Supplement Information

5-HTTLPR genotyping

The 5-HTTLPR region was amplified in a 20µl reaction: 1x Optimized Buffer A, 1x PCR enhancer, 0.25µM of each primer [FAM-ATCGCTCCTGCATCCCCCATTAT (forward primer), GAGGTGCAGGGGGATGCTGGAA (reverse primer)], 0.125µM of dNTP, 10ng of DNA, 1.25u of Platinum Taq polymerase (all from Invitrogen Corp., Carlsbad, CA). The PCR conditions were: 95°C (5 min), 40 cycles of 94°C (30sec), 52 °C (30sec), 68°C (1 min), and a final elongation, 68°C (10 min). S and L genotypes were discriminated directly from the PCR reaction products. The rs25531 LA and LG genotypes were determined by digesting 5µl PCR mix with 100 units of MspI, 1x NEB restriction buffer 1, incubated at 37°C for 1 hour (New England Biolabs). Samples were mixed with deionized formamide and GeneScan™-500 ROX Size Standard (Applied Biosystems, Foster City, CA), and the genotypes were resolved on a 3730 DNA Analyzer, data analyzed using GeneMapper 4.0 software (Applied Biosystems, Foster City, CA). Genotyping accuracy was determined empirically by duplicate genotyping of 25% of the samples selected randomly. The error rate was <0.005, and the completion rate was >0.98. Triallelic genotyping revealed the following allele frequencies: S = 0.25, LA = 0.51, LG = 0.24. Alleles were grouped as low activity (SS, SLG, LGLG) (0.22) medium activity (SLA, LALG) (0.51) and high activity (LALA) (0.28) variants.

MRI data acquisition

Structural images were acquired axially using T1-weighted anatomical MRI (multi-echo magnetization-prepared rapid gradient echo [MEMPRAGE], voxel size 1 × 1 × 1 mm; repetition time [TR] 400 ms; echo time [TE] 1.69 ms; echo spacing 9.8 ms; number of echoes 4; bandwidth

650 Hz/Px; inversion time 1,100 ms; flip angle 7°; acceleration factor 2; matrix size 176 × 256 × 256; field of view [FoV] 256 mm, acquisition time: 6 minutes 2 seconds). Subjects were instructed to rest silently with their eyes closed while remaining "awake, trying to avoid thinking about anything in particular" during the collection of a T2-weighted multi-echoplanar imaging sequence (voxel size 3 × 3 × 3 mm; TR 2,000 ms; TE 11/22/33 ms; flip angle 70°; FoV 210 mm, phase FoV 87.5%, acceleration factor 3, number of slices 34; interleaved, bandwidth 2,552 Hz/Px; repetitions: 180) for 6 minutes. Two runs were collected per participant.

MRI Data Processing

For each run, the averaged echos were analyzed with uber_subject.py. After preprocessing (including despiking, time shift, motion correction, and conversion to percentage signal change) we normalized the echoplanar imaging volumes to the MNI_caez_N27 template and smoothed them with a 4.5-mm full-width-half-maximum Gaussian kernel. The 2 runs were concatenated within the anaticor step, which removed the local white matter artifacts, to obtain the final time series used in the analyses described below.

Resting-State Functional Connectivity Analysis

For the whole-brain seed-based connectivity analysis, the right amygdala was selected as *a priori* region of interest (ROI), based on a previous study reporting increased reactivity to salient emotional stimuli in the right amygdala in FMD patients (8). The seed for the amygdala was defined using a 5-mm radius sphere centered on Talairach coordinates for the right amygdala (x = 23, y = 5, z = −15). Group level seed-based correlation maps were computed using the AFNI tool 3dGroupInstaCorr. Between-group 2-sample *t* tests were performed to detect significant differences in amygdala-frontal connectivity between genotype groups (homozygous for the wild

type allele vs. homozygous and heterozygous for the variant allele), after controlling for age, gender, AIMs score, CTQ total score and psychiatric symptoms. Cluster-level significance was set at p <0.01 (uncorrected) and a minimum cluster extent threshold of 30 contiguous voxels.

To rule out differences due to hypoactivity in the amygdala seed region, we compared spontaneous neuronal activity in the right amygdala in the genotype groups. To accomplish this, we performed between-group 2-sample *t* tests of the amplitude of low-frequency fluctuation (ALFF).

To test whether group differences in amygdala-frontal connectivity were specifically associated with allele status, we also examined resting state functional connectivity (rsFC) data collected in a sample of age- and gender-matched healthy controls (n =38) processed in the same fashion as the FMD patients. First, we created a mask of the amygdala-frontal connectivity cluster showing significant genotypic group difference; z-scores were extracted from each voxel in this significant clusters and averaged to get a mean cluster connectivity score for each subject (patients and controls). Mean cluster z-scores were compared using *post-hoc* two-sample *t* tests between controls versus patients homozygous for the wild type allele, controls versus patients homozygous and heterozygous for the variant allele, and patients homozygous for the wild type allele versus patients carrying the variant allele. Significance threshold was set at p <0.05. These contrasts sought to determine whether there was a distinct amygdala-frontal connectivity alteration within the patient group carrying the variant allele relative to both controls and patients carrying the wild type allele.

To test for correlation between amygdala-frontal connectivity and levels of childhood trauma, we performed linear regression analysis of mean z connectivity to CTQ score. To explore

the relationship between FMD phenotype (age of onset and symptom severity) and amygdala-frontal connectivity, we calculated Pearson' correlation coefficients.

Across the rest of the brain, for completeness, and to obviate bias and generate new hypotheses, we showed group differences in amygdala connectivity at voxel p <0.01 and a minimum cluster size of 40 contiguous voxels.