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Neural Mechanism of a Sex-Specific Risk Variant for Posttraumatic Stress Disorder in the Type I Receptor of the Pituitary Adenylate Cyclase Activating Polypeptide

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ABSTRACT

BACKGROUND: Posttraumatic stress disorder (PTSD) is a frequent anxiety disorder with higher prevalence rates in female patients than in male patients (2.5:1). Association with a single nucleotide polymorphism (rs2267735) in the gene *ADCYAP1R1* encoding the type I receptor (PAC1-R) of the pituitary adenylate cyclase activating polypeptide has been reported with PTSD in female patients. We sought to identify the neural correlates of the described PAC1-R effects on associative learning.

METHODS: In a reverse genetic approach, we examined two independent healthy samples (N_1 = 112, N_2 = 73) using functional magnetic resonance imaging during cued and contextual fear conditioning. Skin conductance responses and verbal self-reports of arousal, valence, and contingency were recorded.

RESULTS: We found that PAC1-R modulates the blood oxygenation level–dependent response of the hippocampus. Specifically, we observed decreased hippocampal activity during contextual, but not during cued, fear conditioning in female participants carrying the PAC1-R risk allele. We observed no significant differences in conditionability for skin conductance responses, verbal reports, or activation in other brain regions between the genotype groups in female participants.

CONCLUSIONS: Our results suggest that impaired contextual conditioning in the hippocampal formation may mediate the association between PAC1-R and PTSD symptoms. Our findings potentially identify a missing link between the involvement of PAC1-R in PTSD and the well-established structural and functional hippocampal deficits in these patients.

Keywords: *ADCYAP1R1*, Amygdala, Fear conditioning, Functional magnetic resonance imaging, Hippocampus, PTSD

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Learning to cope with environmental stressors is a highly adaptive process. However, this process can become maladaptive, such as in patients with anxiety disorders such as posttraumatic stress disorder (PTSD). Women have a higher prevalence than men for many anxiety disorders (female-to-male ratio for PTSD = 2.5:1) (1). Why only some individuals (and women to a higher degree) develop anxiety symptoms after a traumatic experience, whereas others readjust is poorly understood. Interindividual differences, such as prior learning history or genetic background, likely influence the ability to deal with stressful experiences. Heredity has a partial role in the development of PTSD (38%–41%) (2), and several genes have been found to be associated with this disorder (3). A single nucleotide polymorphism (rs2267735) in the type I receptor (PAC1-R) of the pituitary adenylate cyclase activating polypeptide (PACAP) was found to be predictive of PTSD symptoms in female patients, but not male patients. It could also be associated with impaired fear discrimination in healthy female, but not male, participants (4).

PACAP exerts pleiotropic functions in the body. Most importantly, it has been found to regulate neuroendocrine stress circuits (5) and has been implicated in associative learning processes such as context conditioning (6,7) that have also been implicated in PTSD (8). The reported sex-specific effects may occur via the regulation of the PAC1-R encoding gene *ADCYAP1R1* by estrogen (4). Compatible with its role in coping with stress and learning, PAC1-R is predominantly expressed in brain structures such as the neocortex or the limbic system. The hippocampus is one of the limbic structures with the highest PAC1-R concentrations (9), and dysfunction in the hippocampus has been related to PTSD (10). The hippocampus also influences the bodily stress response (11) and is involved in associative learning, specifically in contextual conditioning (12). Impairments in hippocampus-dependent contextual fear conditioning have been shown in PAC1-R-deficient mice (7,13). Most probably PAC1-R modulates associative hippocampal learning through

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its involvement in a form of long-term potentiation of mossy fiber-CA3 synapses (13). Later experiments additionally implicated PAC1-R in anxiety-like behavior in rats and mice and in healthy humans using cued fear conditioning paradigms (4). Women, but not men, with higher PACAP blood levels showed higher overall acoustic startle reflexes and impaired discrimination of cues signaling safety or danger during the acquisition of cued fear conditioning, which is in line with findings in patients with PTSD (14) and could additionally be replicated in healthy female participants carrying the PAC1-R risk allele (4). For mice, increased PACAP and PAC1-R messenger RNA (mRNA) expression was reported in the amygdala during consolidation, which reflects acute fear responses and is assumed to model PTSD (15). Similar to the hippocampus, the amygdala is one of the limbic structures with high PAC1-R concentrations (9), which is involved in the regulation of the bodily stress response (11), is essential for associative learning (i.e., cued and contextual fear conditioning) (16) and is related functionally as well as structurally to PTSD (10,17).

In the present study, we tested the hypothesis that PAC1-R influences learning relevant for PTSD. We employed an imaging genetics approach using contextual and cued fear conditioning (heritability 35%–45% (18)) in healthy volunteers. During fear conditioning, an originally neutral stimulus or context is presented several times together with an unconditioned threat stimulus (US) and subsequently becomes a conditioned stimulus (CS) or context (CXT), which elicits conditioned fear or anxiety responses (CR). Conditioned cues evoke phasic fear responses, whereas contexts lead to sustained anxiety responses (19). Patients with PTSD show contextual deficits (20), which also represents a vulnerability factor for the development of anxiety disorders such as PTSD (21). During cued fear conditioning, heightened physiologic reactions were observed in patients with PTSD (14).

If dysfunctional fear conditioning is relevant for PTSD, patients with PTSD should show alterations in the brain circuits involved in contextual and cued fear conditioning, and these changes should be apparent in risk allele carriers. Both patients with PTSD and PAC1-R-deficient mice show impairments in hippocampus-dependent contextual fear conditioning (7,8,13), with reduced and potentially compensatory enhanced activation. We expected to see altered activity in the hippocampus in female risk allele carriers during contextual fear conditioning in an additive model. For cued fear conditioning, we expected heightened amygdalar activation in female risk allele carriers, given the described animal research showing increased PACAP and PAC1-R mRNA expression in the amygdala, which reflects acute fear responses and is assumed to model PTSD (15). In addition, amygdalar activation in cued fear conditioning has been shown to be enhanced in PTSD (22). We were interested in the effect of PACAP on brain activations in male participants, which we tested in an explorative manner.

METHODS AND MATERIALS

Participants

The initial study comprised 112 participants (39 female) of Caucasian descent (discovery sample). The volunteers were recruited from training schools for rescue workers (who have a heightened risk to experience traumatic events) in the course of a longitudinal study on predictors of PTSD. Imaging data from the entire sample have already been published with a focus on genetic polymorphisms for alcoholism (cue conditioning (23)) and for schizophrenia (context conditioning (24)). The second study cohort of 72 participants (24 female) took part in the same longitudinal study as a second wave (replication sample). Participants with a mental disorder, as determined by the German version of the Structured Clinical Interview for DSM-IV (25), were excluded from the study. Sample characteristics are presented in Table S1 in Supplement 1.

The study was approved by the Ethics Committee of the Medical Faculty Mannheim of Heidelberg University, and written informed consent was obtained before participation. The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki, sixth revision, 2008).

DNA Extraction and Genotyping

Based on standard procedures, genomic DNA was prepared from whole blood. Using a TaqMan 5′ nuclease assay, rs12807809 was genotyped. By duplicating 15% of the original sample, we assessed accuracy, and reproducibility was 100%. The allele frequencies for the female discovery sample (GG, $n = 15$; GC, $n = 18$; CC, $n = 6$) and for the male sample (GG, $n = 19$; GC, $n = 38$; CC, $n = 16$) did not deviate from Hardy-Weinberg equilibrium ($p_{female} = 1.0$ and $p_{male} = 1.0$). The same was true for the entire sample ($p_{all} = 1.0$). The allele frequencies for the replication sample (female participants, GG, $n = 9$; GC, $n = 8$; CC, $n = 7$; male participants, GG, $n = 17$; GC, $n = 24$; CC, $n = 7$) did not deviate from Hardy-Weinberg equilibrium ($p_{female} = .11$ and $p_{male} = 1.0$). The same applied to the entire sample ($p_{all} = .47$).

Neuropsychological and Clinical Assessment

Neuropsychological assessments were conducted to ensure comparability between the groups. We screened intelligence using the German version of the Culture Fair Intelligence Test (26). Memory performance was assessed by the California Verbal Learning Test (27). To control for trait anxiety as a potential confounder of conditioning, we used the trait version of the State–Trait Anxiety Inventory (28).

Experimental Procedure

Unconditioned Threat Stimulus. An electrical stimulus served as US and was delivered by a copper electrode to the right thumb, using an electrical stimulus generator (DS7A; Digitimer Ltd, Welwyn Garden City, United Kingdom). We administered increasingly intense stimuli (50-ms bursts, 12 Hz) starting with a mild stimulus until each participant indicated it as “painful” (pain threshold) and then further until the pain became unbearable (pain tolerance). After threefold repetition, we averaged values of the last two trials. Next, each volunteer received a painful stimulus, starting at 80% between the threshold and the tolerance level, which had to be rated with respect to the intensity and unpleasantness on two Likert scales ranging from 0 (not painful or unpleasant) to 10 (extremely painful or unpleasant). The stimulation intensity

was adjusted until the ratings on the intensity and on the unpleasantness scale reached a level of ~7.

To guarantee the safety of participants, all were extensively informed beforehand and monitored constantly during the entire pain procedure (quick abortion was possible at all times without any negative consequences). Also, the stimulus generator has a special certification within the boundaries of the law for medical products for medical use with very high safety standards. The procedure has been used in earlier work from our laboratory (12,23,24).

Contextual Fear Conditioning. As described earlier (24), contextual fear conditioning comprised four phases: habituation, early acquisition, late acquisition, and extinction. In line with previous studies, two different spatial contexts (CXT+/CXT−) were represented using two colors (orange and blue), which "surrounded" the subject by illuminating the entire scanner (Figure S1 in Supplement 1). To enhance a complex processing of the employed stimuli and to reinforce the feeling of context, the colors were slowly blended in. After having reached their full spectrum (for 3–12 seconds), the respective contextual color stimulus changed into the next color. The CXT+/CXT− succession was randomly assigned (but identical for each subject), and the colors serving as CXT+ were counterbalanced across volunteers. Using a mirror system to project the stimuli into the magnetic resonance tomograph, a surround color (i.e., an actual context) was realized.

During each context conditioning phase, CTX+ and CTX− were displayed (3–12 seconds) 10 times in random order. The US (2.9 seconds) occurred during the habituation phase 10 times in the interstimulus interval (4–12 seconds); during acquisition, the US was coupled to half of the CTX+ (50% reinforcement rate). To maximize unpredictability, US onset was randomly assigned over the time course of the CXT+ (3–8 seconds after CS+ onset), which produces greater context conditioning (29,30). CTX− was never accompanied by the US (safe condition). During extinction, no US was presented. The participants were told to view the stimuli passively and were uninformed about the CXT-US contingency.

Cued Fear Conditioning. The study participants took part in contextual and cued fear conditioning in counterbalanced order with structural brain measurements interspersed. As for contextual fear conditioning, the protocol consisted of habituation, early acquisition, late acquisition, and extinction. In contrast, two colored geometric shapes (square and diamond) were used as CS+/CS−, which had a clear onset and offset. Colors were counterbalanced across participants, and the CS+/CS− sequence was pseudorandomized.

During habituation, 10 CS+ and CS− were presented for 6 seconds, and 4 US (during the interstimulus interval, 7–12 seconds) were presented for 2.9 seconds. The acquisition and extinction phases comprised 18 presentations of each CS. During acquisition, the US was coupled to 50% of the CS+ (starting 3.1 seconds after cue onset). Stimuli were projected on a screen in the scanner room visible to the participants via a mirror system.

Data Acquisition and Analysis

Skin conductance response (SCR) data were analyzed according to standard procedures as described earlier (24). The self-report scales, for arousal and emotional valence, were based on the Self-Assessment Manikin (31). Additionally, perceived contingency between the CS and US (1 = no CS-US contingency to 9 = perfect CS-US contingency) was rated after each conditioning phase. Functional scans for the discovery and replication sample were acquired with 1.5-tesla and 3-tesla scanners (Siemens AG, Erlangen, Germany), respectively, using an echo planar imaging sequence and analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm). We used the general linear model to investigate genotype effects on the neural response to cue and context conditioning. For the hippocampus and amygdala, region of interest masks from the Wake Forest University (Automated Anatomical Labeling atlas) PickAtlas v3.0.3 (32) were employed. Details on the methods, analyses, and results are provided in Supplement 1.

RESULTS

Neuropsychological and Clinical Assessments and Magnetic Resonance Imaging Data

Although we counterbalanced the order of the two conditioning procedures, we tested for potential differences between the genotype groups, which yielded nonsignificant results for female [$\chi^2_2 = 1.34$, $p = .51$] and male [$\chi^2_2 = 2.21$, $p = .33$] participants for the entire sample. Separated for the two studies, we observed in the discovery sample nonsignificant results for female [$\chi^2_2 = .19$, $p = 1.00$] and male [$\chi^2_2 = .91$, $p = .63$] participants. In the replication sample, a comparable picture emerged for the female [$\chi^2_2 = 2.53$, $p = .35$] and male [$\chi^2_2 = 1.88$, $p = .41$] participants.

In the discovery sample, the three genotype groups did not differ significantly with respect to age or sex ratio. Levels of intelligence (Culture Fair Intelligence Test), trait anxiety (State–Trait Anxiety Inventory), and hippocampus-dependent memory (California Verbal Learning Test) were comparable between the groups (Tables S2 and S3 in Supplement 1). For the general task-related brain activations during late acquisition of contextual fear conditioning, we observed significant whole-brain activations (familywise error corrected, $p < .05$) for the CXT+ > CXT− contrast in a network comprising the cerebellum and parietal, occipital, and frontal lobes (Table S4 in Supplement 1 presents general task-related brain activations during cued and contextual fear conditioning). We found significant hippocampal and amygdalar responses in the entire discovery sample (Figure S2 in Supplement 1).

SCRs and Self-Report Data

Successful fear conditioning was shown by SCRs and verbal self-reports and did not differ between allele groups (Supplement 1).

Functional Magnetic Resonance Imaging Results During Fear Conditioning

During contextual fear conditioning, significant hippocampal and amygdalar activation was present in all participants ($N = 112$, 39 females). Female carriers of the PAC1-R risk allele for PTSD showed a significantly reduced gene-dose–dependent activation in the left hippocampus during late

acquisition ($t = 4.06$, $p = .038$) (Figure 1A,B). In contrast, we did not find any significant association of PAC1-R with amygdalar activation during contextual conditioning in female participants. During early acquisition and extinction, we observed no significant effects of genotype for female participants. We replicated the results from the discovery sample. Female carriers of the PAC1-R risk allele in the replication sample again showed significantly reduced left-sided hippocampal activation during late acquisition ($t = 4.92$, $p < .001$) (Figure 1C,D). Additionally, no other brain region was found to be significantly influenced by PAC1-R genotype at an exploratory threshold ($p_{uncorrected} < .001$) for female participants, suggesting regional specificity of our result.

For the male participants, we observed a dose-dependent effect in the right hippocampus ($t = 3.85$, $p = .036$) (Figure 2A,B) during late acquisition (i.e., male carriers of the PAC1-R risk allele showed significantly increased right-sided hippocampal activation). In contrast, we did not find any significant association of PAC1-R with amygdalar activation during contextual conditioning in male participants. During early acquisition and extinction, we observed no significant effects of genotype for male participants. Additionally, no other brain region was found to be influenced by PAC1-R genotype at an exploratory threshold ($p_{uncorrected} < .001$) for male participants, suggesting regional specificity of this result. For cued fear conditioning, we did not observe any significant influence of PAC1-R on amygdalar or hippocampal activity in male participants. In the replication analysis with 48 healthy participants of Caucasian descent (from a second cohort), we found only a trend toward significance in the right hippocampus ($t = 2.54$, $p = .086$) (Figure 2C,D) during late acquisition.

Based on the observed findings in female and male participants, we decided also to investigate sex-genotype interactions in a general model using gender as a covariate

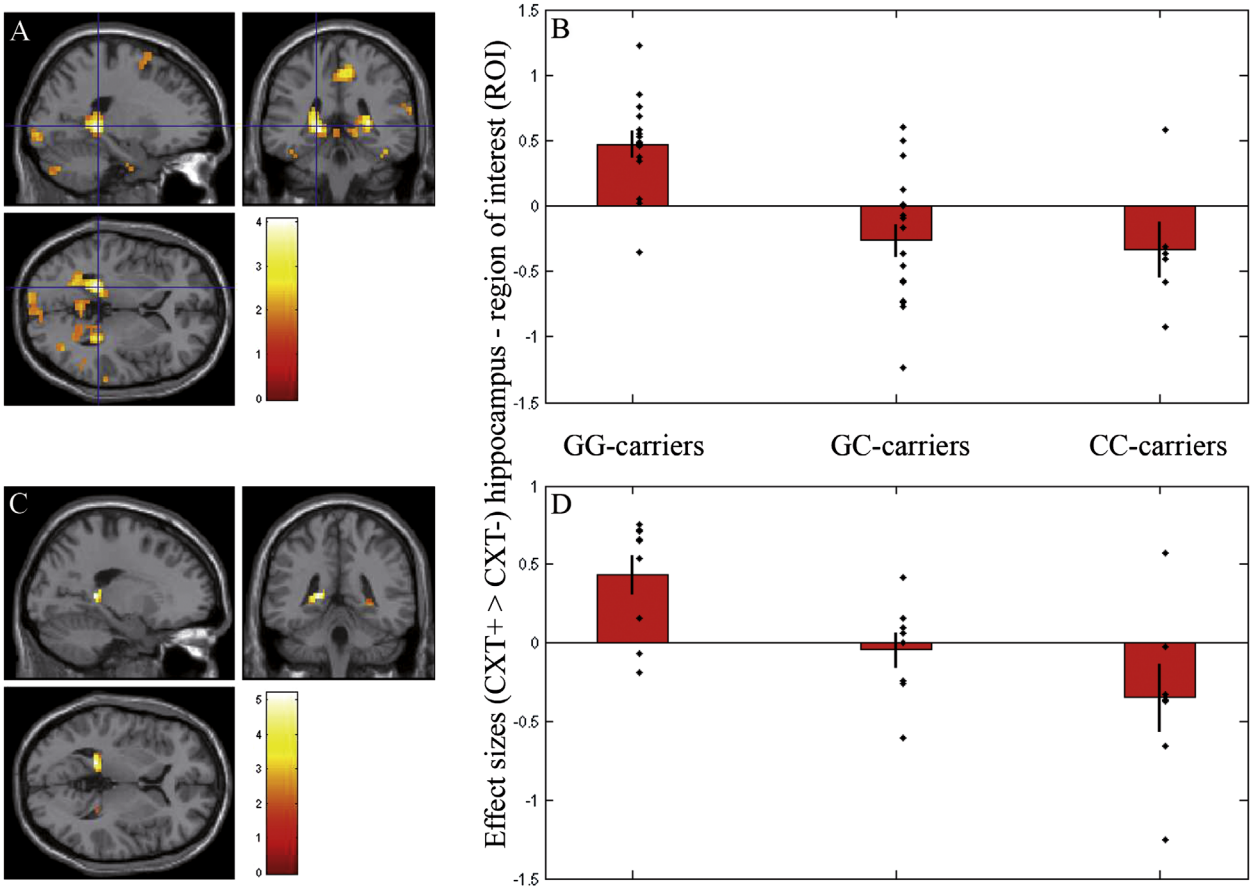

Figure 1. Relationship between the type I receptor of the pituitary adenylate cyclase activating polypeptide (PAC1-R) and hippocampal activation in female participants. **(A)** Discovery study ($n = 39$). Significant gene-dose effect during late acquisition of contextual fear for the CXT+ > CXT− contrast with carriers of two rs2267735 risk alleles (CC) showing the least hippocampal activation and GG carriers showing the most hippocampal activation. The peak voxel at x = −20, y = −37, z = 2, k = 20; $t = 4.06$, $p = .038$ is familywise error corrected for the region of interest. Colors indicate *t* scores, shown at a threshold of $p_{uncorrected} < .05$. **(B)** Discovery study. Effect sizes (CXT+ > CXT−) in the hippocampus region of interest separately for the three PAC1-R allele groups during late acquisition (error bars indicate SEM). **(C)** Replication study ($n = 24$). Significant gene-dose effect during late acquisition of contextual fear for the CXT+ > CXT− contrast with carriers of two rs2267735 risk alleles (CC) showing the least hippocampal activation and GG carriers showing the most hippocampal activation. The peak voxel at x = −18, y = −41, z = 4, k = 36; $t = 4.92$, $p < .001$ is familywise error corrected for a 6-mm sphere around the peak voxel from the discovery study. **(D)** Replication study. Effect sizes (CXT+ > CXT−) in the hippocampus sphere separately for the three PAC1-R allele groups during late acquisition (error bars indicate SEM). ROI, region of interest.

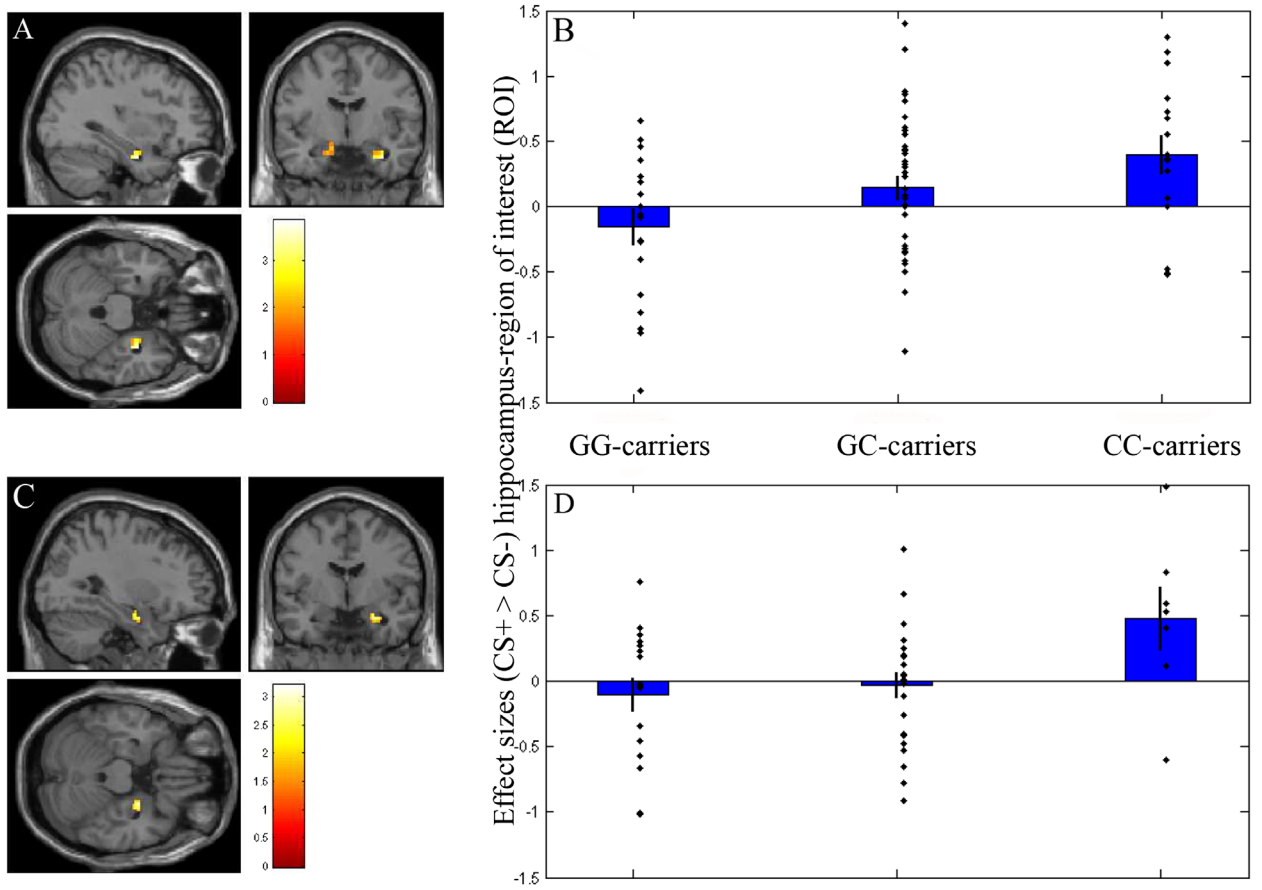

Figure 2. Relationship between type I receptor of the pituitary adenylate cyclase activating polypeptide (PAC1-R) and hippocampal activation in male participants. **(A)** Discovery study ($n = 72$). Significant gene-dose effect during late acquisition of contextual fear for the CXT+ > CXT− contrast with carriers of two rs2267735 CC alleles showing the highest hippocampal activation and GG carriers showing the lowest hippocampal activation. Significantly increased hippocampal activation during late acquisition of contextual fear in carriers of the rs2267735 risk allele (CC, $n = 16$) compared with heterozygotes (CG, $n = 38$) and GG carriers ($n = 19$) for the CXT+ > CXT− contrast. The peak voxel at x = 34, y = −7, z = −26, k = 15; $t = 3.85$, $p = .036$ is familywise error corrected for the region of interest. Colors indicate t scores, shown at a threshold of $p_{uncorrected} < .05$. **(B)** Discovery study. Effect sizes (CXT+ > CXT−) in the hippocampus region of interest separately for the three PAC1-R allele groups during late acquisition (error bars indicate SEM). **(C)** Replication study ($n = 48$). Gene-dose effect (trend level only) during late acquisition of contextual fear for the CXT+ > CXT− contrast with carriers of two rs2267735 CC alleles showing the highest hippocampal activation and GG carriers showing the lowest hippocampal activation. The peak voxel at x = 30, y = −4, z = −23, k = 5; $t = 2.54$, $p = .086$ is familywise error corrected for a 6-mm sphere around the peak voxel from the discovery study. **(D)** Replication study. Effect sizes (CXT+ > CXT−) in the hippocampus sphere separately for the three PAC1-R allele groups during late acquisition (error bars indicate SEM). ROI, region of interest.

in SPM. This model yielded no significant result for the hippocampus or any other brain region (familywise error corrected $p > .94$). For cued fear conditioning, no significant influence of PAC1-R on amygdalar or hippocampal activity in female or male participants was observed.

DISCUSSION

The development of PTSD after a traumatic experience is thought to depend critically among other factors on the genetic vulnerability of an individual. In the present study, we investigated the effect of a known vulnerability gene in participants without PTSD. Although participants experienced no trauma, we expected that risk gene carriers would react differently to nonrisk carriers. Although no effect was seen on the behavioral level, we found that female carriers of the PAC1-R risk allele for PTSD showed gene-dose–dependent, diminished hippocampal activation during contextual fear conditioning in both studies, which is well in line with the previously reported increased PTSD symptoms in female risk allele carriers (4) and the more recent notion that genetic variants are more penetrant at the level of brain measures (33). Additionally, our findings indicate specificity of the neural mechanism mediating the PAC1-R–PTSD association. Despite modulation of hippocampal activity by the PAC1-R genotype, we found no evidence for other relevant brain regions, even on an exploratory threshold.

We explored if PAC1-R genotype would have an impact on neural activity in male participants. We found the opposite pattern—risk allele carriers showed gene-dose–dependent, significantly increased hippocampal activity during late contextual acquisition. However, this result was reproducible only on a trend level in the replication sample. The test for a potential sex-genotype interaction yielded no significant

results. This outcome most likely was due to the different locations in men (right-sided) and women (left-sided). We conclude that the reported PACAP-PTSD association (4) is mediated via the hippocampus in women in a dose-dependent manner, whereas this link could not be found in male patients. Nevertheless, PAC1-R genotype has an impact on hippocampal activation in men, but possibly via a different mechanism. Further studies are needed to address this question, which is beyond the scope of this study.

Although we observed PAC1-R-dependent hippocampal activation differences in both sexes during late contextual fear acquisition, we found no significant differences during habituation, early acquisition, or extinction. This finding is well in line with earlier work from our group on this paradigm, where the strongest hippocampal activation was observed during this phase (12). During cued fear conditioning, participants learn to differentiate CS+/CS− already during early acquisition (23), most probably owing to the fact that US onset is unpredictable in a context conditioning paradigm compared with a cue conditioning paradigm. Our data suggest that PAC1-R plays a role in the acquisition of fear rather than extinction learning; this might be related to the observation that not "pure" hippocampal activations, but hippocampal-prefrontal interactions, are important for contextual extinction (34). We found no evidence for an impact of PAC1-R on prefrontal areas.

In contrast to our hypotheses, we did not observe any association between PAC1-R and cued fear conditioning in acquisition or extinction. Although this finding is not in line with the data from Ressler *et al.* (4), it replicates earlier research showing impairments in contextual fear conditioning but intact cued fear learning in PAC1-R-deficient mice (13). Ressler *et al.* (4) recorded startle responses to the CS+ and CS−, whereas in the present study (owing to the scanner environment), only SCRs were used. This methodologic difference might have contributed to discrepancies in the results on the relationship of the PAC1-R polymorphism and cued fear conditioning because acoustic startle responses could potentially be more sensitive to detect subtle differences (35). We found only a genetic influence on hippocampal activity, a brain structure not essential for simple cue conditioning paradigms (16).

Since their initial report in 2011, Ressler *et al.* replicated (36) and extended (37) their findings. Male and female children carrying the PAC1-R risk allele showed pronounced dark-enhanced startle responses suggesting genetic vulnerability to be evident in both sexes during development but only in women after adolescence because of changes in the estrogen system. The first replication attempts from an independent laboratory using two large population-based samples were unsuccessful (38); however, a less traumatized cohort (39) was used. The authors also studied gene-environment interaction with the assessment of childhood maltreatment in a large sample of American women and tested for interaction effects with the PAC1-R polymorphism to predict past month PTSD and PTSD severity. They observed a significant PAC1-R × childhood maltreatment interaction in the hypothesized direction—that is, women with a history of childhood maltreatment are at a higher risk to develop PTSD if they are risk allele carriers (40). These authors also reported a relationship between the risk genotype and increased reactivity of the amygdala and hippocampus in traumatized African-American women to fearful versus neutral faces in a functional magnetic resonance imaging investigation (41). Another independent study in Chinese earthquake survivors with PTSD observed higher emotional numbing symptoms in female risk allele carriers (42). Our results contribute to these findings by providing a neural mechanism for the PAC1-R–PTSD association in female risk allele carriers with the hippocampus as key component and by extending the role of the PAC1-R in fear learning to male subjects as well.

This study has several limitations. Given the small sample size in the group of female risk allele carriers, one might question the robustness of the results. However, the replicability of our initial finding argues in favor of reliable differences. Still, we cannot rule out the possibility that we might have detected additional significant differences in other brain regions or on a behavioral level with larger sample sizes. Although we counterbalanced the order of the cued and contextual fear conditioning paradigms, one cannot rule out the possibility of transfer effects from the first to second learning or even interactions of this transfer with variables such as age or intelligence. A more detailed analysis (trial by trial) of the limited number of SCRs (63 of $N = 112$) was impossible because of a technical artifact in the scanner resulting in missing values during acquisition and extinction. Also, although we were able to replicate our results, the second cohort was measured in a different scanner (1.5-tesla vs. 3-tesla), limiting the direct comparability of the two cohorts. Although we investigated healthy participants without PTSD, this allowed the investigation of a sample unconfounded by factors typically present in patients, such as medication or different disorder subtypes. We did not control for phase of menstrual cycle or contraceptive medication in female participants. Future studies should address this issue, given that estrogen functions as a potential regulator of *ADCYAP1R1* (4) and can potentially influence fear conditioning (43).

In conclusion, our data revealed that hippocampal activity during contextual fear conditioning is affected by a sex-specific PAC1-R genotype effect. The results might form the missing link between the reported hippocampal impairments (e.g., hippocampal hypoactivation in PTSD (10)) and the previous finding that female patients with PTSD carrying the PAC1-R risk allele have higher total symptom scores (4). Because healthy female risk allele carriers show diminished hippocampal activation during fear learning, but no significant behavioral effect could be detected, it is assumed that this difference to noncarriers of the risk allele becomes more pronounced and behaviorally relevant after traumatic experiences. This assumption would fit the postulated higher penetrance of genetic variants on brain measures (33) and behavior differences in female risk allele carriers with PTSD (4). These findings contribute to our understanding of the neural mechanisms behind the association of PAC1-R and PTSD and sex-specific differences in PTSD and aversive contextual associative learning processes in general. Because this was a healthy sample, we do not know what the observed altered brain activation patterns in the high-risk groups mean with respect to clinical populations. Although our results fit the data

in patients with PTSD, it is possible that we recruited a biased sample, as suggested by previous studies on fire workers who were more resilient than the general population (44). Further research is needed to understand how other individual and environmental factors influence PAC1-R functioning and its relationship to PTSD.

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