Study Exclusion Criteria

1) Medical diagnoses of cancer, stroke, diabetes requiring insulin treatment, chronic kidney or liver disease, or a lifetime history of psychotic symptoms; (2) use of psychotropic, glucocorticoid, or cardiovascular (e.g., antihypertensive, antiarrythmic) medication; (3) conditions affecting cerebral blood flow and metabolism (e.g., hypertension); and (4) any current DSM-IV Axis I disorder as assessed by the Structured Clinical Interview for DSM-IV (SCID), non-patient edition.

FAAH C385A Genotyping

High molecular weight DNA was isolated from EDTA anticoagulated whole blood samples obtained from all participants using the Puregene kit (Gentra Systems, Minneapolis, MN). Each sample was genotyped by the polymerase chain reaction using forward primer F:5'-CCAACTGTGTGACCTCCTAT-3' and reverse primer R:5'-GCCTCTGGACCTGATACCTC-3', followed by fluorescence polarization (1) using detection primer 5'-GACTCAGCTGTCTCAGGCC-3' to produce a 220 bp fragment containing rs324420. PCR was carried out with a final Mg concentration of 2.5 mM and 35 cycles at annealing temperature 54.5 C. Subsequent products were cleaned by 1.5 h incubation with Exo-SAP reaction mixture (USB). Fluorescence was read on the LJL AnalystHT (Molecular Devices) and analyzed using the AlleleCaller software.

Population Stratification

The following ancestry informative markers, which are unlikely to be related to phenotypes of interest, were genotyped for this analysis: rs1022106, rs1335995, rs1439564, rs1502812, rs1860300, rs548146, rs705388, rs715994, rs720517, rs722743, rs730899, rs734204, rs9059966, rs1328994, rs1485405. We ran STRUCTURE assuming a model with admixture, correlated allele frequencies, individual parameters

and independent F_{ST} for all subpopulations. We tested models with 1, 2, 3 and 4 subpopulations using a burn-in of 40,000 followed by 80,000 repetitions and compared the likelihoods of models fitting the data.

Amygdala Reactivity Paradigm

During the face processing task, subjects viewed a trio of faces (expressing either anger or fear) and selected one of two faces (bottom) identical to a target face (top). Angry and fearful facial expressions can represent honest indicators of ecologically-valid threat, especially that relate to conspecific challengers (2). Within this context, we interpret the amygdala activation elicited by our task as being threat-related. Each face processing block consisted of six images, balanced for sex and target affect (angry or fearful) all derived from a standard set of pictures of facial affect (3). During the sensorimotor control blocks, subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of two shapes (bottom) identical to a target shape (top). Each sensorimotor control block consisted of six different shape trios. All blocks were preceded by a brief instruction ("Match Faces" or "Match Shapes") lasting 2 seconds. In the face processing blocks, each of the six face trios was presented for 4 seconds with a variable inter-stimulus interval of 2-6 sec (mean = 4 sec) for a total block length of 48 seconds. In the sensorimotor control blocks, each of the six shape trios was presented for 4 seconds with a fixed inter-stimulus of 2 seconds for a total block length of 36 seconds. Total task time was 390 seconds. As we were not interested in neural networks associated with face-specific processing per se, but rather in eliciting a maximal amygdala response across all subjects that we could then interrogate for genotype effects, we chose not to use neutral faces as control stimuli because neutral faces can be subjectively experienced as affectively laden or ambiguous and thus engage the amygdala (4; 5). However, the variable inter-stimulus

interval within blocks allowed for the determination of expression-specific (i.e., anger or fear) amygdala reactivity.

Ventral Striatum Reactivity Paradigm

Participants were told that their performance on the card game would determine a monetary reward to be received at the end of the game. During each trial, participants had 3 seconds to guess, via button press, whether the value of a visually presented card was higher or lower than 5 (index and middle finger, respectively). After a choice was made, the numerical value of the card was presented for 500 ms and followed by appropriate feedback (green upward-facing arrow for positive feedback; red downward-facing arrow for negative feedback) for an additional 500 msec. Upon receiving positive feedback (i.e., green arrow), subjects were required to respond via button press (either index or middle finger). No response was required upon negative feedback (i.e., red arrow). A crosshair was then presented for 3 seconds, for a total trial length of 7 seconds. Each block was comprised of 5 trials, with 3 blocks each of predominantly positive feedback (75% correct) and 3 of predominantly negative feedback (25% correct) interleaved with 3 control blocks. During control blocks, participants were instructed to simply make alternating button presses during the presentation of an "x" (3 sec) which was followed by an asterisk (500 msec) and a yellow circle (500 msec). Each block was preceded by a 2 second instruction of "Guess Number" (for positive or negative feedback blocks) or "Press Button" (for control blocks), resulting in a total block length of 38 seconds and a total task length of 342 seconds. Participants were unaware of the fixed outcome probabilities associated with each block and were led to believe that their performance would determine their net monetary gain. Instead, all participants received $10. We included one incongruent trial within each task block (e.g., 1 of 4 trials during positive feedback blocks was incorrect, resulting in negative feedback) to prevent

participants from anticipating the feedback for each trial and to maintain participants' engagement and motivation to perform well. While the presence of a button press during positive feedback but not negative feedback represents a potential motor confound unrelated to reward-specific characteristics of our paradigm, the pattern of VS activation associated with our paradigm is highly consistent with that of the original event-related paradigm from which our task was derived (6), as well as several other reward incentive paradigms and stimuli (cf., (7; 8) that were not confounded by a differential motor component.

BOLD fMRI Data Acquisition & Analyses

All scanning parameters were selected to optimize the quality of the BOLD signal while maintaining a sufficient number of slices to acquire whole-brain data. Before the collection of fMRI data for each participant, we acquired a reference EPI scan that we visually inspected for artifacts (e.g., ghosting), as well as for good signal across the entire volume of acquisition, including the amygdala and ventral striatum. Additionally, an autoshimming procedure was conducted before the acquisition of BOLD data in each subject to minimize field inhomogeneities. The fMRI data from all 82 participants included in this study met these quality standards.

For each scan, images for each participant were realigned to the first volume in the time series to correct for head motion. Data sets were then selected for their high quality (scan stability) as demonstrated by small (<2 mm translational and <1 rotational) motion correction. Based on this criterion, data from all participants were included in subsequent analyses. Realigned images were spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model. These normalized images were then smoothed to minimize noise and residual

difference in gyral anatomy with a Gaussian filter, set at 6 mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean.

For the amygdala reactivity paradigm we calculated condition-specific effects for (1) all faces > shapes, (2) angry faces > shapes and (3) fearful faces > shapes. For the VS reactivity paradigm we calculated main effects of reward: (positive feedback > control) > (negative feedback > control). Statistical maps of these condition effects for each subject were then used to determine *FAAH* C385A genotype effects on condition-specific amygdala and VS reactivity using ANCOVAs with sex as a covariate.

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