Divergent Effects of Genetic Variation in Endocannabinoid Signaling on Human Threat- and Reward-Related Brain Function

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Background: Fatty acid amide hydrolase (FAAH) is a key enzyme in regulating endocannabinoid (eCB) signaling. A common single nucleotide polymorphism (C385A) in the human FAAH gene has been associated with increased risk for addiction and obesity.

Methods: Using imaging genetics in 82 healthy adult volunteers, we examined the effects of *FAAH* C385A on threat- and reward-related human brain function.

Results: Carriers of *FAAH* 385A, associated with reduced enzyme and possibly increased eCB signaling, had decreased threat-related amygdala reactivity but increased reward-related ventral striatal reactivity in comparison with C385 homozygotes. Similarly divergent effects of *FAAH* C385A genotype were manifest at the level of brain-behavior relationships. The 385A carriers showed decreased correlation between amygdala reactivity and trait anxiety but increased correlation between ventral striatal reactivity and delay discounting, an index of impulsivity.

Conclusions: Our results parallel pharmacologic and genetic dissection of eCB signaling, are consistent with the psychotropic effects of Δ^9-tetrahydrocannabinol, and highlight specific neural mechanisms through which variability in eCB signaling impacts complex behavioral processes related to risk for addiction and obesity.

Key Words: Amygdala, endocannabinoids, fMRI, genetics, individual differences, reward, threat, ventral striatum

Prompted by the well-described psychotropic effects of Δ^9-tetrahydrocannabinol (THC), the active chemical of the medicinal plant *Cannabis*, modern neuroscience methodologies have greatly advanced our understanding of the intrinsic mechanisms mediating and regulating cannabinoid signaling in the central nervous system (CNS) (1). Such endogenous cannabinoid or endocannabinoid (eCB) signaling has emerged as a potent modulator of neural circuitries mediating both basic physiological (2,3) and advanced behavioral responses (4–6). Experimental manipulation of these mechanisms has revealed significant behavioral effects, especially in threat- and reward-related domains, that are generally consistent with the effects of *Cannabis* intoxication in humans (7). Furthermore, robust effects of eCB signaling on complex emotion- and reward-related behaviors and their underlying neural substrates, which are often abnormal in diseases such as addiction, depression, anxiety, and obesity, have spurred efforts to develop novel therapeutic agents targeting this neuromodulatory system (8,9).

After their biosynthesis from arachidonic acid, eCBs such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) typically modulate synaptic neurotransmission through stimulation of cannabinoid receptor, type 1 (CB1), the principal CNS cannabinoid receptor widely expressed on multiple neuronal subtypes and their distributed circuitries. In turn, the duration and intensity of eCB signaling, especially for AEA, is regulated by two complementary mechanisms: enzymatic degradation via fatty acid amide hydrolase (FAAH) (10) and active synaptic clearance via the AEA transporter (11). The psychotropic and THC-like effects of AEA, however, appear to be coupled with fatty acid amide hydrolase but not AEA transporter function (12). Thus, FAAH, an integral membrane enzyme, may uniquely regulate behaviorally relevant eCB signaling by mediating the hydrolytic breakdown of AEA into arachidonic acid and ethanolamine.

The elucidation of these molecular mechanisms has motivated attempts to understand their possible contribution to the emergence of stable individual differences in behavioral attributes (e.g., anxious or impulsive temperament) associated with increased risk for psychiatric disorders. Common genetic variation (i.e., polymorphisms) affecting the functioning of components involved in eCB neurotransmission (e.g., AEA, CB1, FAAH) may represent a significant potential source of interindividual variability in eCB signaling that mediates emergent differences in emotion- and reward-related behaviors (13). In the last 5 years, significant progress has been made in describing the contributions of such common genetic polymorphisms to individual differences in complex behavioral phenotypes—in particular, by identifying effects of functional genetic variation on the neural processes that mediate behavioral responses to environmental challenge (14,15).

In the current study, we used an imaging genetics (16,17) strategy to explore the contribution of genetic variation affecting eCB signaling to interindividual variability in threat- and reward-related human brain function. Because of its critical role in regulating the signaling duration and intensity of AEA (10) and its selective contribution to the psychotropic effects of AEA (12), we focused on a common variant in the human gene for FAAH. Specifically, we examined a functional nonsynonymous single nucleotide polymorphism (C385A; rs324420), resulting in the

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Received August 20, 2008; revised October 23, 2008; accepted October 31, 2008.

0006-3223/09/$36.00
doi:10.1016/j.biopsych.2008.10.047

conversion of a conserved proline residue to threonine (P129T) in the amino acid sequence of FAAH (18). In human lymphocytes, *FAAH* 385A, with an allele frequency of ~25% in populations of Caucasian ancestry, is associated with normal catalytic properties but reduced cellular expression of FAAH, possibly through enhanced sensitivity to proteolytic degradation (18,19). Moreover, the C385A is the only common mutation in *FAAH* (20) and the 385A, which putatively augments AEA signaling via decreased enzymatic degradation, has been associated with reward-related pathologies including street drug use and problem drug/alcohol abuse, as well as being overweight and obese (18,20).

In animal models, both pharmacologic and genetic disruption of FAAH function result in decreased anxiety-like behaviors, as well as increased consumption and preference for ethanol (12,21–24). Consistent with these divergent effects, we hypothesized that the *FAAH* 385A would be associated with relatively decreased threat-related amygdala reactivity but increased reward-related reactivity in the ventral striatum (VS). Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) was employed to assay threat-related amygdala and reward-related VS reactivity using challenge paradigms that have successfully identified the impact of genetically driven variability in serotonin and dopamine neurotransmission on these same brain functions (25–29).

Methods and Materials

Participants

A total of 103 participants were recruited from the Adult Health and Behavior (AHAB) project, an archival database encompassing detailed measures of behavioral and biological traits among a community sample of 1379 nonpatient, middle-aged volunteers. Written informed consent, according to the guidelines of the University of Pittsburgh Institutional Review Board, was provided by all participants prior to their participation in our neuroimaging subcomponent of AHAB. All participants included in our analyses were in good general health and free of study exclusions (Supplement 1). In the current study, overlapping *FAAH* genotype and threat-related amygdala reactivity data were available in 82 adult Caucasian volunteers, while overlapping genotype and reward-related VS reactivity were available in 71 of these same volunteers.

Genotyping

FAAH C385A was genotyped using published methods (Supplement 1). In addition, the serotonin-transporter-linked polymorphic region (5-HTTLPR), monoamine oxidase A (*MAOA*) 30-base pair (bp) variable number tandem repeat (VNTR), tryptophan hydroxylase 2 (*TPH2*) G(-844)T, dopamine transporter (DAT1), dopamine D2 receptor (*DRD2*)-141C insertion/deletion (ins/del), and dopamine D4 receptor (*DRD4*) exon3 48-bp VNTR were all genotyped using published protocols (25–29). All of these genotypes were scored by two independent readers by comparison with sequence-verified standards and all call rates were >95%. No additional polymorphisms in *FAAH* or any other eCB-related genes (e.g., CB1) were examined in our study.

Consistent with analyses in the parent study from which our subjects were recruited (30), we used the program STRUCTURE (http://pritch.bsd.uchicago.edu/structure.html) (31) to evaluate presence of genetic substructure in the sample using 15 ancestry informative markers (see Supplement 1 for details).

Amygdala Reactivity Paradigm

The experimental fMRI paradigm consisted of four blocks of a face-processing task interleaved with five blocks of a sensorimotor control task (25,32–34). Subject performance (accuracy and reaction time) was monitored during all scans. Details of our paradigm are provided in Supplement 1.

Ventral Striatum Reactivity Paradigm

Our blocked-design paradigm consisted of pseudorandom presentation of trials wherein participants played a card guessing game and received positive or negative (i.e., win or loss) feedback for each trial (27,35). Details of our paradigm are provided in Supplement 1.

BOLD fMRI Data Acquisition, Processing, and Analysis

Each participant was scanned using a Siemens 3T MAGNETOM (Siemens AG, Medical Solutions, Erlangen, Germany). Allegra developed specifically for advanced brain imaging applications and characterized by increased T2* sensitivity and fast gradients that minimize echo spacing, thereby reducing echo-planar imaging (EPI) geometric distortions and improving image quality. Blood oxygenation level-dependent functional images were acquired with a gradient-echo EPI sequence (repetition time [TR]/echo time [TE] = 2000/25 msec, field of view [FOV] = 20 cm, matrix = 64 × 64) that covered 34 interleaved axial slices (3 mm slice thickness) aligned with the anterior commissure-posterior commissure (AC-PC) plane and encompassing the entire cerebrum and the majority of the cerebellum (Supplement 1).

Whole-brain image analysis was completed using SPM2 (http:fil.ion.ucl.ac.uk/spm/software/spm2/). Following preprocessing (Supplement 1), linear contrasts employing canonical hemodynamic response functions were used to estimate condition-specific (e.g., faces > shapes) blood oxygenation level-dependent activation for each individual and scan. These individual contrast images (i.e., weighted sum of the beta images) were then used in second-level random effects models that account for both scan-to-scan and participant-to-participant variability to determine *FAAH* C385A genotype effects on condition-specific regional responses (Supplement 1). All analyses were thresholded at a voxel level of $p < .05$, false discovery rate (FDR)-corrected for multiple comparisons within an inclusive mask of activations of interest and an extent threshold of at least 10 contiguous voxels. These statistical thresholds have recently been demonstrated to effectively limit false-positive associations in imaging genetics studies below 5% (.2%–4.1%) and are, in fact, conservative (36).

Both our amygdala and VS regions of interest (ROI) were constructed using the WFU PickAtlas Tool (v1.04, http://www.fmri.wfubmc.edu/cms/software#PickAtlas). Our bilateral amygdala ROI was based on the Talairach Daemon option of the PickAtlas but with additional three-dimensional (3-D) dilation (1×) to encompass the dorsal extended amygdala. The VS ROI was defined as a sphere of 20 mm in radius and centered on the Talairach coordinates of x = 0, y = 10, z = −10. Visual inspection confirmed that this ROI encompassed the entire VS as well as adjacent regions of the caudate nucleus (i.e., head of caudate) in both right and left hemispheres. All neuroimaging data are reported using the coordinate system of Talairach and Tournoux.

Behavioral Assessments

The Spielberger State-Trait Anxiety Inventory (STAI) trait version was used to assess the general tendency with which individuals perceive encountered situations to be threatening