

PKC α is genetically linked to memory capacity in healthy subjects and to risk for posttraumatic stress disorder in genocide survivors

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Abstract: Strong memory of a traumatic event is thought to contribute to the development and symptoms of posttraumatic stress disorder (PTSD).

Emotional experiences are typically well remembered, but there is a large, partly genetically controlled, variability for this phenomenon (1). On the one hand, enhanced memory for emotionally arousing events can be seen as an adaptive mechanism, which helps us to remember important information (2). On the other hand, strong memory of an extremely aversive event may contribute to the development and symptoms of posttraumatic stress disorder (PTSD) (3–6). In a previous study we reported that a deletion variant of the *ADRA2B* gene was significantly associated with emotional memory in healthy humans and with traumatic memory in a traumatized population, but not significantly with the risk for PTSD (1). Thus, so far, there is no evidence indicating that genetic factors that predispose individuals to build strong aversive memories could also be risk factors for PTSD.

Considerable evidence suggests that protein kinases, in particular protein kinase A (PKA), protein kinase C (PKC), Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), and mitogenactivated protein kinase (MAPK), play an important role in the formation of emotional memory in animals (7, 8). To study whether the genes encoding these protein kinases are also related with human emotional memory, we applied a behavioral genetics approach and captured the variability of these genes with 2,005 singlenucleotide polymorphisms (SNPs) (*Methods* and *SI Appendix*).

The 2,005 selected SNPs were analyzed in an initial sample of 723 young healthy Swiss adults (476 females, 247 males; median age, 22 y; range, 18–35 y), who underwent memory testing. Subjects were presented 24 neutral, 24 positive, and 24 negative photographs in a random order. The photographs were taken from the international affective picture system (IAPS) (9) and presented for 2.5 s each. Immediately following the presentation of each photograph, subjects were asked to rate it for valence and arousal using the IAPS rating scales. After a delay of 10 min, during which subjects performed an *n*-back working memory task, subjects underwent a surprise free recall test of the previously presented pictures. Because we were interested in the link between aversive memory and PTSD, our target phenotype for the genetic association study was the number of freely recalled negative (aversive) pictures.

Results

Hypothesis Testing and Replication Sample

The analysis including all 2,005 SNPs revealed that SNP rs4790904, which is located within *PRKCA* (encoding protein kinase C α), was significantly associated with memory for negative pictures after Bonferroni correction for multiple comparisons ($P_{\text{uncorrected}} = 0.000002$, $P_{\text{corrected}} = 0.004$; Table 1 and

Table S1). There were no further Bonferroni-corrected significant SNPs. SNP rs4790904 was also significantly associated with memory for positive and neutral pictures (Table 1). Sex did not influence the association of rs4790904 with negative memory (sex \times SNP interaction $P = 0.5$). This SNP was not associated with valence or arousal ratings of the pictures ($P \geq 0.8$; Table S2), indicating that the genotype-dependent differences in memory for negative pictures were not due to genotype-dependent differences in emotional arousal. Furthermore, rs4790904 was not associated with attention or working memory performance ($P \geq 0.6$; Table S3). In addition to the findings on short-term memory, analysis of data on free recall 24 h after picture presentation (available in the hypothesis-testing sample only) also revealed significant genotype-dependent differences in memory performance (Table S4).

SNP rs4790904 was further investigated in an independent sample of 394 healthy Swiss subjects, who performed the same tasks in a brain scanner (Methods). In this population, we could replicate the association of rs4790904 with memory for negative information ($P = 0.028$; Table 2). SNP rs4790904 was also significantly associated with memory for positive pictures ($P = 0.005$; Table 2), whereas the association with memory for neutral pictures was not significant ($P = 0.284$; Table 2). The direction of effect and the genetic model used (i.e., additive) were the same as in the initial discovery sample.

Table 1. Genotype-dependent memory performance in the hypothesis-testing sample ($n = 723$)

Genotype, rs4790904	Negative pictures, mean \pm SEM	Positive pictures, mean \pm SEM	Neutral pictures, mean \pm SEM	All pictures, mean \pm SEM
AA, $n = 459$	11.1 \pm 0.1	11.7 \pm 0.1	6.9 \pm 0.1	29.7 \pm 0.4
AG, $n = 232$	10.0 \pm 0.2	11.1 \pm 0.2	6.0 \pm 0.2	27.1 \pm 0.5
GG, $n = 32$	9.3 \pm 0.5	10.2 \pm 0.5	5.8 \pm 0.4	25.3 \pm 1.2
	$P = 0.000002$	$P = 0.006$	$P = 0.0004$	$P = 0.00001$

Table 2. Genotype-dependent memory performance in the replication sample ($n = 394$)

Genotype, rs4790904	Negative pictures, mean \pm SEM	Positive pictures, mean \pm SEM	Neutral pictures, mean \pm SEM	All pictures, mean \pm SEM
AA, $n = 234$	11.3 \pm 0.2	12.3 \pm 0.2	6.9 \pm 0.2	30.5 \pm 0.5
AG, $n = 139$	10.7 \pm 0.3	11.6 \pm 0.3	6.5 \pm 0.2	28.7 \pm 0.7
GG, $n = 21$	10.1 \pm 0.6	10.6 \pm 0.6	6.5 \pm 0.7	27.1 \pm 1.5
	$P = 0.028$	$P = 0.005$	$P = 0.284$	$P = 0.014$

Functional Brain Imaging

In the next step, we used fMRI to identify genotype-independent and genotype-dependent differences in brain activity related to aversive memory in this population of 394 healthy subjects. The event-related fMRI design allowed us to investigate brain regions involved in memory formation by analyzing differential activity during encoding of subsequently remembered vs. subsequently forgotten events (subsequent memory analysis). Independently of genotype, this analysis revealed activation of a large network of neocortical and limbic

brain regions, including the frontal, temporal, parietal, and occipital cortex; amygdala; hippocampus; insular cortex; and anterior cingulum (Fig. S1). These results are largely consistent with the findings of a recent meta-analysis of functional magnetic resonance imaging (fMRI) studies of successful emotional memory encoding (10). *PRKCA* rs4790904 genotype-dependent, subsequent memory analyses for negative information revealed significant [$P < 0.05$, false discovery rate (FDR) corrected for whole brain] gene dose-dependent (with increasing number of A alleles) activity increases in the lateral and medial prefrontal cortex (Table 3 and Fig. 1). An additional region of interest (ROI) analysis for the amygdala and the hippocampus did not reveal significant (small-volume FDR corrected for the corresponding ROI) genotype-dependent activation differences in these brain regions. There were no significant activity increases with increasing number of G alleles. Thus, the present fMRI experiment revealed that the A allele, which was associated with increased memory for negative information, was also robustly related to increased brain activity in the lateral and medial prefrontal cortex during successful memory encoding of negative pictures. Previous studies have shown that these brain regions belong to a network involved in emotional memory encoding (10–12). Subsequent memory analyses for positive and neutral information did not reveal any significant genotype-dependent activation differences (at the same significance threshold as used for negative information, i.e., $P < 0.05$, FDR corrected for whole brain). This finding indicates that at the level of brain activation, significant PKC α genotype-dependent differences were observed only for negative information.

Because we used two different scanners in the fMRI study (*Methods*), we reanalyzed the data including scanner type as a covariate. This analysis revealed similar results to those described above.

Table 3. *PRKCA* rs4790904 genotype-dependent, subsequent memory analysis for negative information (gene dose- dependent activity increases with increasing number of A alleles)

Region	BA	No. voxels	L/R	MNI coordinates			T
				x	y	z	
Middle frontal gyrus	6	51	L	-33	3	40	4.56*
Middle frontal gyrus	9	47	L	-47	25	36	4.45*
Inferior frontal gyrus	46/47	41	L	-44	38	0	4.24*
Superior frontal gyrus	8	36	L	-6	33	52	3.83*

BA, Brodmann area; L/R, left/right hemisphere.

*Thresholded at $P < 0.05$, FDR corrected for whole brain.

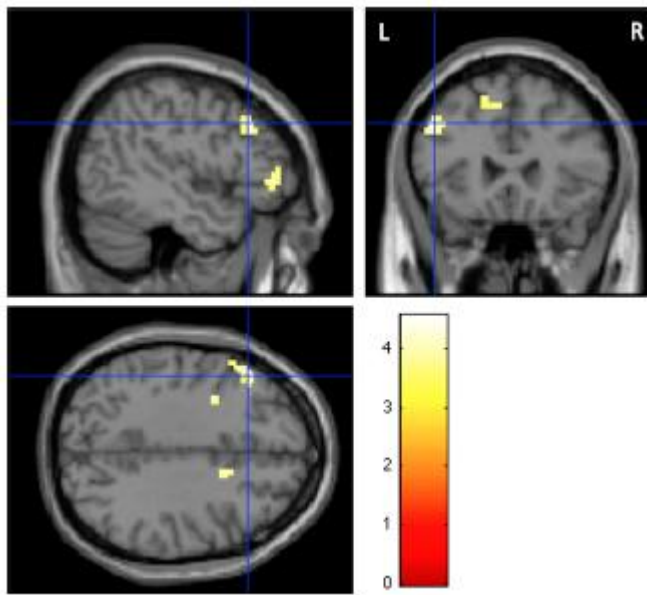


Fig. 1. *PRKCA* rs4790904 genotype-dependent differences in brain activity related to successful encoding of memory for negative information in 394 healthy young subjects. Displayed are gene dose-dependent (with increasing number of A alleles) increases in activity in the lateral and medial prefrontal cortex. The blue cross indicates the peak activation in the left dorsolateral prefrontal cortex at (-47 25 36). Activations are overlaid on sagittal (*Upper Left*), coronal (*Upper Right*), and axial sections of a T1-weighted magnetic resonance image of SPM5, displayed at a whole brain FDR-corrected threshold of $P < 0.05$ and using color-coded t values. L, left side of the brain; R, right side of the brain.

Genetic Study in Traumatized Survivors of the Rwandan Genocide

We hypothesized that the *PRKCA* polymorphism, which predisposes individuals to build strong emotional memory, may also predispose to build strong traumatic memories after an aversive event and, possibly, also increase the risk for PTSD. We tested this hypothesis in 347 refugees who have fled from the Rwandan civil war and have been living in the Nakivale refugee camp in Uganda during the time of investigation (184 females, 163 males; median age, 34 y; range, 17–68 y). All subjects had experienced highly aversive situations and were examined by trained experts with a structured interview based on the Posttraumatic Diagnostic Scale (13) with the help of trained interviewers chosen from the refugee community. Traumatic events were assessed using a checklist of 36 war- and non-war-related traumatic event types (e.g., injury by a weapon, rape, accidents). The population consisted of 134 subjects fulfilling the diagnostic criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (5) for current PTSD and 213 subjects without current PTSD. *PRKCA* SNP rs4790904 was significantly associated with symptoms of reexperiencing the traumatic event (traumatic memory) with the same direction of effect as with memory for negative information in the healthy population ($P = 0.033$; Table 4). Furthermore, the A allele of rs4790904 was associated with increased avoidance symptoms ($P = 0.037$; Table 4) and with increased risk for PTSD ($P = 0.009$; Table 5). Sex did not influence the genotype effect on

traumatic memory or PTSD risk (sex \times SNP interaction $P \geq 0.7$). SNP rs4790904 was not associated with hyperarousal symptoms or with the number of experienced event types ($P > 0.5$). Of note, the A allele, which was the major allele in the Swiss population, was the rare allele in the Rwandese population. Such differences in allele and genotype frequency are commonly observed between genetically distinct populations. In the case of the present study, no conclusions can be drawn with regard to any possible functional relevance of this difference.

Table 4. Correlation of the A allele of rs4790904 with PTSD-related symptoms in the Rwanda sample

	Intrusions	Avoidance	Hyperarousal
Spearman ρ	0.115	0.112	0.030
Significance P	0.033	0.037	0.579
n	347	347	347

Table 5. Association between PRKCA SNP rs4790904 and risk for PTSD in the Rwanda sample

Genotype	No PTSD	PTSD	Total
GG	90, 42.3%	37, 27.6%	127, 36.6%
AG	99, 46.5%	76, 56.7%	175, 50.4%
AA	24, 11.2%	21, 15.7%	45, 13.0%

$$\chi^2 = 6.8, df = 1, P_{\text{additive}} = 0.009$$

Discussion

The present results indicate that PKC α is genetically linked to memory capacity (including aversive memory) in nontraumatized individuals and to traumatic memory and the risk for PTSD in heavily traumatized genocide survivors. Unlike the previously reported *ADRA2B* deletion variant (1), PKC α seems to be related to memory independent of emotional valence, because the genetic association in healthy individuals was not consistently restricted to emotional information.

Free recall of pictures in healthy subjects as assessed in the present study involves voluntary retrieval of image-based memory. Encoding of this memory depends on a large network of neocortical and limbic brain regions, including the frontal, temporal, parietal, and occipital cortex; amygdala (especially for emotionally arousing information); hippocampus; insular cortex; and anterior cingulum (10). In PTSD, intrusive reexperiencing of the traumatic event consists of a primarily involuntary activation of representations (emotional memories) that contain detailed sensory and perceptual images. At the same time, it has been proposed that due to a reduced hippocampal involvement, these emotional memories are not well contextualized (14) (for review see ref. 15). In the nontraumatized population the neuroimaging findings revealed that the A allele went along with more activation in prefrontal regions, but, interestingly,

not with increased activation in medial temporal structures, such as the amygdala and hippocampus. There is compelling evidence that the amygdala plays an important role in enhancing the formation of memory for emotionally arousing information (11) and that the hippocampus is crucial for binding memory items to their context and for the successful transfer of this information into long-term memory (8, 16). It is important to note that because scanning in the present study was restricted to the encoding phase, we cannot exclude that genotypedependent activation differences in medial temporal lobe regions may have occurred later in the memory formation process.

Although theoretical considerations might have implied that individuals who are prone to build strong emotional memories are at risk for developing PTSD after a traumatic experience, there is only weak empirical evidence supporting this assumption. Several studies have found increased implicit and explicit emotional memory functions in patients with PTSD (17–19), but also the opposite has been reported (20). It is important to note that data on memory performance acquired in patients with PTSD are difficult to interpret, because changes in mood, motivation, attention, or arousal can indirectly affect memory performance in both directions. Furthermore, even specific memory changes could be a consequence of the disease or preexisting risk factors. The same difficulty regarding interpretation exists for imaging data acquired in PTSD patients, because neuroanatomical or neurofunctional abnormalities may reflect an underlying causal factor or a consequence of the disorder. For example, it has been found that patients with PTSD have reduced hemodynamic responses in the medial prefrontal cortex (21), which fits with the idea that decreased prefrontal activity, through amygdala disinhibition, could lead to increased formation of traumatic memories and predispose to PTSD. However, another study found that encoding of later remembered negative words vs. baseline was associated with increased activations in the cingulate cortex and dorsomedial prefrontal cortex in complex PTSD compared with healthy controls (22). Moreover, a recent study has demonstrated that damage to the medial prefrontal cortex protects against PTSD (23). Also in the present study, we found that the *G* allele, which was related to decreased emotional memory and decreased prefrontal activity in healthy humans, was related to decreased risk for PTSD.

Considerable evidence indicates that PKC plays an important role in learning and memory (7). For example, it has been shown that PKC targets phosphorylation sites on the C-terminal domain of NR2B, which can modulate NMDAR conductance (24). PKCs include several isoforms and it has been shown that mice with a specific deletion of PKC β have impaired fear conditioning when tested 24 h after training (25). Furthermore, it has been shown that the α 1-adrenergic receptor pathway is capable of activating PKC α , which may represent a mechanism for enhancing memory (26). In our study, a *PRKCA* genotype-dependent difference was observed as early as 10 min after learning in the Swiss cohort of healthy young individuals, suggesting that short-

term memory processes were already affected. The analysis of additional data on free recall 24 h after picture presentation, reflecting long-term memory performance in the Swiss cohort, also revealed significant genotype-dependent differences in memory performance. Furthermore, the genotype was also related to long-term traumatic memories in individuals who experienced life-threatening situations, suggesting a role of *PRKCA* in both short- and long-term memory processes.

In summary, the present results provide evidence for a role of *PRKCA* in memory, including aversive and traumatic memory. Our study also points to a genetic link between the predisposition to build strong memory and the risk for PTSD and indicates differential genetic risks for different clusters of PTSD symptoms. Genetic analyses may thus help to uncover PTSD dimensions with different symptom patterns, a subtyping that may be necessary to improve understanding and treatment of posttrauma psychopathology.

Methods

Emotional and Working Memory Testing

Picture task

Stimuli consisted of 72 pictures that were selected from the IAPS (9) as well as from in-house standardized picture sets that allowed us to equate the pictures for visual complexity and content (e.g., human presence). On the basis of normative valence scores (from 1 to 9), pictures were assigned to emotionally negative (2.3 ± 0.6), emotionally neutral (5.0 ± 0.3), and emotionally positive (7.6 ± 0.4) conditions, resulting in 24 pictures for each emotional valence. Four additional pictures showing neutral objects were used to control for primacy and recency effects in memory. Two of these pictures were presented in the beginning and two at the end of the picture task. They were not included in the analysis. In addition, 24 scramble pictures were used. The background of the scrambled pictures contained the color information of all pictures used in the experiment (except primacy and recency pictures), overlaid with a crystal and distortion filter (Adobe Photoshop CS3). In the foreground, a mostly transparent geometrical object (rectangle or ellipse of different sizes and orientations) was shown. Pictures were presented in the scanner using MR-compatible liquid crystal display goggles (Visuastim XGA; Resonance Technology). Eye correction was used when necessary.

The pictures were presented for 2.5 s in a quasi-randomized order so that at maximum four pictures of the same category occurred consecutively. A fixation cross appeared on the screen for 500 ms before each picture presentation. Trials were separated by a variable intertrial period of 9–12 s (jitter) that was equally distributed for each stimulus category. During the intertribal period, participants subjectively rated the picture showing scenes according to valence (negative, neutral, positive) and arousal (large, medium, small) on a three-point scale (self assessment manikin, SAM) by pressing a button with a finger of their

dominant hand. Participants were not told that they had to remember the pictures for later recall.

Ten minutes after picture presentation, memory performance was tested using a free-recall task, which required participants to write down a short description (a few words) of the previously seen pictures. Remembered primacy and recency pictures as well as training pictures were excluded from the analysis. No time limit was set for this task. A picture was scored as correctly recalled if the rater could identify the presented picture on the basis of the subject's description. Two trained investigators independently rated the descriptions for recall success (interrater reliability >99%). A third independent rater decided on pictures, which were rated differently.

Subjects (from the hypothesis-testing sample) performed a similar freerecall test 24 h after picture presentation.

Working memory task

Between picture presentation and recall, participants performed on the 0- and 2-back versions of the n-back working memory task (27). In this task, letters are presented successively in the center of the screen. In the 0-back condition, subjects had to respond to the occurrence of the letter "x", which is a baseline measure of general attention, concentration, and reaction time. The 2-back task requires subjects to respond to a letter repetition with one intervening letter (g – S – f – s). The latter condition required both the maintenance of the last two letters in memory and updating of these remembered stimuli as each new stimulus was presented.

Subjects were free of any lifetime neurological or psychiatric illness and did not take any medication at the time of the experiment. The experiments were approved by the ethics committee of the Canton of Basel. Written informed consent was obtained from all subjects before participation.

fMRI Experiment

Participants

A total of 394 healthy young subjects (241 females, 153 males; median age, 22 y; range, 18–30 y; 234 AA genotype carriers of SNP rs4790904, 139 AG genotype carriers of SNP rs4790904, and 21 GG genotype carriers of SNP rs4790904) were included in the study. All subjects were right-handed, free of any lifetime neurological or psychiatric illness, and did not take any medication at the time of the experiment. The experiments were approved by the ethics committees of the Cantons of Zurich and Basel. Written informed consent was obtained from all subjects before participation. Subjects were scanned either in Zurich (n = 86) or in Basel (n = 308), using the identical design.

Emotional and working memory task

We used identical tasks to those described above.

Procedure

After receiving general information about the study and giving their informed consent, participants were instructed and then trained on the picture task they later performed in the scanner. After training, they were positioned in the scanner. The participants received earplugs and headphones to reduce scanner noise. Their heads were fixated in the coil using small cushions, and they were told not to move their heads. Functional MR images were acquired during the performance of the picture task in two separate sessions (total scanning time ~30 min). After finishing the tasks, participants left the scanner and were taken to a separate room for free recall of the pictures. Finally, participants filled out questionnaires, gave saliva for genotype analysis, and were debriefed. The total length of the experimental procedure was ~3 h. Participants received 25 Swiss francs/h for participation. Saliva samples were obtained from each person, using an Oragene DNA Self-Collection Kit (DNA Genothek). DNA was extracted from saliva using standard protocols.

fMRI data acquisition and processing

Zurich site

Measurements were performed on a Philips Intera 3 T whole-body MR unit equipped with an eight-channel Philips SENSE head coil. Functional time series were acquired with a sensitivity encoded (28) single-shot echo-planar sequence (SENSE-sshEPI). We used the following acquisition parameters: echo time (TE) = 35 ms, field of view (FOV) = 22 cm, acquisition matrix = 80×80 , interpolated to 128×128 , voxel size = $2.75 \times 2.75 \times 4 \text{ mm}^3$, and SENSE acceleration factor $R = 2.0$. Using a midsagittal scout image, 32 contiguous axial slices were placed along the anterior–posterior commissure (AC–PC) plane covering the entire brain with a repetition time (TR) = 3,000 ms ($\alpha = 82^\circ$). The first two acquisitions were discarded due to T1 saturation effects.

Basel site

Measurements were performed on a Siemens Magnetom Verio 3 T whole-body MR unit equipped with a 12-channel head coil. Functional time series were acquired with a single-shot echo-planar sequence using parallel imaging (GRAPPA). We used the following acquisition parameters: TE = 35 ms, FOV = 22 cm, acquisition matrix = 80×80 , interpolated to 128×128 , voxel size = $2.75 \times 2.75 \times 4 \text{ mm}^3$, and GRAPPA acceleration factor $R = 2.0$. Using a midsagittal scout image, 32 contiguous axial slices were placed along the AC–PC plane covering the entire brain with a TR = 3,000 ms ($\alpha = 82^\circ$). The first two acquisitions were discarded due to T1 saturation effects.

Mapping; Wellcome Department of Cognitive Neurology, London, United Kingdom) implemented in MATLAB 2008a (MathWorks). Volumes were slice-time corrected to the first slice, realigned to the first acquired volume, normalized into standard stereotactic space (template provided by the Montreal Neurological Institute), and smoothed using an 8-mm full-width-at-half-maximum Gaussian kernel. A 128-s cutoff high-pass filter was added to the confound partition of the design matrix to account for low-frequency drifts, and a correction for intrinsic autocorrelations was included in the analysis. For each subject, evoked hemodynamic responses to event types were modeled with a delta (stick) function corresponding to presentation of each stimulus category (negative, positive, neutral, and scrambled pictures, respectively) convolved with a canonical hemodynamic response function within the context of a general linear model (GLM). The pictures accounting for possible primacy and recency effects as well as button presses during valence and arousal ratings were modeled separately. In addition, six movement parameters from spatial realigning were included as regressors of no interest. The contrast between brain activity during encoding of pictures subsequently remembered and brain activity during encoding of pictures subsequently forgotten was calculated individually using a fixed-effects model (first-level analysis). The resulting contrast parameters were then used for genotype-dependent analyses in a randomeffects model (second-level analysis). Specifically, we used a regression model to analyze gene-dose-dependent differences in brain activity (with the number of A alleles as a covariate). We used a threshold of $P < 0.05$, FDR corrected for whole brain, and a minimum number of 30 adjacent voxels for an exploratory analysis of the whole brain. For the hippocampus and the amygdala, we additionally used the left and right amygdala and the left and right hippocampus as ROIs, where small volume correction was applied with a threshold of $P < 0.05$ (FDR corrected). The hippocampus and amygdala ROIs were defined by the Talairach atlas with the categorization in Brodmann areas (29), implemented in the software WFU PickAtlas v2.4 (30).

Rwanda Sample

As the Nakivale refugee camp has grown over the last decade and is spread over a large area, participants were sampled proportionally to the population size from each zone. To exclude genetic relatives in the samples, only one person per household was interviewed. Interviewers had been trained to detect current alcohol abuse and acute psychotic symptoms; candidates exhibiting these signs were excluded. All subjects had experienced highly aversive traumatic situations and were examined in 2006/ 2007 by trained experts, using a structured interview based on the Posttraumatic Diagnostic Scale (PDS) (13) with the help of trained interpreters. Traumatic events were assessed with a checklist of 36 war-

and non-war– related traumatic event types, e.g., injury by weapon, rape, accident (1, 31). Traumatic load was estimated by assessing the number of different traumatic event types experienced or witnessed. This measure is considered more reliable than assessing the frequency of traumatic events (31). Depressive symptoms were assessed with the depression section of the Hopkins Symptom Checklist (HSCL-D) (32). A subset of this sample has been analyzed in previous studies (1, 33). The procedures were approved by the Ethics Committees of the University of Konstanz and the Mbarara University of Science and Technology, Mbarara, Uganda.

The PDS and event list were completed in the form of a standardized interview. Interviewers were first trained in a 6-wk course on principles of quantitative data collection and interviewing techniques. Instruments were translated into Kinyarwanda, using several steps of translations, blind backtranslations, and subsequent corrections by independent groups of translators (34). Following the translations, the psychometric properties of the translated scales were investigated in a validation study including a retest spanning a 2-wk period and a cross-validation with expert rating (35). To avoid known ceiling effects (36), subjects were selected to have experienced no more than 16 traumatic event types.

Saliva samples were obtained from each person, using an Oragene DNA Self-Collection Kit (DNA Genotek). DNA was extracted from saliva using standard protocols.

SNP Selection

To capture the variability of the genes encoding PKA, PKC, CaMKII, MAPK, and their subunits, 2,005 intragenic SNPs that are present on the Affymetrix Human SNP Array 6.0 were selected. In addition to the SNPs being intragenic, the following inclusion criteria for each SNP were set: minor allele frequency (MAF) $\geq 5\%$; nondeviance from Hardy–Weinberg equilibrium ($P_{\text{Fisher}} \geq 0.01$); and call rate $\geq 95\%$, i.e., a SNP was excluded if more than 5% of all individuals failed to have genotypic information for this SNP (*SI Appendix*).

Array-Based SNP Genotyping

Samples were processed as described in the Genome-Wide Human SNP Nsp/Sty 6.0 User Guide (Affymetrix). Briefly, genomic DNA concentration was determined by fluorometry (Qubit dsDNA BR Assay Kit; Invitrogen) in a Qubit 1.0 fluorometer and adjusted to 50 ng/ μL in water. Two hundred fifty nanograms of DNA was digested in parallel with 10 units of StyI and

NspI restriction enzymes (New England Biolabs) for 2 h at 37 °C. Enzyme-specific adaptor oligonucleotides were then ligated onto the digested ends with T4 DNA Ligase for 3 h at 16 °C. After adjustment to 100 µL with water, 10 µL of the diluted ligation reactions were subjected to PCR. Three PCR reactions of 100 µL were performed for Sty-digested products and four PCR reactions for Nsp. PCR was performed with Titanium Taq DNA Polymerase (Clontech) in the presence of 4.5 µM PCR primer 002 (Affymetrix), 350 µM each dNTP (Clontech), 1 M G-C Melt (Clontech), and 1× Titanium Taq PCR Buffer (Clontech). Cycling parameters were as follows: initial denaturation at 94 °C for 3 min; amplification at 94 °C for 30 s and 60 °C for 45 s and then extension at 68 °C for 15 s, repeated a total of 30 times; and final extension at 68 °C for 7 min. Reactions were then verified to migrate at an average size between 200 and 1,100 bp, using 2% Tris-borate-EDTA (TBE) gel electrophoresis [2 g agarose (Sigma A9539; Sigma-Aldrich) in 100 mL of 1× TBE buffer]. PCR products were combined and purified with the Filter Bottom Plate (Millipore; P/N MDRLN0410), using Agencourt AMPure XP Beads (Beckman Coulter). Purified PCR products were quantified on a Zenith 200rt microplate reader (Anthos-Labtec). Four to 5 µg/µL was obtained on average for each sample. From this stage on, the SNP Nsp/Sty 5.0/6.0 Assay Kit (Affymetrix) was used. Around 250 µg of purified PCR products were fragmented using 0.5 unit of DNase I at 37 °C for 35 min. Fragmentation of the products to an average size less than 180 bp was verified using 4% TBE gel electrophoresis. Following fragmentation, the DNA was end-labeled with 105 units of terminal deoxynucleotidyl transferase at 37 °C for 4 h. The labeled DNA was then hybridized onto the Genome-Wide Human SNP 6.0 Array at 50 °C for 18 h at 60 rpm (GeneChip Hybridization Oven 645, Affymetrix, Santa Clara, CA). The hybridized array was washed, stained, and scanned according to the manufacturer's (Affymetrix) instructions, using the Affymetrix GeneChip Command Console (AGCC, version 3.2.0.1515). Generation of SNP calls and Array quality control were performed using the command line programs of the Affymetrix Power Tools package (version apt-1.14). According to the manufacturer's recommendation, contrast quality control (QC) was chosen as QC metric, using the default value of greater than or equal to 0.4. Mean call rate for all samples averaged >98.5%. All samples passing QC criteria were subsequently genotyped using the Birdseed (v2) algorithm.

Statistical Analyses

All nongenetic statistical analyses were done with a standard software package (SPSS Statistics, version 19). Wherever appropriate, nonparametric methods (e.g., rank correlation) were used. The nominal significance threshold was set to $P \leq 0.05$. The corrected significance threshold (i.e., Bonferroni correction for the analysis of 2,005 SNPs) was set to $P < 0.000025$. Golden Helix SNP and Variation Suite 7 (SVS7, version 7.5.3) was used for statistical analysis of genetic data. Analyses were run under the assumption of an additive model. Population stratification was assessed with EIGENSTRAT (37) by analyzing all genome-wide, array-based autosomal SNPs passing QC criteria. Principal component analysis (PCA) was first applied to reduce genetic variation to a few dimensions. For PCA, default parameters were used (i.e., definition of 10 principal components in five iterations; outlier criterion was 6 SDs). We also applied the Genomic Control program that is implemented in the EIGENSTRAT package to compute the inflation factor λ (38).

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Supporting Information

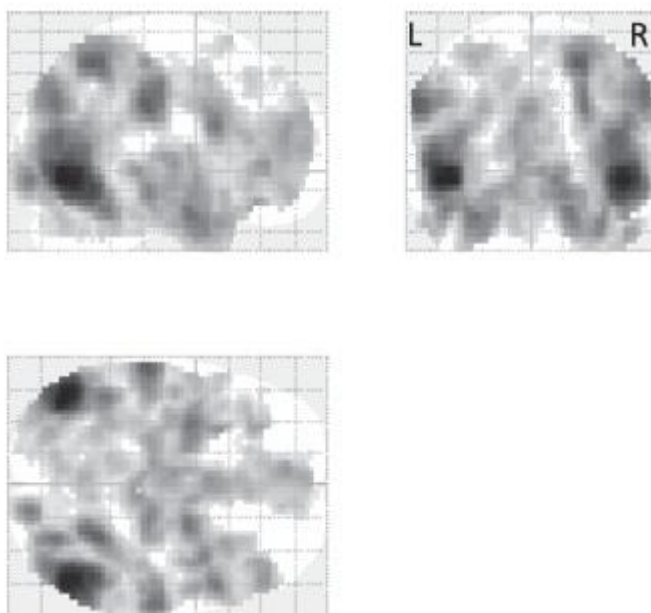


Fig. S1. Genotype-independent, subsequent memory analysis for negative information, thresholded at $P < 0.05$, FDR corrected for whole brain. Sagittal, coronal, and axial SPM glass-brain projections are shown. L, left side of the brain; R, right side of the brain.

Table S1. Nominal significance of associated SNPs (negative pictures free recall, $P_{\text{uncorrected}} < 0.01$) in the hypothesis-testing sample ($n = 723$)

Affymetrix marker	Chromosome	Position	dbSNP RS ID	Associated gene	Cytoband	Call rate	MAF	HWE P	Association P
SNP_A-8703022	17	62,209,616	rs4790904	PRKCA	q24.2	0.997	0.20	0.697077012	0.000002
SNP_A-1995659	10	12,875,051	rs928337	CAMK1D	p13	1.000	0.41	0.353067038	0.00078
SNP_A-1995661	10	12,875,594	rs2493766	CAMK1D	p13	0.999	0.37	0.716531688	0.00081
SNP_A-1806035	10	12,887,844	rs4750267	CAMK1D	p13	0.997	0.37	0.80609225	0.0012
SNP_A-1936417	10	12,880,848	rs1556408	CAMK1D	p13	0.997	0.37	0.797212222	0.0013
SNP_A-8659980	10	12,891,662	rs12358381	CAMK1D	p13	1.000	0.27	0.049411446	0.0014
SNP_A-1985684	6	36,127,314	rs17714205	MAPK14	p21.31	0.997	0.13	0.524852836	0.0017
SNP_A-8281277	10	12,874,932	rs928336	CAMK1D	p13	0.994	0.43	0.170919012	0.0019
SNP_A-2111454	10	12,890,474	rs10906229	CAMK1D	p13	0.999	0.35	0.714469219	0.0020
SNP_A-2108498	6	36,184,912	rs3804452	MAPK14	p21.31	0.999	0.13	0.550959233	0.0024
SNP_A-2222070	17	62,147,088	rs16960114	PRKCA	q24.2	0.993	0.20	0.780677016	0.0028
SNP_A-8281665	10	12,876,558	rs1929389	CAMK1D	p13	1.000	0.43	0.18044124	0.0030
SNP_A-1816092	10	49,265,870	rs17697960	MAPK8	q11.22	1.000	0.11	0.148347676	0.0033
SNP_A-2000443	10	49,312,216	rs7086275	MAPK8	q11.22	0.999	0.42	0.628650983	0.0041
SNP_A-4248684	10	49,290,273	rs9888128	MAPK8	q11.22	1.000	0.43	0.729951509	0.0045
SNP_A-2000441	10	49,312,152	rs11101320	MAPK8	q11.22	0.994	0.42	0.661763446	0.0047
SNP_A-1781918	10	49,264,246	rs10857560	MAPK8	q11.22	0.999	0.43	0.395575993	0.0048
SNP_A-8637914	17	61,737,476	rs7210446	PRKCA	q24.2	0.990	0.44	0.87454757	0.0049
SNP_A-4229112	17	64,035,235	rs3785906	PRKAR1A	q24.2	1.000	0.23	0.815795241	0.0054
SNP_A-8550960	7	151,186,459	rs6961830	PRKAG2	q36.1	0.994	0.18	0.93078015	0.0056
SNP_A-1931100	10	12,872,599	rs4747999	CAMK1D	p13	1.000	0.41	0.250623163	0.0056
SNP_A-8470906	17	64,023,836	rs4281788	PRKAR1A	q24.2	0.999	0.23	0.770857219	0.0061
SNP_A-2163878	10	49,261,724	rs2698761	MAPK8	q11.22	1.000	0.43	0.747074632	0.0062
SNP_A-4222841	10	49,220,700	rs6537561	MAPK8	q11.22	1.000	0.43	0.864461361	0.0064
SNP_A-8676867	6	161,418,462	rs9458114	MAP3K4	q26	0.997	0.07	0.45633922	0.0077
SNP_A-4292124	10	6,539,234	rs4750491	PRKCQ	p15.1	0.994	0.34	0.052291675	0.0081
SNP_A-2146600	17	62,196,905	rs4381631	PRKCA	q24.2	0.999	0.47	0.596302414	0.0082
SNP_A-2055994	17	62,148,632	rs4465636	PRKCA	q24.2	0.994	0.20	0.584940644	0.0093
SNP_A-2237869	10	12,686,538	rs7906212	CAMK1D	p13	0.990	0.07	0.752773631	0.0095
SNP_A-8330608	17	61,738,935	rs4577128	PRKCA	q24.2	1.000	0.44	0.583248971	0.0096
SNP_A-8366935	6	161,338,632	rs625977	MAP3K4	q26	1.000	0.17	0.586805235	0.0099

Bonferroni-corrected SNPs are in boldface type. HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; dbSNP RS, reference SNP sequence number according the National Center for Biotechnology Information SNP data base.

Table S2. Genotype-dependent differences in arousal and valence ratings ($n = 716$)

Genotype, rs4790904	Arousal negative pictures, mean \pm SEM	Valence negative pictures, mean \pm SEM
AA, $n = 456$	1.67 \pm 0.02	2.78 \pm 0.01
AG, $n = 229$	1.67 \pm 0.02	2.79 \pm 0.01
GG, $n = 31$	1.64 \pm 0.07	2.76 \pm 0.04
	$P = 0.9$	$P = 0.8$

Table S3. Genotype-dependent performance in the 0- and 2-back task ($n = 678$)

Genotype, rs4790904	Accuracy, 0-back, mean \pm SEM	Accuracy, 2-back, mean \pm SEM
AA, $n = 428$	0.964 \pm 0.002	0.869 \pm 0.005
AG, $n = 219$	0.962 \pm 0.002	0.873 \pm 0.006
GG, $n = 31$	0.971 \pm 0.04	0.874 \pm 0.016
	$P = 0.9$	$P = 0.6$

Table S4. Genotype-dependent memory performance (24-h delay) in the hypothesis-testing sample ($n = 719$)

Genotype, rs4790904	Negative pictures, mean \pm SEM	Positive pictures, mean \pm SEM	Neutral pictures, mean \pm SEM	All pictures, mean \pm SEM
AA, $n = 456$	7.7 \pm 0.2	8.1 \pm 0.2	4.8 \pm 0.1	20.7 \pm 0.4
AG, $n = 231$	7.0 \pm 0.2	7.8 \pm 0.2	4.2 \pm 0.2	19.0 \pm 0.5
GG, $n = 32$	5.6 \pm 0.4	7.1 \pm 0.4	3.7 \pm 0.5	16.4 \pm 0.8
	P = 0.0001	P = 0.07	P = 0.0006	P = 0.0004