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ORIGINAL PAPER

Impact of FAAH genetic variation on fronto-amygdala function during emotional processing

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Abstract

Recent translational studies identified a common endocannabinoid polymorphism, *FAAH* C385A, in the gene for the fatty acid amide hydrolase (FAAH). This polymorphism alters endocannabinoid anandamide levels, which are known to be involved in the fronto-amygdala circuitry implicated in mood regulation and anxiety-like behaviors. While it has been shown that the variant that selectively enhances fronto-amygdala connectivity at rest is associated with decreased anxiety-like behaviors, no study so far has investigated whether this finding of *FAAH*-related differential plasticity extends to task-related differential functional expression and regulation during negative emotional processing. Using an imaging genetics approach, this study aimed to replicate and extend prior findings by examining functional activity and task-related connectivity in fronto-amygdala regions during emotion reactivity and emotional down-regulation of negative affect. Therefore, 48 healthy young adults underwent a functional MRI resting state measurement, completed an emotion regulation paradigm and provided self-reports on anxiety and use of emotion regulation strategies. In line with previous studies, preliminary evidence suggests that A-allele carriers demonstrate stronger fronto-amygdala connectivity during rest. In addition, exploratory whole-brain analyses indicate differential functional activity of A-allele carriers during emotion reactivity and emotion regulation. There were no associations with anxiety-related self-reports and use of emotional regulation strategies. Further research using larger samples and polygenic approaches is indicated to clarify the precise role and its underlying mechanisms in emotion processing.

Keywords *FAAH* C385A · Fronto-amygdala · Amygdala · Emotion reactivity · Emotion regulation · Individual differences · Functional connectivity · fMRI · Genetics

Introduction

Being able to accurately process and regulate emotional information is of crucial importance for appropriate social interactions and an essential feature of mental health. Disrupted emotion processing is proposed as a liability spectrum underlying different mental disorders such as mood and anxiety disorders [43, 53, 76]. Growing evidence has shown that the endogenous cannabinoid (eCB) system is involved in normal emotional processing [46], specifically in the anxiety and stress response [11, 32, 65, 77, 84], and in affective disturbances such as anxiety disorders and depression [2, 37, 63]. Recent studies have highlighted in particular a plasticity regulating gene that affects individual differences in fear expression and anxiety, that is *FAAH* C385A [20, 29].

The integral enzyme fatty acid amide hydrolase (FAAH; Cravatt et al. [15]; Patricelli and Cravatt [74]) is a catabolic enzyme and primary regulator of anandamide (AEA) signaling in the brain [14], an important eCB agonist on the cannabinoid receptor 1 (CB1). Studies have shown that in mice with a targeted disruption in the *FAAH* gene (*FAAH*$^{-/-}$), endogenous brain levels of AEA and related fatty acid amides are increased over tenfold [14, 54]. Within the human *FAAH* gene exists a single nucleotide polymorphism (SNP; rs324420) with a minor allele frequency of 21% [88]. This missense mutation (385C to A) leads to a substitution of an

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evolutionary conserved proline to threonine at amino-acid position 129 (Pro129Thr) resulting in reduced FAAH protein expression and enzymatic activity as well as increased AEA levels in the brain [12, 20, 79, 80].

Regarding the differential impact of *FAAH* C385A on brain circuitry and function, recent studies developed a knock-in mouse model that biologically recapitulates the human *FAAH* C385A SNP and linked the A-allele to greater fear extinction, enhanced fronto-limbic connectivity and decreased anxiety-like behaviors in both mouse and humans [20, 29]. In detail, Gee et al. [29] demonstrated that enhanced structural fronto-limbic connectivity in the uncinate fasciculus (a white matter tract that connects limbic structures with the prefrontal cortex) is associated with decreased anxiety in humans. In a similar vein, Dincheva et al. [20], performed a functional resting state connectivity analysis focusing on connectivity between amygdala and subgenual ventromedial prefrontal cortex (vmPFC) and dorsal anterior cingulate cortex (dACC), respectively, two key regions within the fronto-amygdala circuitry. They found that A-allele carriers show an increased correlation between BOLD signals in the vmPFC and amygdala as well as decreased anxiety-like behaviors. Further evidence comes from studies demonstrating that *FAAH* C385A predicts individual differences in amygdala habituation to threat and stress-reactive personality traits [32, 33] as well as a decreased threat-related amygdala reactivity associated with reduced trait anxiety in A-allele carriers compared to C/C homozygotes [35]. In sum, phenotypic differences in anxiety-like behaviors may result from functional effects of the A-allele on prefrontal regulation of the amygdala during fear [33, 35] through stronger fronto-amygdala connectivity.

Besides the impact of *FAAH* C385A on structural and functional plasticity in the brain, there might be a second functional mechanism that influences genotype-related anxiety and stress responses: normally, high levels of AEA are associated with anxiogenic effects, whereas low levels are associated with anxiolytic effects. However, it has been shown that the inhibition of AEA degradation through FAAH inhibitors seems to circumvent these biphasic effects by enhancing CB1 receptor signaling in a temporarily and spatially restricted manner, resulting in reduced anxiety-like behaviors (for review see Moreira and Wotjak [66]). Therefore, given that *FAAH* C385 results in reduced FAAH expression and activity and, thus, elevated AEA levels in the brain, the polymorphism might influence brain circuitry and function through immediate, phasic effects associated with the degradation of AEA and CB1 receptor signaling. This notion is backed up by a recent study showing that the A-allele is associated with (phasic) increased serum AEA levels throughout a stress challenge compared to C/C homozygotes, accompanied by a decreased subjective anxiety response to the stress challenge [82]. Furthermore, a recent PET study has shown that A-allele carriers show a longer in vivo binding of a FAAH labeling ligand, supporting the effect of this genetic variation on central FAAH levels [5].

Given these well-established effects of *FAAH* C385A on fronto-limbic connectivity that impacts fear expression and anxiety, the current study sought to examine whether this finding of differential plasticity extends to task-related differential functional expression and regulation during negative emotional processing. Considering the prominent role of amygdala, dACC and vmPFC in expression, extinction, and regulation of fear and emotional processing [26, 78] and following up on the rationale by Dincheva et al. [20], we examined exactly these brain regions. Regarding emotion regulation, studies have consistently shown that voluntary down-regulation of negative affect is associated with increased activation of the dorsolateral and medial prefrontal cortex (mPFC) and that this activity increase is negatively correlated with amygdala activity [40, 71, 85]. Regarding emotion reactivity, complementary evidence can be found in analyses on task-related functional connectivity, showing both positive and negative connectivity of the amygdala with dorsal and ventral ACC and mPFC, respectively [25]. In addition, primarily negative connectivity between amygdala and ventral ACC and mPFC was observed during emotion regulation [3, 24, 25]. This implies not only that amygdala and prefrontal regions work in parallel to facilitate downregulation, but that functional coupling between those areas contributes to successful emotion regulation.

Based on these findings, the goal of this study was (a) to replicate the findings of Dincheva et al. showing stronger functional fronto-amygdala connectivity for A-allele carriers during resting state, and (b) extend these findings on task-related functional connectivity by examining the impact of *FAAH* C385A on emotion reactivity and emotion regulation. Furthermore, we were interested whether (c) *FAAH* C385A modulates functional activity in fronto-amygdala regions during emotion reactivity and emotion regulation (task-related activation). We hypothesized that A-allele carriers show stronger resting state connectivity between amygdala and the target regions of interest (ROI) examined by Dincheva et al. [20], that is, dACC and vmPFC. Furthermore, we hypothesized that stronger fronto-amygdala connectivity in these individuals is associated with decreased emotional reactivity during passively viewing of emotional pictures as well as more successful top-down regulation of negative affect during volitional emotion regulation.

Methods and materials

Participants

The sample comprised 48 healthy young adults (22 male, aged 18–36 years, $M_{\text{age}} \pm$ SD 24.7 ± 4.0 years) of German/Middle European descent recruited at the TU Dresden. All participants were right-handed and pre-screened for magnetic resonance imaging (MRI) contraindications (for example, metal plates or implants). None of the participants did report any current or prior medical, neurological or psychiatric illness or treatment. The experimental protocol was approved by the ethics committee of the TU Dresden. The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the German Psychological Association. All participants provided written informed consent and received compensation for expenses. Data of one participant was only available for the resting state session. Therefore, functional activity and connectivity analyses included 47 participants.

Experimental paradigm and procedure

The paradigm was based on previous designs to study emotion reactivity and regulation [62, 85]. Emotion reactivity was operationalized as the task condition in which participants were asked to permit any emotions arising in response to a set of negative and neutral pictures, without manipulating their emotional response. Emotion regulation was operationalized as the task condition in which participants were asked to decrease any emotional response which may arise towards negative and neutral pictures by adopting the position of a detached observer who is not involved in the scene presented in the picture. Other possible strategies to detach themselves were imagining to see the pictures through a glass or imagining the pictures shrink, spin or fade in the background. Consequently, four task conditions were presented (strategies: permit, detach; valence: negative, neutral).

Because the study was part of a larger project (cf. [19]), half of the participants also underwent an additional experimental condition, that is, trying to increase any emotions arising in response to negative and neutral pictures. The data of this condition are not analyzed and reported here. However, overall stimuli durations for this group (referred to as group B, $n = 26$) were somewhat shorter than for the other group (group A, $n = 22$). To exclude the potential influence of varying conditions and trial durations, a dichotomous group variable was included as covariate in all subsequent analyses.

To ensure understanding of the instructions and familiarity with the procedure, all participants underwent a training session outside the MR scanner, which took about 10–15 min and consisted of 16–24 trials. The subsequent functional magnetic resonance imaging (fMRI) measurement lasted approximately 60 min (group A) or 70 min (group B). In the beginning of the session, 5 min were spent with preparatory technical scans, which allowed the participants to accommodate themselves with the scanning environment. Four experimental runs followed with a duration of 9 min (group A) or 11 min (group B) each. At the beginning of each trial, a picture was presented for 10 s (group A) or 8 s (group B). During the initial 2 s of this period, a semi-transparent overlay was presented across the center of the picture, which contained—as a single word—the instruction for either the "permit" or the "detach" condition (instruction phase). Afterwards, the picture was presented alone for the remaining 8 s or 6 s, respectively (regulation phase). Subsequently, a fixation cross was presented with a jitter of 16–24 s (20 s on average) or 12–20 s (16 s on average), respectively (fixation phase). Similar to previous studies [41, 62], we used an event-related design to avoid multiple repetitions of stimuli of the same experimental condition and possibly ensuing increased habituation effects. After each of the four experimental runs, participants rated their current emotional arousal for the four conditions ("permit negative", "permit neutral", "detach negative", "detach neutral") using a slider, ranging from "very low" to "very high" without time limit.

After all experimental runs, an anatomical scan was acquired, which lasted approximately 8 min, followed by a passive viewing task of previously presented pictures (data not analyzed here). After completing the scanning session, participants were debriefed about the experiment. All participants returned to a second session approximately one week later, which included an 8-min resting state session. Furthermore, participants were asked to fill in questionnaires aimed at investigating individual differences in anxiety, negative emotionality, and use of emotion regulation strategies.

Stimuli

Stimuli were selected from the International Affective Picture System (IAPS; Lang et al. [48]) and the Emotional Picture Set (EmoPicS; Wessa et al. [86]; see Supplementary Material for picture numbers). In group A, we used 32 negative (valence $M \pm SD$ 2.68 ± 0.76; arousal $M \pm SD$ 5.77 ± 0.85) and 32 neutral pictures (valence $M \pm SD$ 5.15 ± 0.56; arousal $M \pm SD$ 2.95 ± 0.59). In group B, we used 48 negative (valence $M \pm SD$ 2.67 ± 0.75; arousal $M \pm SD$ 5.69 ± 2.44) and 48 neutral pictures (valence $M \pm SD$ 5.16 ± 0.56; arousal $M \pm SD$ 2.92 ± 0.49). The assignment of the pictures to either the "permit" or "detach" condition was counterbalanced across participants and experimental runs with no more than two successive stimuli of one condition. To rule out any stimulus- or content-related confounds, one part of negative and neutral pictures was used for one-half of the participants, and the other part was used for the other half of the participants. All stimuli were presented onto a back-projection screen located at the rear end of the scanner and were viewed through a mirror attached to the head coil.

Genotyping

Genomic DNA was extracted from EDTA anti-coagulated venous blood samples according to standard procedures. Rs324420 was genotyped using a TaqMan 5′ nuclease assay. Genotype frequencies were 58.3% for C/C ($n = 28$), 41.7% for C/A ($n = 18$), and 4.2% for AA ($n = 2$). The genotypes did not deviate from Hardy–Weinberg equilibrium (Chi-square tests with $df = 1$; $\chi^2 = 0.18$, $p = 0.67$). Because there were only two A/A homozygotes, analyses were conducted using two genotype groups: A-allele carriers ($n = 20$) and C/C homozygotes ($n = 28$).

Behavioral trait measures

Anxiety and negative emotionality

The German version of the Spielberger State-Trait Anxiety Inventory (STAI) trait version was used to assess anxiety [49]. To examine individual differences in self-reported negative affect and emotionality, negative affect was rated on the German version of the Positive and Negative Affect Schedule (PANAS-NA; Krohne et al. [44]), and Neuroticism was rated on the German version of the NEO Five-Factor Inventory (NEO-FFI; Borkenau and Ostendorf [6]).

Emotion regulation

Because individual differences in the use of cognitive emotion regulation strategies may modulate task performance in the emotion regulation task, the German version of the Emotion Regulation Questionnaire (ERQ; Abler and Kessler [1]) was used to assess the habitual use of reappraisal and suppression.

Statistical analyses of behavioral data

Analyses on emotional arousal ratings and trait measures were conducted using SPSS (version 24; SPSS Inc.). All variables were normally distributed (Shapiro–Wilk tests, $p > 0.05$) and checked for outliers. A $2 \times 2 \times 2$ mixed-model ANOVA was calculated including the factors valence (negative and neutral) and strategy (permit and detach) as within-subjects variable and *FAAH* genotypes as between-subjects variable to examine possible differences in emotion reactivity and regulation between genotype groups. Independent *t*-tests were conducted to examine potential differences in self-reported anxiety, negative emotionality and habitual use of emotion regulation strategies between genotype groups. In addition, we tested potential differences in possible confounding factors such as age, sex, and testing group. *FAAH* A-allele carriers did not differ from C/C homozygotes in these factors (all $p \geq 0.211$). Statistical analyses of the behavioral data were thresholded at $p < 0.05$. For the STAI, data were missing from ten participants, for NEO-FFI Neuroticism from four participants and for the emotional arousal ratings from one participant due to technical problems.

Data acquisition

MRI images were acquired using a 3-T Trio-Tim MRI whole-body scanner (Siemens, Erlangen, Germany), using a 12 channel head coil. Functional ($T2^*$) MR images during resting state and the emotion regulation task were acquired using an EPI sequence with 42 axial slices per volume and a slice thickness 2 mm (TR 2410 ms; TE 25 ms; flip angle 80°; slice gap 1 mm; field of view 192 mm; matrix size 64×64; voxel size: $3 \times 3 \times 2$ mm^3). In addition, anatomical (T1) images were acquired using an MPRAGE sequence that consisted of 176 sagittal slices with a thickness of 1 mm (TR 1900 ms; TE 2.26 ms; flip angle 9°; field of view 256 mm; matrix size 256×256; voxel size: $1 \times 1 \times 1$ mm^3).

Resting state functional connectivity data preprocessing and analysis

Preprocessing and further processing steps were implemented in the CONN toolbox (version 16.b) pipeline [87] and performed with SPM 12 (Wellcome Trust Center for Neuroimaging, UCL, London, UK). All functional scans of the resting state session were spatially realigned and unwarped to correct for interscan movement. The structural image was segmented and normalized to the MNI (Montreal Neurological Institute, Quebec, Canada) reference brain. Functional images were normalized to the reference brain followed by outlier detection (ART-based scrubbing), and smoothed using an 8 mm Gaussian kernel. To avoid confounding signals from surrounding regions, time series were extracted from the unsmoothed spatially normalized data. Prior to first-level analyses, a denoising procedure was applied to remove motion artifacts, physiological and other artifactual effects from the fMRI-signal. This procedure included the component-based correction method (CompCor; Behzadi et al. [4]) and temporal band-pass filtering of 0.008–0.09 Hz.To avoid potential ramping effects at the beginning of the session, CONN models the entire acquisition and includes an additional confounding variable as covariate in the denoising procedure. The six movement parameters and a matrix containing the ART-detected outliers were included as first-level nuisance covariates. For time series extraction, we first defined the respective regions of interest (ROIs). For the amygdala ROIs, maximum probability maps were created using the SPM Anatomy toolbox v2.2c [22], containing basolateral, centromedial, and the superficial divisions from the left and right amygdala. The ROI masks for dACC and subgenual vmPFC were build using the WFU Pickatlas [59]. Two 4 mm spherical ROIs were created at exactly the same locations examined by Dincheva et al. [20] based on previous literature, i.e., Milad et al. [64] with regard to dACC [− 3, 23, 18 Tal] and Schiller and Delgado [78] with regard to vmPFC [0, 40, − 3 MNI]. ROI-to-ROI analyses were performed for each participant and a general linear model was used to calculate connectivity of both amygdalae to dACC and subgenual vmPFC, respectively. *FAAH* C385A genotypes were included as second-level covariate to test for voxel-wise correlations between the ROI-to-ROI connectivity values and *FAAH* C385A genotypes. For all ROI-to-ROI connectivity analyses, the significance threshold was set to $p<0.05$, family-wise error (FWE) corrected for multiple comparisons. Note that we refer to one-sided p values because of our strong hypothesis driven approach, but two-sided p values are also provided (see "Discussion").

Functional activity data preprocessing and analysis

Imaging data analysis was performed using Matlab 2010b (Math-Works, Natick, MA, USA) and SPM12. The first four volumes of each run were discarded, and preprocessing consisted of slice-time correction, motion correction, coregistration of individual functional and anatomical data, spatial normalization of the anatomical images to the MNI template, application of the estimated transformation parameters to the coregistered functional images using a resampling resolution of $2\times2\times2$ mm, and spatial smoothing of the functional images (FWHM 8 mm). First-level statistical analysis was performed using a general linear model with eight regressors ("permit negative", "permit neutral", "detach neutral", "detach negative"; each for regulation and fixation phase; group A) and 12 regressors ("permit negative", "permit neutral", "detach negative", "detach neutral", "intensify negative", "intensify neutral"; each for regulation and fixation phase; group B), respectively, modeled as an event with a duration of 10 s (group A) and a duration of 8 s (group B), respectively. All above regressors were convolved with the canonical HRF. In addition, six motion regressors of no interest were included. The resulting parameter estimates for the contrasts of interest (emotion reactivity: permit negative > permit neutral; emotion regulation: detach negative > permit negative, and permit negative > detach negative) during each run were averaged across runs and submitted to a second-level, random-effects analysis. At the second-level, differences in activation in predefined ROIs between *FAAH* C385A genotypes were analysed using a two-sample *t*-test. Based on our a priori hypotheses, we examined four regions of interest: dACC, subgenual vmPFC, and left and right amygdala, as defined above. For these analyses, the significance threshold was set at $p<0.05$, FWE-corrected for small volume. To investigate possible effects outside our small ROI masks, we conducted an exploratory whole-brain analysis using a less stringent threshold ($p<0.001$ uncorrected with $k>10$ voxel extent threshold) selected according to Lieberman and Cunningham [55], demonstrating that this threshold produces a desirable balance between Types I and II error rates and is thereby comparable to the effective FDR in typical behavioral science studies. Regions were labeled according to the Anatomical Automatic Labeling atlas (AAL, [83]) and the Anatomy toolbox for SPM [22].

Task-related effective connectivity analysis

We performed a generalized psycho-physiological interaction (gPPI) analysis as implemented in CONN [87] to assess differences in amygdala effective connectivity during emotion reactivity and emotion regulation with dACC and vmPFC and investigated whether this task-related connectivity differed between *FAAH* C385A genotype groups. A gPPI analysis is the recommended PPI model to analyze task-related connectivity in the presence of multiple task conditions [61]. In general, in a PPI analysis the seed region of interest is chosen on the basis that this region was active in a certain contrast of interest [73]. Since only the right amygdala showed increased activity during emotion reactivity and decreased activity during emotion regulation (in contrast to our dACC or vmPFC ROI), we created a functional 5 mm spherical ROI around the center of activation in the right amygdala for the contrast permit negative > detach negative across all participants (cf. Kanske et al. [41]). This region was chosen as a seed region for the gPPI analysis. Then, the deconvolved time series of our seed region was extracted in each participant as the first regressor in the gPPI analysis (physiological variable). The second regressor represented the experimental condition (emotion reactivity and emotion regulation, respectively). The regressor of interest was the interaction between the time series of the seed region and the experimental condition (gPPI). Note that the gPPI model in CONN follows the implementation in FSL, where the interaction factor is convolved with the HRF and the interactions are modeled on the resulting BOLD-level signal.

Also note that the PPI effects are relative to a baseline state, thereby providing a relative measure of connectivity (vs. an absolute measure of connectivity as estimated in standard functional connectivity measures). Finally, the individual gPPI contrast images were entered into a second-level random-effects analysis to compare amygdala coupling between *FAAH* genotypes using a two-sample *t*-test. For a priori ROI analyses, we used a significance threshold of $p < 0.05$ FWE-corrected for small volume. For whole-brain analyses, voxel-level two-sample *t*-tests were considered significant at an initial height threshold of $p < 0.001$ uncorrected and a cluster extent threshold of $k > 10$ voxels (see Lieberman and Cunningham [55]).

Results

Behavioral results

Self-reported anxiety, negative emotionality and emotion regulation

The *FAAH* genotype groups did not differ regarding self-reported trait anxiety and negative emotionality, respectively, STAI-T: $t(36) = -0.54$, $p = 0.594$, $d = 0.18$; Neuroticism: $t(42) = -1.37$, $p = 0.178$, $d = 0.42$; PANAS-NA: $t(46) = 0.66$, $p = 0.512$, $d = 0.19$, nor regarding the habitual use of reappraisal and suppression, ERQ-R: $t(46) = 1.02$, $p = 0.314$, $d = 0.30$; ERQ-S: $t(46) = -0.19$, $p = 0.849$, $d = 0.06$ (see Table 1).

Emotional arousal ratings

Regarding the emotional arousal ratings in the emotion regulation task, a repeated measures ANOVA including *FAAH* genotypes as between-subjects factor revealed a significant main effect of valence, $F(1, 44) = 186.34$, $p < 0.001$, $\eta_p^2 = 0.81$. Planned contrasts revealed significant higher arousal for negative than neutral pictures. Moreover, a significant main effect of strategy emerged, $F(1, 44) = 41.01$, $p < 0.001$, $\eta_p^2 = 0.48$. Planned contrasts revealed significant higher arousal for the permit than detach condition. There was also a significant ordinal interaction between valence and strategy, $F(1, 45) = 5.91$, $p = 0.019$, $\eta_p^2 = 0.12$, indicating a stronger increase in arousal from neutral to negative pictures in the permit compared to the detach condition. Regarding the influence of *FAAH* C385A, there was no significant interaction between genotypes and valence, $F(1, 44) = 0.18$, $p = 0.672$, $\eta_p^2 = 0.01$, and no three-way interaction between genotypes and valence and strategy, $F(1, 44) = 0.44$, $p = 0.835$, $\eta_p^2 = 0.01$, but a marginal significant interaction between genotypes and strategy, $F(1, 44) = 3.43$, $p = 0.071$, $\eta_p^2 = 0.07$. Planned contrasts revealed slightly decreased levels of self-reported arousal for A-allele carriers compared to C/C homozygotes during permit compared to detach across negative and neutral pictures (see Fig. 1). Descriptive statistics are detailed in Table 1.

Table 1 Descriptive statistics for behavioral results

	FAAH C385A A-allele carriers $n = 20$		*FAAH* C385A C/C homozygotes $n = 28$	
	M	*SD*	*M*	*SD*
Sex (female/male)	13/7		13/15	
Age	24.8	3.6	24.6	4.4
STAI-T	42.7	6.5	41.4	7.7
PANAS-NA	21.9	6.4	22.9	5.0
NEO-N	23.3	7.0	20.6	6.6
ERQ reappraisal	27.6	5.6	29.2	5.4
ERQ suppression	14.9	4.1	14.6	4.9
Emotional arousal ratings				
Negative permit	23.1	7.4	25.5	3.9
Negative detach	19.9	7.2	20.5	6.0
Neutral permit	10.9	6.9	12.6	6.3
Neutral detach	9.4	5.9	9.1	5.0

STAI-T, State Trait Anxiety Inventory [49]; PANAS-NA, Positive Affect Negative Affect Schedule-Negative Affect [44]; NEO-N, NEO Five-Factor Inventory Neuroticism [6]; ERQ, Emotion Regulation Questionnaire [1]; Slider: 0 = very calm, 40 = very excited

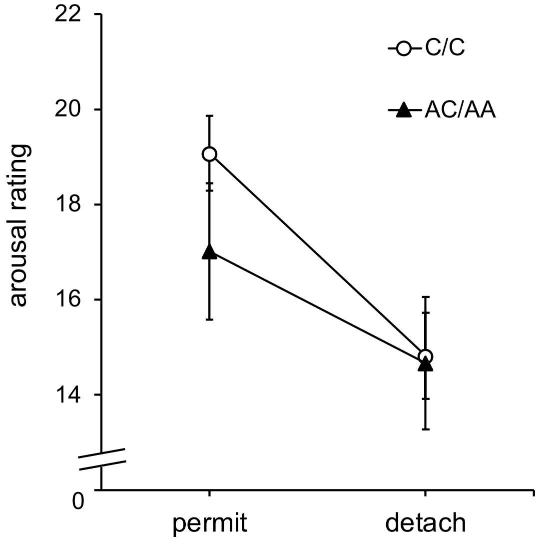

Fig. 1 Self-reported arousal compared between permit vs. detach negative and neutral pictures in *FAAH* A-allele carriers relative to C/C homozygotes. Scale ranging from 0 ("not aroused at all") to + 40 ("extremely aroused"). Error bars represent standard errors of the means

fMRI results

FAAH genotype effects on resting state connectivity

First, we examined resting state connectivity between our ROIs in the amygdala, vmPFC and dACC. Overall, there was a significant positive correlation in resting state activity between dACC and left [$t(47)=3.16$, $p_{\mathrm{FWE}}=0.011$] and right amygdala [$t(47)=2.60$, $p_{\mathrm{FWE}}=0.049$], respectively. Moreover, there was a significant positive correlation between vmPFC and left [$t(47)=2.98$; $p_{\mathrm{FWE}}=0.018$] and right amygdala [$t(47)=3.39$; $p=0.008$]. Whole-brain seed-to-voxel connectivity maps can be seen in Supplementary Figure S1.

Regarding *FAAH* C385A, A-allele carriers showed a significantly increased correlation between right amygdala and dACC compared to C/C homozygotes [$t(46)=2.43$, $p_{\mathrm{FWE}}=0.038$, one-sided; $p_{\mathrm{FWE}}=0.075$, two-sided; see Fig. 2 and Supplementary Figure S2 for Bayesian statistics]. This pattern was selective to dACC–amygdala connectivity, with no genotypic difference in right amygdala–vmPFC connectivity ($p_{\mathrm{FWE}}=1$). Furthermore, there were no genotype differences in connectivity between left amygdala and dACC and vmPFC, respectively ($p_{\mathrm{FWE}}=1$; see Supplementary Table 1 for further information).

FAAH genotype effects on functional activity

Regions significantly involved in emotion reactivity (permit negative > permit neutral) were the right inferior frontal gyrus, right precentral gyrus, left and right occipital and ventral temporal cortices, left cerebellum (Crus2), and right amygdala after small volume correction (see Supplementary Table 2). Regions significantly involved in emotion regulation (detach negative > permit negative) were the left and right angular gyrus and right frontal cortices. Also, there was extensive activation in the posterior cingulum and significantly decreased activation in the right amygdala (see Supplementary Table 3).

Neural responses of *FAAH* genotypes were compared regarding emotion reactivity and emotion regulation within our a priori regions of interest in dACC, vmPFC, and left and right amygdala. Regarding both, emotion reactivity and emotion regulation, we found no significant differences in activation in our regions of interest. To investigate possible effects outside our small ROI masks, we conducted an exploratory whole-brain analysis using a less stringent threshold ($p<0.001$ uncorrected with $k>10$ voxel extent threshold, see Lieberman and Cunningham [55]). For emotion reactivity, we found significant higher activation in the right gyrus rectus for A-allele carriers relative to C/C homozygotes (see Table 1; Supplementary Figure S3). For emotion regulation, we observed significantly higher activation in the left and right anterior cingulate; and significantly decreased activation in the insula for A-allele carriers compared to C/C homozygotes (see Table 2; Supplementary Figure S4).

FAAH genotype effects on task-related effective connectivity

Regarding emotion reactivity (permit negative > permit neutral) and emotion regulation (detach negative > permit negative), we found no differences in effective connectivity between the amygdala and our target ROIs. To investigate possible effects outside our small ROI masks, we conducted an exploratory seed-to-voxel analysis ($p<0.001$ uncorrected with $k>10$ voxel extent threshold, see Lieberman and Cunningham [55]). However, in a direct comparison between *FAAH* C385A genotype groups, we found no significantly increased or decreased connectivity between right amygdala and our target ROI, neither in a ROI-to-ROI nor seed-to-voxel analysis.

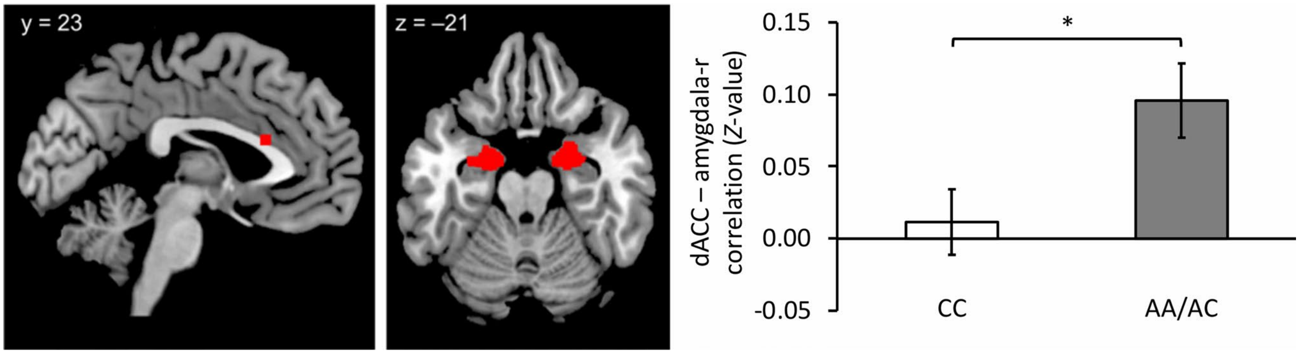

Fig. 2 (Left) ROI mask in Montreal Neurological Institute standard space of dorsal anterior cingulate (dACC) and left and right amygdala. (Right) Resting state functional connectivity of dACC and right amygdala in *FAAH* A-allele carriers relative to C/C homozygotes. * $p_{\mathrm{FWE}} < .05$

Table 2 Peak voxels and cluster sizes from whole-brain analyses of *FAAH* genotype effects on emotion reactivity and emotion regulation

Region	H	*x*	*y*	*z*	*k*	*Z*	*p* (uncorr)
Emotion reactivity (permit negative > permit neutral)							
FAAH-A > *FAAH-C*							
Gyrus rectus	R	12	44	− 18	13	3.49	< 0.001
FAAH-C > *FAAH-A*							
No suprathreshold voxels							
Emotion regulation (detach negative > permit negative)							
FAAH-A > *FAAH-C*							
Anterior cingulum	R	22	44	0	95	3.62	< 0.001
Anterior cingulum	L	− 14	42	12	10	3.35	< 0.001
White matter	L	− 14	36	0	22	3.55	< 0.001
FAAH-C > *FAAH-A*							
No suprathreshold voxels							
Emotion regulation (permit negative > detach negative)							
FAAH-A > *FAAH-C*							
No suprathreshold voxels							
FAAH-C > *FAAH-A*							
Insula/inferior orbital frontal cortex	L	− 18	34	0	76	3.87	< 0.001
White matter	R	24	42	0	39	3.65	< 0.001

Cluster with $p < 0.001$ (uncorrected with $k > 10$ voxel extent threshold). Coordinates are given in MNI space. H, Hemisphere; R, right; L, left

Discussion

In the light of the findings on the influence of *FAAH* C385A on fronto-amygdala function at rest by Dincheva et al. [20] and Gee et al. [29], this study aimed to replicate (a) and extend previous findings by examining whether *FAAH* C385A impacts on functional activity (b) and task-related effective connectivity (c) in fronto-amygdala regions during negative emotional processing. Our data provide preliminary evidence that A-allele carriers show stronger connectivity between amygdala and dACC at resting state. In addition, exploratory whole-brain analyses revealed differential functional activity of A-allele carriers during emotion reactivity and emotion regulation, although these effects survived only a less stringent threshold. We found no differences in task-related effective connectivity and no influence of *FAAH* C385A on self-reported anxiety, negative affect and use of emotion regulation strategies.

Behavioral results

The finding that *FAAH* C385A did not influence self-reported anxiety, negative emotionality, and use of emotion regulation strategies is in contrast to the study by Dincheva et al. [20], who found lower self-reported trait anxiety as measured by the STAI-T questionnaire in two independent samples (one with a comparable sample size of $N = 40$). However, the finding is in line with three other studies who found no effects on self-reported anxiety [13, 35, 50]. Regarding the emotional arousal ratings in the emotion regulation task, we found the typical effect of less self-reported negative affect during regulation of negative pictures compared to permit [21, 41]. However, we found no meaningful effect of *FAAH* C385A genotypes on arousal ratings, which is in line with a previous study [13], although A-allele carriers reported a slightly decreased arousal during permit vs. detach of both negative and neutral pictures compared to C/C homozygotes. In line with Conzelmann et al. [13] and Hariri et al. [35], an explanation for the missing effects might be that ratings and self-reports are prone to biases by normative behavior and expectancies, and endophenotypes such as emotion reactivity and emotion regulation may be better revealed by biopsychological measures [30]. Furthermore, we examined a truly healthy sample, which might underestimate possible gene-related effects because of variance homogeneity. In addition, no environmental effects were assessed, but these might help in unmasking genetically driven effects on behavior [34].

FAAH genotype effects on resting state connectivity

We found stronger resting state connectivity between right amygdala and dACC in A-allele carriers compared to C/C homozygotes. Note, however, that we used one-tailed tests to predict the directional hypothesis (i.e., stronger resting state connectivity between dACC and vmPFC with amygdala for A-allele carriers compared to C/C homozygotes; see "Methods and materials"). We did this because our hypotheses followed up on the rationale by Dincheva et al. [20], who selected both regions of interest based on findings that emphasize their crucial role in expression and regulation of fear [64, 78]. In addition, a long line of research has highlighted both dACC and vmPFC to be fundamentally involved in the regulation of emotions and emotional responses (reviewed in Buhle et al. [9]; Dörfel et al. [21]; Etkin et al. [25]; Kalisch [39]; Ochsner and Gross [70]; Ochsner et al. [72]). Thus, we predicted stronger (vs. less or the same) connectivity because of the proposed stronger prefrontal regulation of emotional responses in A-allele carriers compared to C/C homozygotes as reported in the literature [20, 29, 32, 35]. Using this approach, we replicated the general pattern of gene-related differential plasticity in fronto-limbic function. Nevertheless, while Dincheva et al. [20] found enhanced connectivity between vmPFC and amygdala, we found enhanced connectivity between dACC and amygdala. Applying a two-tailed test reveals a marginally significant effect with $p = 0.075$ (corrected for multiple comparisons). Based on previous findings, the effect seems highly reasonable, given that the dACC has been reliably implicated in fear expression and threat appraisal [26, 64], cognitive control and conflict monitoring [7, 42, 75] and in autonomic control [16, 17]. However, the findings have to be interpreted with caution. Firm conclusions about the underlying relationship of the involved brain areas await further research.

A final comment concerns statistical power: assuming a true genotype effect of size $d = 0.73$ on vmPFC-amygdala connectivity as implicated by the findings of Dincheva et al. [20; $N = 35$], the probability in the present study ($N = 48$) was 79% (1-beta) to observe an effect that surpasses the threshold of alpha = 0.05 (one-sided), and 95% to observe an effect that surpasses alpha = 0.2 (one-sided) as calculated in G*Power 3.1 [27]. Because our study did not reveal a one-sided alpha level smaller than 0.2 for vmPFC-amygdala connectivity, the true effect size is likely smaller than 0.73.

FAAH genotype effects on functional activity and task-related effective connectivity during emotional processing

We found the general pattern of increased activation in the amygdala and other cortical regions (e.g., occipital and ventral temporal cortices, inferior frontal gyrus) for viewing negative vs. neutral pictures, as well as increased activation in the right frontoparietal regulation network and posterior cingulum for regulating vs. permit negative pictures. Therefore, the overall results for emotion reactivity and emotion regulation in this sample are comparable to the findings of other studies [21, 41, 62] and allow to look for potential effects of *FAAH* genetic variation. When applying our ROI masks for dACC, vmPFC and amygdala, we found no significant differences between *FAAH* C385A genotypes. However, given their small size (4 mm radius), we were interested in potential effects outside of these regions and conducted exploratory whole-brain analysis using a less stringent threshold. For emotion reactivity, we observed significant higher activation in the right gyrus rectus for A-allele carriers relative to C/C homozygotes. The gyrus rectus lies within the medial orbitofrontal cortex and has been shown to be implicated in successful operant extinction [28, 31]. Although our results need to be replicated in future research, they provide preliminary evidence that *FAAH* C385A might impact activation in the medial orbitofrontal cortex during emotion reactivity. For emotion regulation, we observed significantly higher activation in the left and right ACC and decreased activation in the insula for A-allele carriers compared to C/C homozygotes. Studies have reliably shown that negative stimuli elicit a strong response in affective regions (most prominently amygdala, but also insula) and that reappraisal of negative stimuli results in increased activation of frontoparietal regions, including the ACC [40, 69]. Therefore, these results provide preliminary evidence that A-allele carriers of *FAAH* C385A show not only increased connectivity between amygdala and dACC at rest but also increased activity of dACC during emotion regulation, which might reflect increased recruitment of top down control on limbic regions such as the insula. However, we recognize that the ACC region differs from our a priori dACC ROI mask and was only found with a less stringent threshold. Therefore, the results need to be confirmed in further studies.

We found no effects of *FAAH* C385A on task-related effective connectivity between amygdala and dACC and vmPFC, respectively. However, one should also keep in mind that resting state and task-related connectivity measures differ both methodological and regarding their functional implications (e.g., resting state connectivity analyses focus on spontaneous fluctuations in a low frequency range; PPI analyses are not generally filtered and can also include a higher frequency range). Given our relatively small sample size and the event-related design that tends to have generally smaller effect sizes than block designs [73], this issue should be addressed in larger samples using a block design with a higher proportion of task time in the condition of interest.

Developmental challenges

Another issue that deserves attention is the fact that eCB signaling peaks in adolescence and then declines, with AEA, CB1 receptor and FAAH expression fluctuating reciprocally across development [10, 52, 57]. In detail, animal studies revealed that the onset of adolescence is marked by the highest expression of the CB1 receptor in both cortical and subcortical regions, and a subsequent decline to adult levels throughout adolescence, especially in limbic regions [18, 23, 36, 23, 36]. Conversely, FAAH expression shows a dip at the onset of adolescence, a subsequent increase throughout adolescence and a subsequent decline to adult levels [52]. Consistent with the regulatory role of FAAH, AEA levels show an inverse pattern, that is, the highest expression at the onset of adolescence with a subsequent decline throughout adolescence and a subsequent increase to adult levels [51, 52]. These dynamic changes in AEA, CB1 receptor and FAAH expression reflect decreasing endocannabinoid signaling during adolescence, associated with increasing risk for anxiety (for review see Hill and Patel [38]). Although our sample comprised mainly young adults (M_{age} 24.7 ± 4.0 years), participants in the samples by Dincheva et al. [20] and Gee et al. [29] were somewhat younger (Dincheva: M_{age} 21.2 ± 3.7 years and M_{age} 20.1 ± 2.2 years; Gee: M_{age} 15.1 ± 1.7 years and M_{age} 16.3 ± 2.5 years), demonstrating a slightly shifted age-distribution towards adolescence. Given the dynamical changes and the fact that the effects of *FAAH* C385A on fronto-limbic circuitry and anxiety emerge during adolescence (as AEA levels decrease), but not before as revealed by the study of Gee et al. [29], one might speculate that the impact of this polymorphism peaks in adolescence and potentially declines in later life. As the expression of key genes in the eCB system changes (and partially decreases) with maturation of cortical function, it might be promising to examine this temporal specificity more closely and investigate potential differences across multiple age groups starting from adolescence.

Limitations and future research

A limitation of our study is the comparably small sample size in the context of molecular genetics. Therefore, further studies using larger samples are clearly needed to confirm the results. In addition, although we found *FAAH*-related differences in fronto-amygdala connectivity, further studies should extend these findings regarding the underlying neurobiological mechanisms that contribute to these differences. Related to that, a recent study identified another player in the eCB system to be implicated in development of anxiety, the cannabinoid CB1 receptor [47]. Other genes related to anxiety disorders are the monoamine oxidase A (*MAOA*) gene, the oxytocin receptor (*OXTR*) gene [68, 89] and genes in the well-established monoaminergic system [8, 58, 60]. Based on these findings, further studies should investigate polygenic effects of these reasonable candidates to enhance power and to account for complex gene–gene interactions.

Regarding our study design, the resting state measurement took place in a separate session approximately one week after the emotion regulation task. Although there was no dropout and the investigated effects are supposed to be independent from each other (resting state vs. task-related), unknown effects of time cannot be excluded since the experimental protocol was not randomized. Additionally, about half the participants underwent another experimental condition ('increase' as additional strategy) in the emotion regulation task, resulting in a somewhat shorter picture presentation. However, we included a group variable as additional covariate in all respective analyses to control for the potential impact of these small discrepancies in the study design. Furthermore, to stay close to the protocol (e.g., McRae et al. [62], Walter et al. [85]), we did not include a physiological measure to assess whether the participants were always looking directly to the pictures. However, we explicitly instructed the participants to pay close attention to the task and the presented stimuli and to not look away or close their eye. Retrospective ratings revealed that of all participants, $n = 2$ turned a blind eye somewhere during the permit condition; and $n = 3$ somewhere during the detach condition. Although we find it highly unlikely that the results are influenced by this, taking additional measures like eye-tracking into account would be advantageous to ensure that participants are looking directly to the pictures. In addition, all participants were healthy and screened for previous medical, neurological or psychiatric illness or treatment. Thus, we likely reduced the variance of the phenotype and consequently reduced the power to detect genotype effects. However, Dincheva et al. [20] and Conzelmann et al. [13] also examined a truly healthy sample. Therefore, the sample characteristics are very unlikely to explain some of the findings. Moreover, we did not collect physiological parameters (e.g., respiration rate, pulse) to control for them during the noise correction procedure. However, the de-noising procedure in the CONN toolbox allows for an approximate correction of physiological noise and does not require external monitoring of physiological fluctuations [4]. Nevertheless, given the findings that especially in resting-state studies a sufficient form of noise correction is crucial to reduce type I errors [45, 67], further studies should include additional physiological parameters to directly assess physiological noise and control for its influence.

Conclusion

In summary, our investigation revealed preliminary support for a link between *FAAH C385A* and fronto-amygdala connectivity during rest and provides initial evidence for differential functional activity during emotion reactivity and emotion regulation depending on *FAAH* C385A genotypes. Future research is needed to validate and extend these findings using further paradigms on emotional processing. Furthermore, gene × environment interactions and epigenetic mechanisms play a profound role in phenotype manifestation, thereby representing promising approaches for further investigations. Larger sample sizes and polygenic approaches as pursued by Sipe et al. [81] and Lieb et al. [56] may enhance power and enable more precise predictions on the role of *FAAH* C385A on fronto-amygdala circuitry and its underlying mechanisms in emotion processing.

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Author contributions AG, KD, BB and AS designed the research; KD performed the measurements; AG and KD conducted the analyses; SHW was responsible for DNA extraction and genotyping; AG, DD, BB, AS and SHW wrote the paper. The final version of the paper was approved by all authors.

Compliance with ethical standards

Conflict of interest None of the authors has a conflict of interest to declare.

References

1. Abler B, Kessler H (2009) Emotion regulation questionnaire—Eine deutschsprachige Fassung des ERQ von Gross und John. Diagnostica 55(3):144–152. https://doi.org/10.1026/0012-1924.55.3.144
2. Ashton CH, Moore PB (2011) Endocannabinoid system dysfunction in mood and related disorders: endocannabinoids in mood disorders. Acta Psychiatr Scand 124(4):250–261. https://doi.org/10.1111/j.1600-0447.2011.01687.x
3. Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007) Amygdala–frontal connectivity during emotion regulation. Soc Cogn Affect Neurosci 2(4):303–312. https://doi.org/10.1093/scan/nsm029
4. Behzadi Y, Restom K, Liau J, Liu TT (2007) A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. NeuroImage 37(1):90–101. https://doi.org/10.1016/j.neuroimage.2007.04.042
5. Boileau I, Tyndale RF, Williams B, Mansouri E, Westwood DJ, Foll BL, Tong J et al (2015) The fatty acid amide hydrolase C385A variant affects brain binding of the positron emission tomography tracer [^{11} C]CURB. J Cereb Blood Flow Metab 35(8):1237–1240. https://doi.org/10.1038/jcbfm.2015.119
6. Borkenau P, Ostendorf F (1993) NEO-Fünf-Faktoren Inventar (NEO-FFI) nach Costa und McCrae. Z Klin Psychol Psychother 28(2):145–146. https://doi.org/10.1026//0084-5345.28.2.145
7. Botvinick MM, Braver TS, Barch DM, Carter CS, Cohen JD (2001) Conflict monitoring and cognitive control. Psychol Rev 108(3):624–652
8. Brühl AB, Kaffenberger T, Herwig U (2010) Serotonergic and noradrenergic modulation of emotion processing by single dose antidepressants. Neuropsychopharmacology 35(2):521–533. https://doi.org/10.1038/npp.2009.159
9. Buhle JT, Silvers JA, Wager TD, Lopez R, Onyemekwu C, Kober H, Ochsner KN et al (2014) Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. Cereb Cortex 24(11):2981–2990. https://doi.org/10.1093/cercor/bht154
10. Casey BJ, Glatt CE, Lee FS (2015) Treating the developing versus developed brain: translating preclinical mouse and human studies. Neuron 86(6):1358–1368. https://doi.org/10.1016/j.neuron.2015.05.020
11. Chhatwal JP, Ressler KJ (2007) Modulation of fear and anxiety by the endogenous cannabinoid system. CNS Spectr 12(03):211–220. https://doi.org/10.1017/S1092852900020939
12. Chiang KP (2004) Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet 13(18):2113–2119. https://doi.org/10.1093/hmg/ddh216
13. Conzelmann A, Reif A, Jacob C, Weyers P, Lesch K-P, Lutz B, Pauli P (2012) A polymorphism in the gene of the endocannabinoid-degrading enzyme FAAH (FAAH C385A) is associated with emotional–motivational reactivity. Psychopharmacology 224(4):573–579. https://doi.org/10.1007/s00213-012-2785-y
14. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci 98(16):9371–9376. https://doi.org/10.1073/pnas.161191698
15. Cravatt BF, Giang DK, Mayfield S, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384(6604):83–87. https://doi.org/10.1038/384083a0
16. Critchley HD (2004) The human cortex responds to an interoceptive challenge. Proc Natl Acad Sci 101(17):6333–6334. https://doi.org/10.1073/pnas.0401510101
17. Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar B-K, Dolan RJ et al (2003) Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 126(10):2139–2152. https://doi.org/10.1093/brain/awg216
18. Fonseca de FR, Ramos JA, Bonnin A, Fernández-Ruiz JJ (1993) Presence of cannabinoid binding sites in the brain from early postnatal ages. NeuroReport 4(2):135–138. https://doi.org/10.1097/00001756-199302000-00005
19. Diers K, Weber F, Brocke B, Strobel A, Schönfeld S (2014) Instructions matter: a comparison of baseline conditions for cognitive emotion regulation paradigms. Front Psychol. https://doi.org/10.3389/fpsyg.2014.00347
20. Dincheva I, Drysdale AT, Hartley CA, Johnson DC, Jing D, King EC, Lee FS et al (2015) FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nature Commun 6:6395. https://doi.org/10.1038/ncomms7395
21. Dörfel D, Lamke J-P, Hummel F, Wagner U, Erk S, Walter H (2014) Common and differential neural networks of emotion regulation by detachment, reinterpretation, distraction, and expressive suppression: a comparative fMRI investigation. NeuroImage 101:298–309. https://doi.org/10.1016/j.neuroimage.2014.06.051
22. Eickhoff SB, Paus T, Caspers S, Grosbras M-H, Evans AC, Zilles K, Amunts K (2007) Assignment of functional activations to probabilistic cytoarchitectonic areas revisited. NeuroImage 36(3):511–521. https://doi.org/10.1016/j.neuroimage.2007.03.060
23. Ellgren M, Artmann A, Tkalych O, Gupta A, Hansen HS, Hansen SH, Hurd YL et al (2008) Dynamic changes of the endogenous cannabinoid and opioid mesocorticolimbic systems during adolescence: THC effects. Eur Neuropsychopharmacol 18(11):826–834. https://doi.org/10.1016/j.euroneuro.2008.06.009
24. Erk S, Mikschl A, Stier S, Ciaramidaro A, Gapp V, Weber B, Walter H (2010) Acute and sustained effects of cognitive emotion regulation in major depression. J Neurosci 30(47):15726–15734. https://doi.org/10.1523/JNEUROSCI.1856-10.2010

25. Etkin A, Büchel C, Gross JJ (2015) The neural bases of emotion regulation. Nat Rev Neurosci 16(11):693–700. https://doi.org/10.1038/nrn4044
26. Etkin A, Egner T, Kalisch R (2011) Emotional processing in anterior cingulate and medial prefrontal cortex. Trends Cogn Sci 15(2):85–93. https://doi.org/10.1016/j.tics.2010.11.004
27. Faul F, Erdfelder E, Buchner A, Lang A-G (2009) Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 41(4):1149–1160. https://doi.org/10.3758/BRM.41.4.1149
28. Finger EC, Mitchell DGV, Jones M, Blair RJR (2008) Dissociable roles of medial orbitofrontal cortex in human operant extinction learning. NeuroImage 43(4):748–755. https://doi.org/10.1016/j.neuroimage.2008.08.021
29. Gee DG, Fetcho RN, Jing D, Li A, Glatt CE, Drysdale AT, the Consortium PING et al (2016) Individual differences in frontolimbic circuitry and anxiety emerge with adolescent changes in endocannabinoid signaling across species. Proc Natl Acad Sci 113(16):4500–4505. https://doi.org/10.1073/pnas.1600013113
30. Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. Am J Psychiatry 160(4):636–645. https://doi.org/10.1176/appi.ajp.160.4.636
31. Gottfried JA, Dolan RJ (2004) Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. Nat Neurosci 7(10):1144–1152. https://doi.org/10.1038/nn1314
32. Gunduz-Cinar O, Hill MN, McEwen BS, Holmes A (2013) Amygdala FAAH and anandamide: mediating protection and recovery from stress. Trends Pharmacol Sci 34(11):637–644. https://doi.org/10.1016/j.tips.2013.08.008
33. Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Holmes A et al (2013) Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry 18(7):813–823. https://doi.org/10.1038/mp.2012.72
34. Halldorsdottir T, Binder EB (2017) Gene × environment interactions: from molecular mechanisms to behavior. Annu Rev Psychol 68(1):215–241. https://doi.org/10.1146/annurev-psych-010416-044053
35. Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Manuck SB et al (2009) Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol Psychiatr 66(1):9–16. https://doi.org/10.1016/j.biopsych.2008.10.047
36. Heng L, Beverley JA, Steiner H, Tseng KY (2011) Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse 65(4):278–286. https://doi.org/10.1002/syn.20844
37. Hill MN, Hillard CJ, Bambico FR, Patel S, Gorzalka BB, Gobbi G (2009) The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends Pharmacol Sci 30(9):484–493. https://doi.org/10.1016/j.tips.2009.06.006
38. Hill MN, Patel S (2013) Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses. Biol Mood Anxiety Disord 3(1):19. https://doi.org/10.1186/2045-5380-3-19
39. Kalisch R (2009) The functional neuroanatomy of reappraisal: time matters. Neurosci Biobehav Rev 33(8):1215–1226. https://doi.org/10.1016/j.neubiorev.2009.06.003
40. Kalisch R, Wiech K, Critchley HD, Dolan RJ (2006) Levels of appraisal: a medial prefrontal role in high-level appraisal of emotional material. NeuroImage 30(4):1458–1466. https://doi.org/10.1016/j.neuroimage.2005.11.011
41. Kanske P, Heissler J, Schönfelder S, Bongers A, Wessa M (2011) How to regulate emotion? Neural networks for reappraisal and distraction. Cereb Cortex 21(6):1379–1388. https://doi.org/10.1093/cercor/bhq216
42. Kerns JG (2004) Anterior cingulate conflict monitoring and adjustments in control. Science 303(5660):1023–1026. https://doi.org/10.1126/science.1089910
43. Kret ME, Ploeger A (2015) Emotion processing deficits: a liability spectrum providing insight into comorbidity of mental disorders. Neurosci Biobehav Rev 52:153–171. https://doi.org/10.1016/j.neubiorev.2015.02.011
44. Krohne HW, Egloff B, Kohlmann CW, Tausch A (1996) Untersuchungen mit einer deutschen Version der “Positive and Negative Affect Schedule” (PANAS). Diagnostica 42:139–156
45. Krüger G, Glover GH (2001) Physiological noise in oxygenation-sensitive magnetic resonance imaging. Magn Reson Med 46(4):631–637
46. Lafenêtre P, Chaouloff F, Marsicano G (2007) The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. Pharmacol Res 56(5):367–381. https://doi.org/10.1016/j.phrs.2007.09.006
47. Lange MD, Daldrup T, Remmers F, Szkudlarek HJ, Lesting J, Guggenhuber S, Pape HC et al (2017) Cannabinoid CB1 receptors in distinct circuits of the extended amygdala determine fear responsiveness to unpredictable threat. Mol Psychiatry 22(10):1422–1430. https://doi.org/10.1038/mp.2016.156
48. Lang PJ, Bradley MM, Cuthbert BN (1999) International affective picture system (IAPS): instruction manual and affective ratings (Vol. Center for Research in Psychophysiology). University of Florida, Gainesville
49. Laux L, Glanzmann P, Schaffner P, Spielberger CD (1981) Das State-Trait-Angstinventar (STAI). Beltz, Weinheim
50. Lazary J, Eszlari N, Juhasz G, Bagdy G (2016) Genetically reduced FAAH activity may be a risk for the development of anxiety and depression in persons with repetitive childhood trauma. Eur Neuropsychopharmacol 26(6):1020–1028. https://doi.org/10.1016/j.euroneuro.2016.03.003
51. Lee TT-Y, Gorzalka BB (2012) Timing is everything: evidence for a role of corticolimbic endocannabinoids in modulating hypothalamic–pituitary–adrenal axis activity across developmental periods. Neuroscience 204:17–30. https://doi.org/10.1016/j.neuroscience.2011.10.006
52. Lee TT-Y, Hill MN, Hillard CJ, Gorzalka BB (2013) Temporal changes in *N*-acylethanolamine content and metabolism throughout the peri-adolescent period. Synapse 67(1):4–10. https://doi.org/10.1002/syn.21609
53. Leppänen JM (2006) Emotional information processing in mood disorders: a review of behavioral and neuroimaging findings. Curr Opin Psychiatry 19(1):34–39. https://doi.org/10.1097/01.yco.0000191500.46411.00
54. Lichtman AH, Shelton CC, Advani T, Cravatt BF (2004) Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 109(3):319–327. https://doi.org/10.1016/j.pain.2004.01.022
55. Lieberman MD, Cunningham WA (2009) Type I and Type II error concerns in fMRI research: re-balancing the scale. Soc Cogn Affect Neurosci 4(4):423–428. https://doi.org/10.1093/scan/nsp052
56. Lieb W, Manning AK, Florez JC, Dupuis J, Cupples LA, McAteer JB, Fox CS et al (2009) Variants in the *CNR1* and the *FAAH* genes and adiposity traits in the community. Obesity 17(4):755–760. https://doi.org/10.1038/oby.2008.608
57. Long LE, Lind J, Webster M, Weickert C (2012) Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. BMC Neurosci 13(1):87. https://doi.org/10.1186/1471-2202-13-87
58. Lövheim H (2012) A new three-dimensional model for emotions and monoamine neurotransmitters. Med Hypotheses 78(2):341–348. https://doi.org/10.1016/j.mehy.2011.11.016

59. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. NeuroImage 19(3):1233–1239. https://doi.org/10.1016/S1053-8119(03)00169-1
60. Martin EI, Ressler KJ, Binder E, Nemeroff CB (2009) The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. Psychiatr Clin N Am 32(3):549–575. https://doi.org/10.1016/j.psc.2009.05.004
61. McLaren DG, Ries ML, Xu G, Johnson SC (2012) A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. NeuroImage 61(4):1277–1286. https://doi.org/10.1016/j.neuroimage.2012.03.068
62. McRae K, Jacobs SE, Ray RD, John OP, Gross JJ (2012) Individual differences in reappraisal ability: links to reappraisal frequency, well-being, and cognitive control. J Res Pers 46(1):2–7. https://doi.org/10.1016/j.jrp.2011.10.003
63. Micale V, Di Marzo V, Sulcova A, Wotjak CT, Drago F (2013) Endocannabinoid system and mood disorders: priming a target for new therapies. Pharmacol Ther 138(1):18–37. https://doi.org/10.1016/j.pharmthera.2012.12.002
64. Milad MR, Quirk GJ, Pitman RK, Orr SP, Fischl B, Rauch SL (2007) A role for the human dorsal anterior cingulate cortex in fear expression. Biol Psychiatr 62(10):1191–1194. https://doi.org/10.1016/j.biopsych.2007.04.032
65. Moreira FA, Kaiser N, Monory K, Lutz B (2008) Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. Neuropharmacology 54(1):141–150. https://doi.org/10.1016/j.neuropharm.2007.07.005
66. Moreira FA, Wotjak CT (2009) Cannabinoids and anxiety. In: Stein MB, Steckler T (eds) Behavioral neurobiology of anxiety and its treatment, vol 2. Springer, Berlin, pp 429–450. https://doi.org/10.1007/7854_2009_16
67. Murphy K, Birn RM, Bandettini PA (2013) Resting-state fMRI confounds and cleanup. NeuroImage 80:349–359. https://doi.org/10.1016/j.neuroimage.2013.04.001
68. Myers AJ, Williams L, Gatt JM, McAuley-Clark EZ, Dobson-Stone C, Schofield PR, Nemeroff CB (2014) Variation in the oxytocin receptor gene is associated with increased risk for anxiety, stress and depression in individuals with a history of exposure to early life stress. J Psychiatr Res 59:93–100. https://doi.org/10.1016/j.jpsychires.2014.08.021
69. Ochsner KN, Bunge SA, Gross JJ, Gabrieli JDE (2002) Rethinking feelings: an fMRI study of the cognitive regulation of emotion. J Cogn Neurosci 14(8):1215–1229. https://doi.org/10.1162/089892902760807212
70. Ochsner KN, Gross JJ (2008) Cognitive emotion regulation: insights from social cognitive and affective neuroscience. Curr Dir Psychol Sci 17(2):153–158. https://doi.org/10.1111/j.1467-8721.2008.00566.x
71. Ochsner KN, Ray RD, Cooper JC, Robertson ER, Chopra S, Gabrieli JDE, Gross JJ (2004) For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. NeuroImage 23(2):483–499. https://doi.org/10.1016/j.neuroimage.2004.06.030
72. Ochsner KN, Silvers JA, Buhle JT (2012) Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion: functional imaging studies of emotion regulation. Ann N Y Acad Sci 1251(1):E1–E24. https://doi.org/10.1111/j.1749-6632.2012.06751.x
73. O'Reilly JX, Woolrich MW, Behrens TEJ, Smith SM, Johansen-Berg H (2012) Tools of the trade: psychophysiological interactions and functional connectivity. Soc Cogn Affect Neurosci 7(5):604–609. https://doi.org/10.1093/scan/nss055
74. Patricelli MP, Cravatt BF (2001) Proteins regulating the biosynthesis and inactivation of neuromodulatory fatty acid amides. In: Vitamins and hormones, vol 62, pp 95–131. Elsevier. http://linkinghub.elsevier.com/retrieve/pii/S0083672901620028
75. Paus T (2001) Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci 2(6):417–424. https://doi.org/10.1038/35077500
76. Phillips ML, Ladouceur CD, Drevets WC (2008) A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. Mol Psychiatry 13(9):833–857. https://doi.org/10.1038/mp.2008.65
77. Ruehle S, Rey AA, Remmers F, Lutz B (2012) The endocannabinoid system in anxiety, fear memory and habituation. J Psychopharmacol 26(1):23–39. https://doi.org/10.1177/0269881111408958
78. Schiller D, Delgado MR (2010) Overlapping neural systems mediating extinction, reversal and regulation of fear. Trends Cogn Sci 14(6):268–276. https://doi.org/10.1016/j.tics.2010.04.002
79. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci 99(12):8394–8399. https://doi.org/10.1073/pnas.082235799
80. Sipe JC, Scott TM, Murray S, Harismendy O, Simon GM, Cravatt BF, Waalen J (2010) Biomarkers of endocannabinoid system activation in severe obesity. PLoS One 5(1):e8792. https://doi.org/10.1371/journal.pone.0008792
81. Sipe JC, Waalen J, Gerber A, Beutler E (2005) Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). Int J Obes 29(7):755–759. https://doi.org/10.1038/sj.ijo.0802954
82. Spagnolo PA, Ramchandani VA, Schwandt ML, Kwako LE, George DT, Mayo LM, Heilig M et al (2016) *FAAH* gene variation moderates stress response and symptom severity in patients with posttraumatic stress disorder and comorbid alcohol dependence. Alcohol Clin Exp Res 40(11):2426–2434. https://doi.org/10.1111/acer.13210
83. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Joliot M et al (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15(1):273–289. https://doi.org/10.1006/nimg.2001.0978
84. Viveros M, Marco E, File S (2005) Endocannabinoid system and stress and anxiety responses. Pharmacol Biochem Behav 81(2):331–342. https://doi.org/10.1016/j.pbb.2005.01.029
85. Walter H, von Kalckreuth A, Schardt D, Stephan A, Goschke T, Erk S (2009) The temporal dynamics of voluntary emotion regulation. PLoS One 4(8):e6726. https://doi.org/10.1371/journal.pone.0006726
86. Wessa M, Kanske P, Neumeister P, Bode K, Heissler J, Schönfelder S (2010) EmoPicS: Subjektive und psychophysiologische Evaluation neuen Bildmaterials für die klinischbiopsychologische Forschung [EmoPicS: Subjective and psychophysiological evaluation of new imagery for clinical biopsychological research]. Z Klin Psychol Psychother Supplement 1:11–77
87. Whitfield-Gabrieli S, Nieto-Castanon A (2012) *Conn*: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect 2(3):125–141. https://doi.org/10.1089/brain.2012.0073
88. Yates A, Akanni W, Amode MR, Barrell D, Billis K, Carvalho-Silva D, Flicek P et al (2016) Ensembl 2016. Nucleic Acids Res 44(D1):D710–D716. https://doi.org/10.1093/nar/gkv1157
89. Ziegler C, Richter J, Mahr M, Gajewska A, Schiele MA, Gehrmann A, Domschke K et al (2016) MAOA gene hypomethylation in panic disorder—reversibility of an epigenetic risk pattern by psychotherapy. Transl Psychiatry 6(4):e773–e773. https://doi.org/10.1038/tp.2016.41