0.666**	**1**
rs17689966	**G/A**	**236/324**	**248/286**	**0.152**	**1**	**173/247**	**190/204**	**0.044**	**0.349**	**63/77**	**58/82**	**0.546**	**1**
rs4792825	G/A	8/64/205	6/63/218	0.773	1	6/46/153	4/45/163	--	--	2/18/52	2/18/55	--	--
rs4792825	G/A	80/474	75/499	0.503	1	58/352	53/371	0.484	1	22/122	22/128	0.883	1

Supplementary Table 7: Association of haplotypes in the Replication Sample, as well as the Replication Female and Male Subsamples with frequencies in cases and controls, nominal p-value, the nominal and the Bonferroni-corrected p-values. Bold indicates p<0.05 in at least one sample.

Block 1:

					Replication Subsample (N=292)			Replication Female Subsample (N=216)			Replication Male Subsample (N=76)		
rs7209436	**rs4458044**	**rs12936181**	**rs3785877**	**rs17689918**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
C	**G**	**T**	**G**	**A**	**0.226/0.172**	**0.009**	**0.072**	**0.253/0.162**	**0.001**	**0.011**	**0.170/0.201**	**0.616**	**1**
C	G	T	A	G	0.031/0.048	0.145	1	0.035/0.046	0.401	1	0.021/0.046	0.459	1
C	G	C	G	G	0.104/0.117	0.468	1	0.110/0.119	0.693	1	0.096/0.115	0.522	1
T	C	T	G	G	0.221/0.232	0.667	1	0.190/0.224	0.228	1	0.312/0.254	0.232	1
C	C	T	G	G	0.025/0.010	0.062	0.493	0.033/0.013	0.051	0.407	-	-	-
T	G	T	G	G	0.189/0.206	0.480	1	0.192/0.209	0.548	1	0.182/0.205	0.625	1
C	G	T	G	G	0.194/0.214	0.417	1	0.188/0.227	0.159	1	0.220/0.180	0.576	1

Block 2:

rs17689966	**rs4792825**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**	**Case/Control Frequencies**	**Nominal P-Value**	**Bonferroni P-Value**
G	G	0.130/0.137	0.734	1	0.127/0.136	0.705	1	0.136/0.138	0.971	1
G	**A**	**0.337/0.287**	**0.076**	**0.612**	**0.355/0.277**	**0.017**	**0.140**	**0.285/0.316**	**0.581**	**1**
A	A	0.534/0.577	0.156	1	0.517/0.587	0.050	0.396	0.579/0.547	0.592	1

Supplementary Table 8: fMRI results differential conditioning

Differential conditioning	**NR(CS+>CS-) > R(CS+>CS-)**						
rs17689918: GG(CS+>CS-) > A(CS+>CS-)		*Coordinates (MNI)*				*voxel*	*No.*
Male & Female	*Hem.*	*x*	*y*	*z*	*T*	*p(unc)*	*voxels*
MFG, IFG(oper.), IFG(tri.)	Left	-46	26	36	4.62	<0.001	563
MFG, IFG(tri.), IFG(oper.)	Right	50	36	30	4.44	<0.001	1071
MFG, SFG, Precentral gyrus	Right	34	16	48	3.92	<0.001	
MFG, IFG(oper.), IFG(tri.)	Right	54	24	38	2.75	0.003	
MCC, SMA, Paracentral gyrus	Left	-16	-24	52	3.82	<0.001	195
MFG, IFG(tri.), Inf. OFC	Right	52	44	8	3.58	<0.001	274
IFG(tri.), MFG, Inf. OFC	Right	44	40	0	3.41	<0.001	
STG, MTG, Rolandic operculum	Left	-50	-32	6	3.58	<0.001	546
Postcentral gyrus, Rolandic operc., Insula	Left	-42	-20	28	3.51	<0.001	
IPL, SPC, Postcentral gyrus	Left	-34	-50	50	3.34	0.001	363
Female							
Postcentral gyrus, Insula, SMG	Left	-38	-20	30	3.54	<0.001	171
IFG(tri.), MFG, IFG(oper.)	Left	-52	30	20	3.49	<0.001	285
IFG(tri.), MFG, IFG(oper.)	Left	-52	28	30	3.15	0.001	
rs17689966: AA(CS+>CS-) > G(CS+>CS-)							
Male & Female							
					n.s.		
Female							
MTG, MOC, AG	Left	-36	-58	14	3.66	<0.001	214
MTG, ITG, Fusiform gyrus	Left	-60	-36	-14	3.61	<0.001	381
ITG, MTG, IOC	Left	-60	-58	-14	3.36	<0.001	
MTG, ITG, IOC	Left	-62	-46	-12	3.33	0.001	
SFG, Precentral gyrus, MFG	Right	28	-4	72	3.48	<0.001	371
MFG, Precentral gyrus, IFG(oper.)	Right	56	14	40	3.38	<0.001	
MFG, Precentral gyrus, SFG	Right	46	-2	60	3.07	0.001	
Rol. operc., Insula, Heschls' gyrus	Left	-32	-34	18	3.48	<0.001	285
Rol. Operc., STG, SMG	Left	-36	-42	20	3.34	0.001	

SPC, Precuneus, MOC	Left	-20	-60	48	3.44	<0.001	553
SPC, SOC, Precuneus	Left	-18	-68	40	3.24	0.001	
SOC, MOC, Cuneus	Left	-22	-74	32	3.10	0.001	
Medial/superior OFC, SFC/MFC	Right	18	44	-8	3.37	<0.001	438
Caudate nucleus, Olfactory cortex	Right	-4	20	6	3.22	0.001	
Olfactory cortex, Rectal gyrus, OFC	Left	-6	18	-14	3.13	0.001	
MFG, Precentral gyrus, IFG(oper.)	Left	-48	16	44	3.32	0.001	574
IFG(tri.), MFG, IFG(oper.)	Left	-40	26	26	3.19	0.001	
Precentral gyrus, MFG, SFG	Left	-38	0	56	3.04	0.001	
PCC, PCC, Precuneus	Left/Right	-2	-42	22	3.21	0.001	376
Lingual gyrus, Precuneus, PCC	Right	8	-44	6	3.04	0.001	
Calcarine gyrus, Precuneus, PCC	Left	-10	-46	6	2.97	0.002	
IPL, SPC, AG	Left	-42	-58	56	3.10	0.001	216
IPL, AG, SPC	Left	-42	-52	48	3.04	0.001	

MFG: Middle frontal gyrus; IFG(oper.): Inferior frontal gyrus, pars.Opercularis; IFG(tri.): Inferior frontal gyrus, pars.Triangularis; IPL: Inferior parietal lobe;MCC: Middle cingulate cortex; PCC: Posterior cingulate cortex; SFG: Superior frontal gyrus; STG: Superior temporal gyrus; MTG: Middle temporal gyrus; ITG: Inferior temporal gyrus; AG: Angular gyrus; SPC: superior parietal cortex; SMG: Supramarginal gyrus; SOC: Superior occipital cortex; MOC: Middle occipital cortex; SMA: Supplemental motor area; IOC: inferior occipital gyrus;OFC: Orbito frontal cortex;

Supplementary Table 9: fMRI results safety learning

Safety learning	**R(CS-) > NR(CS-)**						
rs17689918: A(CS-) > GG(CS-)		*Coordinates (MNI)*			*voxel*	*voxel*	*cluster*
Male & Female		*x*	*y*	*z*	*t-value*	*p(unc)*	*No. vox.*
n.s.					n.s.		
Female							
Amygdala *		-16	-6	-18	2.43	0.008	27
rs17689966: G(CS-) > AA(CS-)							
Male & Female							
Post-/Precentral gyrus, SMG	Right	38	-20	34	4.12	<0.001	1431
SMG, Postcentral gyr., Rol. operc.	Right	52	-30	34	3.41	<0.001	
Post-/Precentral gyrus, IPL	Right	42	-28	54	3.17	0.001	
Insula, Rolandic operc., Putamen	Left	-32	-10	16	3.90	<0.001	1093
Insula, IFG(tri.), IFG(oper.)	Left	-28	20	12	3.79	<0.001	
MCC, SMA, SFG	Left	-14	-4	42	3.55	<0.001	
Amygdala, Hipp., Temp. pole	Right	36	-2	-24	3.82	<0.001	334
Inferior OFC, Temporal pole, Insula	Right	30	12	-22	3.47	<0.001	
Female							
Post-/Precentral gyrus, SMG	Right	38	-22	34	3.96	<0.001	710
SMG, Angular gyrus, IPL	Right	52	-38	32	3.22	0.001	
SMG, Postcentral gyrus, Rol.operc.	Right	58	-28	34	2.98	0.002	
Insula, Rol. operc., Putamen	Left	-32	-10	18	3.66	<0.001	251
MCC, SMA, SFC	Left	-16	-6	44	2.73	0.003	
MCC', Nucleus caudatus, SFC	Left	-20	-6	34	2.73	0.003	
Inf./sup. OFC, Parahippocampus	Right	22	14	-24	3.63	<0.001	210
Temp. pole, Parahipp.,	Right	28	18	-30	3.13	0.001	
Amygdala, Temporal pole, Hippocampus	Right	36	0	-26	2.94	0.002	
IPL, Angular gyr., Postcentral gyr.	Left	-30	-44	36	3.37	<0.001	401
SMG, STG, Rol. operc.	Left	-44	-36	24	3.25	0.001	
Rol. operc., Heschls gyrus, Insula	Left	-30	-30	16	3.04	0.001	

MFG: Middle frontal gyrus; IFG(oper.): Inferior frontal gyrus, pars.Opercularis; IFG(tri.): Inferior frontal gyrus, pars.Triangularis; IPL: Inferior parietal lobe;MCC: Middle cingulate cortex; PCC: Posterior cingulate cortex; SFG: Superior frontal gyrus; STG: Superior temporal gyrus; MTG: Middle temporal gyrus; ITG: Inferior temporal gyrus; AG: Angular gyrus; SPC: superior parietal cortex; SMG: Supramarginal gyrus; SOC: Superior occipital cortex; MOC: Middle occipital cortex; SMA: Supplemental motor area; IOC: inferior occipital gyrus;OFC: Orbito frontal cortex. *Amygdala activation has been explored using a ROI analysis at an uncorrected voxel level threshold of p<.05.

Supplementary Table 10: ASI vs. genotype analysis of associated *CRHR1* SNPs in the Replication Patient and Control sample. Linear regressions assuming an additive genotypic risk of the minor allele (d) was carried out (significant results in bold).

			Patients (n=292)		Controls (n=292)		Combined (n=584)	
SNP	**Allele (d)**	**Sample**	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**
rs17689918	A	Total	0.418	0.728	-0.350	0.642	2.169	**0.033**
		Female	-0.412	0.761	-1.073	0.244	2.423	**0.040**
		Male	2.557	0.333	1.528	0.232	1.249	0.536
rs17689966	G	Total	-0.020	0.985	-0.833	0.138	0.392	0.637
		Female	0.769	0.548	-1.108	0.096	11.233	0.252
		Male	-2.376	0.231	0.225	0.828	-1.798	0.261

Supplementary Table 11: ACQ vs. genotype analysis of associated *CRHR1* SNPs in the Replication Patient and Control sample. Linear regressions assuming an additive genotypic risk of the minor allele (d) was carried out (significant results in bold).

			Patients (n=292)		Controls (n=292)		Combined (n=584)	
SNP	**Allele (d)**	**Sample**	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**
rs17689918	A	Total	-0.018	0.762	-0.049	0.089	0.095	0.205
		Female	-0.065	0.356	-0.075	**0.034**	0.066	0.226
		Male	0.105	0.368	0.031	0.486	0.028	0.749
rs17689966	G	Total	-0.002	0.975	-0.064	**0.002**	-0.001	0.977
		Female	0.002	0.972	-0.079	**0.002**	0.017	0.715
		Male	-0.037	0.680	-0.01	0.766	-0.057	0.417

Supplementary Table 12: Functional characterization of rs17689918, rs17689966 (highlighted grey) and their proxy SNPs in high linkage disequilibrium (r^2 and D´=1). All Transcription Factors (TFs), shown in table has a core similarity of 0.75 and more. TFs expressed in the nervous system are written in bold. Changes in Splicing Regulatory Elements (SREs), like Splicing Enhancers (Srp40, SF2/ASF, SC35, SRp55, 9G8) and Splicing Inhibitors (hnRNP A1), such as potential splice sites was given for the especially minor and major alleles with a match similarity of minimal 70.0 and more.

Proxy SNP	Position (bp)	Allele (d/D)	localisatio	Gene ID	Coding SNPs	Transcription Factor (>70.0)		splicing enhancer/silencer (>70.0)		Splice sites (>70.0)	
						Minor-Allele	major-Allele	minor-Allele	major-Allele	minor	major
rs393152	41074926	G/A	nonsyn	C17orf6	Y>C; unknown	**MEL1.03**	**EVI1.05**	Srp40		Donor	
rs453997	41082844	T/C	intergenic	-			BARBIE.01				
rs241033	41089766	A/G	intergenic	-		ELF3.01	SPI1 PU1.03				
rs241031	41090087	C/T	intergenic	-		**OBOX5.01**, HNF1.01					
rs17760733	41102059	T/G	intergenic	-							
rs17687571	41105793	A/G	intergenic	-		**HRE.02**, **XBP1.01**	**BRACH.01**, NXF ARNT.01, **PAX4 PD.01**				
rs17687667	41109882	A/G	intergenic	-							
rs17687849	41115502	G/A	intergenic	-		THAP1.01, **NF1.01**					
rs17761207	41118038	C/T	intergenic	-		**E4BP4.01**, VMYB.04					
rs17688002	41118377	T/A	intergenic	-		**MEOX1.01**, HOXB6.01, MYBL1.01	BCL6.02, STAT.01				
rs17688056	41119024	T/C	intergenic	-		PAX2.01	HSF2.02, IRF4.03				
rs17688391	41127892	A/C	intergenic	-		OCT1.02					
rs17688434	41128323	A/G	intergenic	-		**TST1.01**, ISRE.01	CEBPB.01				
rs12150547	41131329	G/A	intergenic	-							
rs17688773	41133493	C/T	intergenic	-							
rs17688922	41135134	A/G	intergenic	-		MTF1.02, NMYC0.1, **HIF1.02**, TWIST.01,**DEC2.01**					

rs17688944	41135202	A/T	intergenic	-							
rs968027	41137033	T/C	intergenic	-							
rs17563501	41157478	T/C	intergenic	-			**NF1.03**				
rs2902662	41162708	A/G	intergenic	-		ISRE.01	BARBIE.01				
rs17563599	41163726	C/A	intergenic	-							
rs17563787	41169023	G/C	intergenic	-		**ARNT.01**, SPIB.01					
rs17563800	41173230	T/C	intergenic	-		**AARE.01**					
rs17563827	41173993	A/C	intergenic	-		ISRE.01, BLIMP1.01,IRF7.01	RFX1.01, FLI.01				
rs12150672	41182408	A/G	intergenic	-		**PAX6.01**	ARE.02				
rs17334894	41182980	A/G	intergenic	-							
rs17426195	41188138	A/G	intergenic	-		HNF6.01, LEF1.04, OC2.01	MTF-1.02				
rs11079724	41197680	T/C	intergenic	-							
rs4074462	41210994	T/G	intergenic	-							
rs17689471	41248753	C/T	intronic	CRHR1				2xSF2/ASF, Srp40, SRp55			Acceptor
rs17762769	41249183	A/G	intronic	CRHR1		LEF1.04	**TR2 TR4.01**, **RORA.01**, **COU.02**, SRRB.01				Acceptor, Donor
rs8072451	41249496	T/C	intronic	CRHR1		SSRF.03			SC35	Donor	Acceptor
rs4277389	41251434	G/A	intronic	CRHR1			**MEIS1.01**		Srp40		
rs4566211	41251477	A/G	intronic	CRHR1				Srp40		Acceptor	
rs17762954	41255567	T/C	intronic	CRHR1		CEBPA.01			SF2/ASF	Acceptor	
rs1396862	41258778	A/G	intronic	CRHR1					SF2/ASF, Srp55		
rs17689824	41260178	T/C	intronic	CRHR1		BTEB3.01, MTATA.01		Srp40			
rs17763086	41261262	G/T	intronic	CRHR1		**EGR1.01**					
rs17689882	41262609	A/G	intronic	CRHR1			**SIX.01**, MIF1.01	SC35	hnRNP A1		Acceptor, Donor
rs1876831	41263526	T/C	intronic	CRHR1			**GLIS3.01**				

rs16940665	41263677	C/T	syn	CRHR1		DMTE.01,HDBP1 2.01		SF2/ASF	Srp40		
rs17689918	41265869	A/G	intronic	CRHR1				SC35	SF2/ASF, SRp55		
rs17689966	41266236	G/A	intronic	CRHR1			PSE.01				
rs1876829	41267224	C/T	intronic	CRHR1				SF2/ASF, SC35, Srp40			
rs878886	41268271	G/C	3´-UTR	CRHR1		ZFX.01	TCFAP2B.01	SF2/ASF, 9G8			
rs17763533	41273970	C/T	intergenic	-		MAZ.01					
rs17763596	41276990	T/G	intergenic	-		SMARCA3.01	**FTF.01**, ESRRA.01				
rs17763634	41277534	C/T	intergenic	-		ZNF35.01					
rs242944	41278960	C/T	nonsyn	IMP5	H>R; benign	**AXCREB.02**	ZNF76 14 3.01	SF2/ASF		Acceptor	Acceptor, Donor
rs12185268	41279463	G/A	nonsyn	IMP5	I>V; benign			SF2/ASF, SC35, Srp40,hnRNP A1		Donor	Acceptor
rs12373123	41279853	C/T	nonsyn	IMP5	S>P; possibly	TCFAP2B.01	**MYT1L.01**	SF2/ASF, SC35			
rs12373139	41279910	A/G	nonsyn	IMP5	G>R; possibly	GCM1.03	**OLF1.02**	hnRNP A1			
rs12373168	41280117	C/A	3´-UTR	IMP5			LACTOFERRIN.01	SC35, 9G8	Srp40		
rs916793	41310477	A/G	intergenic	-			STAT6.01				
rs4441322	41310821	G/A	intergenic	-		TCFE2A.02	VBP.01, HOXB3.01				
rs17691328	41311278	T/C	intergenic	-							
rs17691610	41326456	T/G	intergenic	-		**RORA.01**, ER.04					
rs1864325	41333623	T/C	intronic	MAPT		VTATA.01					
rs2082068	41335767	T/C	intronic	MAPT		PSZ1.01					
rs17563986	41347100	G/A	intronic	MAPT		PAX3.01					
rs17649635	41351818	G/A	intronic	MAPT		AML1.01					
rs17649641	41353200	C/T	intronic	MAPT		OVOL1.01					
rs17564223	41353348	T/C	intronic	MAPT		**IRX5.01**	MTF-1.01,HAS.01				
rs17649700	41353729	C/G	intronic	MAPT							
rs1467969	41354156	T/C	intronic	MAPT		PIT1.01	HMX2.02				
rs17564493	41357207	T/C	intronic	MAPT		TEF.01, **PBX**					

rs17649954	41357489	G/A	intronic	MAPT		**BAPX1.01**	**IRX6.01**				
rs17564619	41357966	G/A	intronic	MAPT		SP2.01	FXRE.01				
rs17650063	41358383	G/A	intronic	MAPT							
rs17564703	41358423	T/C	intronic	MAPT		AP2.01	SP1.02				
rs17564780	41361241	G/A	intronic	MAPT		ZID.01					
rs17564829	41362429	C/T	intronic	MAPT		PAX3.02	**PKNOX1.01**				
rs12150111	41369767	G/A	intronic	MAPT							
rs4327091	41377578	A/G	intronic	MAPT			**XBP1.01**				
rs17571718	41388634	C/T	intronic	MAPT							
rs17571739	41388781	C/T	intronic	MAPT			AML3.01				
rs17571857	41391544	G/A	intronic	MAPT		HSF1.03					
rs17650860	41394844	A/G	intronic	MAPT			DINR.01				
rs17650872	41395352	T/G	intronic	MAPT		**WHN.01**					
rs17650901	41395527	G/A	5´-UTR	MAPT							
rs17572169	41401810	T/C	intronic	MAPT							
rs1800547	41407682	G/A	intronic	MAPT							
rs17651213	41407760	A/G	intronic	MAPT		OCT3 4.02	GZF1.01				
rs17572361	41407845	C/T	intronic	MAPT		HIC1.02	HNF1.04				
rs1981997	41412603	A/G	intronic	MAPT		HSF1.01,DMP1.01, ZNF217.01					
rs17651549	41417115	T/C	nonsyn	MAPT	R>W;probably	AP4.02		SRp55			Acceptor
rs17572851	41419603	G/A	intronic	MAPT							
rs17572893	41420045	A/G	intronic	MAPT		**EVX1.01**, HNF6.01, LEF1.04, CLOX.01	NFY.02, **GSH2.01**, PREB.01				
rs1052551	41424761	A/G	syn	MAPT		ZKSCAN3.01, ZNF202.01		Srp40	SC35		
rs17573175	41426926	G/C	intronic	MAPT		**SF1.01**, DBP.01	**ARNT.01**				
rs1052553	41429726	G/A	syn	MAPT							Acceptor
rs17652121	41429810	C/T	syn	MAPT		FLI.01		SRp55			
rs1078269	41431674	C/T	intronic	MAPT		E2F3.02	PLAGL1.02				

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