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Archival Report

Elevated Anandamide, Enhanced Recall of Fear Extinction, and Attenuated Stress Responses Following Inhibition of Fatty Acid Amide Hydrolase: A Randomized, Controlled Experimental Medicine Trial

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ABSTRACT

BACKGROUND: Posttraumatic stress disorder, an area of large unmet medical needs, is characterized by persistence of fear memories and maladaptive stress responses. In rodents, elevation of the endocannabinoid anandamide due to inhibition of fatty acid amide hydrolase (FAAH) facilitates fear extinction and protects against the anxiogenic effects of stress. We recently reported that elevated anandamide levels in people homozygous for a loss-of-function *FAAH* mutation are associated with a similar phenotype, suggesting a translational validity of the preclinical findings.

METHODS: In this double-blind, placebo-controlled experimental medicine study, healthy adults were randomized to an FAAH inhibitor (PF-04457845, 4 mg orally, once daily; $n = 16$) or placebo ($n = 29$) for 10 days. On days 9 and 10, participants completed a task battery assessing psychophysiological indices of fear learning, stress reactivity, and stress-induced affective responses.

RESULTS: FAAH inhibition produced a 10-fold increase in baseline anandamide. This was associated with potentiated recall of fear extinction memory when tested 24 hours after extinction training. FAAH inhibition also attenuated autonomic stress reactivity, assessed via electrodermal activity, and protected against stress-induced negative affect, measured via facial electromyography.

CONCLUSIONS: Our data provide preliminary human evidence that FAAH inhibition can improve the recall of fear extinction memories and attenuate the anxiogenic effects of stress, in a direct translation of rodent findings. The beneficial effects of FAAH inhibition on fear extinction, as well as stress- and affect-related behaviors, provide a strong rationale for developing this drug class as a treatment for posttraumatic stress disorder.

Keywords: Anandamide, Cannabinoid, Fatty acid amide hydrolase, Fear conditioning, PTSD, Stress

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Posttraumatic stress disorder (PTSD) is a common, devastating psychiatric disorder for which limited treatment options are available. It develops following exposure to a traumatic event and is characterized by intrusive memories of the event, avoidance of trauma reminders, emotional numbing, and hyperarousal (1). PTSD has a chronic and often severe time course, with only a minority of patients achieving full remission (2). The therapeutic needs of this group are great, and currently available treatment options are limited (3). Prolonged exposure (PE) therapy, a clinical implementation of extinction learning, has strong support for clinical efficacy in PTSD. PE is aimed at reducing distress responses through repeated exposure to trauma-associated cues. However, PE is not effective for all patients, and its effects are prone to spontaneous renewal of symptoms (4). Pharmacologically potentiating extinction learning and consolidation is an attractive strategy for improving treatment outcomes. Currently, Food and Drug Administration–approved PTSD pharmacotherapies do not target the core pathophysiology of dysregulated fear (5) and are no more effective when used with PE than when used without PE (6).

Accumulating evidence suggests that the endocannabinoid (eCB) system plays a critical role in the pathophysiology of PTSD. Elevation of the endogenous cannabinoid (eCB) anandamide (AEA) via inhibition of its main degradative enzyme, fatty acid amid hydrolase (FAAH), promotes the consolidation of fear extinction memories and protects against anxiogenic effects of stress in preclinical models (7–13). Initial evidence that these findings may have a translational validity comes from studies capitalizing on a loss-of-function mutation at the human locus encoding *FAAH*, a nonsynonymous *FAAH* 385C→A substitution (8,14–18). The A-allele at this locus encodes an FAAH enzyme that is more readily degraded,

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resulting in reduced enzymatic activity and elevated baseline AEA (14,18–20).

The biochemical consequences conferred by the loss-of-function *FAAH* mutation are accompanied by beneficial behavioral effects. Individuals with higher baseline AEA show facilitated extinction training and enhanced extinction recall when tested 24 hours later (14,18). The genetic studies also suggest a broader ability of reduced FAAH activity to promote adaptive stress responses. Elevated AEA does not influence the stress response per se, but AA homozygotes are protected against stress-induced decreases in AEA across species (e.g., humans, mice), and people homozygous for the A-allele show improved emotion regulation following stress exposure (18). Furthermore, patients with comorbid PTSD and alcohol use disorder carrying the loss-of-function mutation show a faster decline in stress-induced anxiety (21). Together, this suggests that, similar to the animal findings, low FAAH activity and elevations in AEA signaling can facilitate fear extinction and more broadly protect against the negative consequences of stress in people.

There is thus a strong rationale for FAAH inhibition as a therapeutic mechanism in PTSD, but only a controlled interventional study can establish a causal relationship between FAAH inhibition and enhanced fear extinction. In particular, pharmacological manipulation of FAAH is essential to address whether the phenotype seen in the behavioral genetic studies is directly caused by elevated AEA or results from neurodevelopmental effects reported in AA homozygotes (16). Here, we used an orally available, brain-penetrant FAAH inhibitor (FAAHi) originally developed for analgesia, PF-04457845 (22–26). PF-04457845 is safe and well tolerated but was discontinued owing to lack of analgesic efficacy (24). To obtain initial proof of principle, we randomized healthy adults to PF-04457845 (4 mg orally once daily) or placebo (PBO) for 10 days. On days 9 and 10, participants completed a test battery to assess fear learning, stress reactivity, and stress-induced affect, using facial electromyography (EMG) and electrodermal activity, as described previously (18). Plasma samples were obtained to evaluate peripheral levels of eCBs, cortisol, and PF-04457845. We hypothesized that FAAH inhibition would produce markedly elevated AEA levels that would be associated with facilitated fear extinction and attenuated stress-induced negative affect, similar to our previous reports using a behavioral genetics approach (18).

METHODS AND MATERIALS

This was a double-blind, placebo-controlled, phase IIa single-center clinical trial approved by the Linköping Regional Ethics Review Board and the Swedish Medical Products Agency. Qualifying participants were originally randomized 1:1 to receive the FAAHi PF-04457845 (4 mg/day) or PBO orally for 10 days. The PF-04457845 dose chosen produces a near complete and long-lasting inhibition of FAAH (23,24,26). On days 9 and 10, participants completed a laboratory paradigm to assess fear learning, affect, and stress reactivity, as described previously (18). Notably, while the methods are identical to our previous report (18), the current study used a separate, nonoverlapping participant cohort. Sessions took place at the Center for Social and Affective Neuroscience at the Linköping University Hospital, Sweden. Supplemental Table S1 and Supplemental Figure S1 contain detailed data collection procedures (2016-005013-47).

Prospective participants were recruited via flyers and online advertisements July 2017 to May 2018 and completed a screening session (Supplement). Those eligible were invited back to complete the informed consent procedure and receive medication. Sessions were conducted on consecutive days 24 ± 1 hours apart with start times at 12:00 noon or later. Participants and study personnel were blinded to drug administration until study completion.

A power analysis based on effect sizes observed in Mayo *et al.* (18) indicated that for a Cohen's $d \geq 0.8$ on the fear recall measure, a sample size of 30 participants per arm would result in a power of 0.86 for an $\alpha = .05$. The study was therefore originally designed to recruit 60 completers. Owing to a pharmacy error, only 16 individuals allocated to the active condition received it (see Supplement). The analyses presented are the per-protocol analyses of participants who received the intervention they were allocated to, confirmed by plasma analysis of drug levels. Results for the entire study, including individuals allocated to the active condition who did not receive PF-04457845, are available in the Supplement; for all outcome measures, those individuals were indistinguishable from the PBO-allocated group. The participant flow is shown in Figure 1.

Drug

PF-04457845 is a highly selective, orally available irreversible inhibitor of FAAH originally developed for pain and insomnia (17,22,24) (see Supplement for detailed drug information). PF-04457845 tablets (4 mg) and visually indistinguishable PBO were provided by Pfizer (Groton, VT). Blinding and randomization were carried out by a contractor (Oriola, Stockholm, Sweden) insulated from the investigators. Randomization was in blocks of 6. Labeled medication boxes were delivered to the Linköping University Hospital pharmacy. Adherence was confirmed by analysis of PF-04457845 in plasma at study completion. The hospital pharmacy was contracted for an emergency code-breaking procedure; no code-breaking events occurred. Study personnel did not have access to the code until completion of study and analysis.

Data Collection

Self-report measures included the State-Trait Anxiety Inventory (27), Profile of Mood States (28), and Positive and Negative Affect Schedule (29). Psychophysiological recordings were obtained as described previously (18) (Supplement). Briefly, bipolar recording electrodes were placed over the zygomatic, corrugator, and orbicularis muscles for facial EMG. Electrocardiography was assessed via electrodes placed at the right supraclavicular fossa and midaxillary on the left side of the abdomen. Electrodermal activity was measured via 2 electrodes on the palm of the hand. Psychophysiological signals were acquired and filtered according to standard practices using an MP150 data acquisition system and AcqKnowledge software, version 5.0 (Biopac Systems, Camino Goleta, CA).

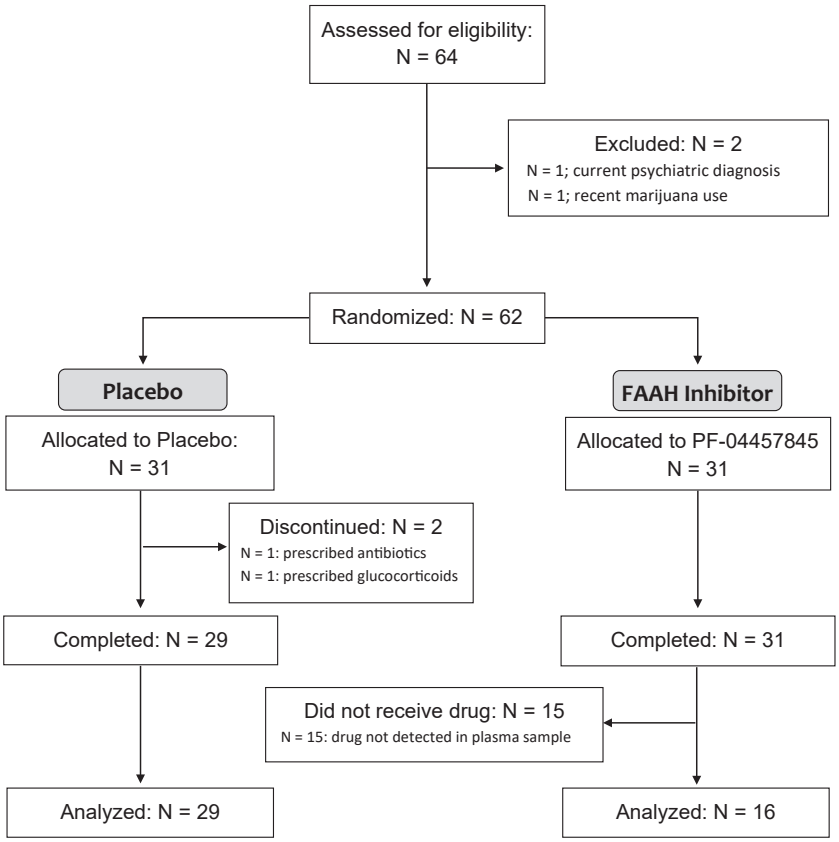

Figure 1. Study flowchart. As a result of a pharmacy error, a number of participants allocated to PF-04457845 (*n* = 15) did not receive active medication (see Supplement for more information). Results presented are from per-protocol analyses of subjects, with exposure biochemically confirmed. Results from the entire study sample (*N* = 60) are available in the Supplement. FAAH, fatty acid amide hydrolase.

Behavioral Tasks

The fear-potentiated startle paradigm (30) consists of 5 phases over 2 days (18) (Supplement). Briefly, day 1 consisted of habituation, acquisition, and extinction; day 2 included recall of fear extinction memory and renewal of fear responding. The eyeblink component of the startle response was measured following a startle probe (50-ms burst of white noise), quantified as the peak-to-peak orbicularis EMG value after probe onset (31). The task had two contexts that each included a lamp; specific lamp colors constituted the conditioned stimuli (CS+, CS−). The unconditioned stimulus was an aversive sound of nails across a chalkboard (32). Tasks were presented using Presentation Software, version 20.3 (Neurobehavioral Systems, Berkley, CA).

The affective image task (18) (Supplement) was completed before and after stress and control tasks. Positive, neutral, and negative images were selected from the International Affective Picture System (33). Facial EMG of zygomatic and corrugator muscles was quantified as the mean EMG amplitude during the 6-second image presentation compared with the preceding 1-second baseline. "Baseline" affective responses were responses obtained during the first task at the first session.

The Maastricht Acute Stress Test is a 10-minute task consisting of alternating hand immersion in ice cold water and mental arithmetic trials with negative socioevaluative feedback. The control version consists of hand immersion in room-temperature water and simple counting (34). Blood samples were collected via an indwelling catheter in the arm not submerged during the task.

Biochemical Analysis

Cortisol levels were obtained from plasma using the DetectX Cortisol Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, MI) according to manufacturer instructions. Plasma levels of AEA, 2-arachidonylglycerol (2-AG), palmitoylethanolamide (PEA), and oleoylethanolamide (OEA) (35), as well as PF-04457845 (26) (lower detection limit: 1 ng/mL) were assessed using mass spectrometry (Supplement).

Statistical Analysis

Primary outcome measures were plasma AEA levels, fear extinction (startle EMG), recall of fear extinction, and stress-induced negative affect (corrugator EMG), based on Mayo *et al.* (18). Secondary outcomes were stress reactivity (skin conductance responses, subjective stress, cortisol, heart rate) and baseline affect (corrugator EMG). Exploratory outcomes included affective responses (zygomatic EMG) (self-reported valence and arousal), acquisition of fear, and renewal of fear, as well as posttreatment assessments of self-reported mood (Profile of Mood States), anxiety (State-Trait Anxiety Inventory–State Anxiety subscale), and affect (Positive and Negative Affect Schedule) and plasma levels of OEA, PEA, and 2-AG.

Table 1. Participant Demographics and Treatment Effects on Baseline Mood and Affect

	Overall (N = 45)	PBO Group (n = 29)	FAAHi Group (n = 16)	Test Statistic
STAI-S	31.1 (6.7)	31.1 (6.2)	31.2 (7.8)	$F_{1,43} < 0.01$, $p = .96$
PANAS				
Positive	27.3 (5.9)	27.4 (5.4)	27.2 (6.9)	$F_{1,43} = 0.02$, $p = .90$
Negative	14.9 (5.1)	14.2 (4.3)	16.1 (6.2)	$F_{1,43} = 1.53$, $p = .22$
POMS				
Friendly	15.4 (3.2)	15.5 (3.4)	15.1 (3.0)	$F_{1,43} = 0.22$, $p = .64$
Anxiety	6.7 (3.8)	6.9 (4.1)	6.3 (3.5)	$F_{1,43} = 0.24$, $p = .63$
Anger	4.8 (3.8)	4.9 (3.5)	4.7 (4.3)	$F_{1,43} = 0.01$, $p = .91$
Fatigue	6.1 (3.6)	6.5 (3.6)	5.4 (3.6)	$F_{1,43} = 0.88$, $p = .35$
Confusion	6.8 (3.3)	7.0 (3.5)	6.5 (3.1)	$F_{1,43} = 0.29$, $p = .59$
Depression	4.6 (4.6)	5.0 (4.1)	4.8 (5.4)	$F_{1,43} = 0.03$, $p = .86$
Vigor	18.3 (5.2)	18.4 (5.6)	18.3 (4.5)	$F_{1,43} < 0.01$, $p = .95$

Values are mean (SD).

FAAHi, fatty acid amide hydrolase inhibitor (PF-04457845); PANAS, Positive and Negative Affect Schedule (State version); PBO, placebo; POMS, Profile of Mood States; STAI-S, Spielberger State-Trait Anxiety Inventory–State Anxiety subscale.

Behavioral and biochemical measures were examined for distribution and homogeneity of variance and analyzed using 1-way or repeated-measures analysis of variance with treatment as a between-subjects factor and $\alpha = .05$. Significant effects were followed up with Tukey honestly significant difference post hoc comparisons. Uncorrected p values are provided and denoted if no longer significant after correction for multiple comparisons. If data violated assumptions of normality, Mann-Whitney U tests or Spearman's ρ were employed.

Acquisition of fear conditioning was assessed with cue (CS+, CS−) as a within-subjects variable. Fear responding during other task phases was assessed using a repeated-measures analysis of variance for extinction, which had 2 phases (early, late), or 1-way analysis of variance (recall of fear extinction memory, renewal of fear responding). Baseline affective responses were analyzed with the within-subjects factor of stimulus type (positive, neutral, negative) for each muscle (corrugator, zygomatic) and rating (valence, arousal) individually. The effect of stress on affective responses was assessed as the change (pre vs. post) in EMG response at each session for each stimulus type. Change scores were analyzed for each variable (corrugator, zygomatic, valence, and arousal) individually. Changes in physiological variables (skin conductance responses, heart rate) and subjective stress were assessed with session as a within-subjects factor. Changes in eCBs and cortisol were calculated as the pre-to-post task (i.e., stress or control), with session as a within-subjects factor.

RESULTS

PF-04457845 Is Safe and Well Tolerated

No serious adverse events occurred. Nonserious adverse events were few and did not differ in frequency between groups (Supplemental Table S3). No differences were present in participant characteristics at baseline (Supplemental Table S4), nor was there an effect of treatment on self-report measures (Table 1).

Baseline AEA Is Markedly Elevated by FAAH Inhibition

Plasma concentrations of PF-04457845 in the FAAHi group were 21.7 ± 3.73 ng/mL (Supplemental Figure S3). FAAH inhibition produced markedly elevated AEA (FAAHi median = 5.99, PBO median = 0.41 [Mann-Whitney $U = 1.00$, $p < .001$]) (Figure 2A) and OEA (FAAHi median = 40.3, PBO median = 4.96 [Mann-Whitney $U = 4.00$, $p < .001$]) (Figure 2B) but not PEA ($p = .79$) (Figure 2C) and 2-AG ($p = .20$) (Figure 2D).

FAAH Inhibition Does Not Affect Acquisition of Conditioned Fear

All participants acquired conditioned fear, evidenced by greater startle response to the CS+ compared with CS− cue at acquisition ($F_{1,43} = 25.7$, $p < .001$, $\eta_p^2 = .38$) (Figure 3A). There was no effect of treatment on fear acquisition (treatment: $p = .28$; interaction: $p = .75$).

FAAH Inhibition Promotes Recall of Fear Extinction

FAAH inhibition did not significantly influence within-session extinction, but did promote recall of extinction memory when tested 24 hours later. Overall, extinction training produced attenuated responding to the CS+ over time (early vs. late extinction [$F_{1,43} = 12.2$, $p = .001$, $\eta_p^2 = .22$]) (Figure 3B, Supplemental Figure S3), but there was no effect of treatment ($p = .66$), and only a trend toward a task phase × treatment interaction ($F_{1,43} = 2.93$, $p = .094$, $\eta_p^2 = .064$). However, when recall of conditioned fear was tested on day 2 (recall of fear extinction memory), responses were markedly lower in the active treatment group, indicating enhanced recall of extinction ($F_{1,43} = 11.6$, $p = .001$, $\eta_p^2 = .21$) (Figure 3B, Supplemental Figure S3). There was no effect of treatment on renewal of fear ($p = .18$).

Baseline Affect Is Not Affected by FAAH Inhibition

Prior to stress exposure, affective images elicited the expected EMG and self-reported responses, but this was not influenced by FAAH inhibition. Specifically, negative images increased corrugator reactivity, whereas positive pictures reduced it

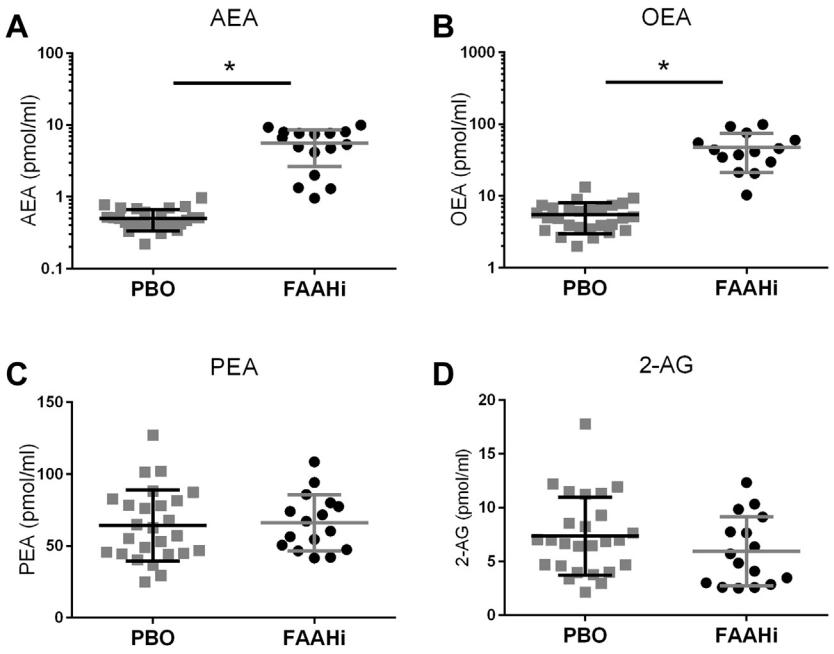

Figure 2. Fatty acid amide hydrolase (FAAH) inhibition selectively increases circulating anandamide (AEA) and oleoylethanolamide (OEA) levels. Ten days of FAAH inhibition produced elevated levels of **(A)** AEA and **(B)** OEA but did not influence **(C)** palmitoylethanolamide (PEA) or **(D)** 2-arachidonylglycerol (2-AG). Symbols represent individual data points; bars represent mean and SD; *$p < .001$ for effect of treatment. Note that the y-axis in panels **(A)** and **(B)** is log-based. FAAHi, fatty acid amide hydrolase inhibitor (PF-04457845); PBO, placebo.

($F_{2,86} = 42.4$, $p < .001$, $\eta_p^2 = .50$) (Figure 4A); positive pictures elicited the greatest zygomatic reactivity ($F_{2,86} = 15.3$, $p < .001$, $\eta_p^2 = .26$). We also found expected effects of stimulus type on ratings of valence and arousal; however, there was no effect of treatment on any of these measures (Supplemental Results).

Stress-Induced Negative Affect Is Attenuated by FAAH Inhibition

FAAH inhibition attenuated stress-induced negative affect, as indexed by decreased corrugator activity (treatment [$F_{1,43} = 4.28$, $p = .045$, $\eta_p^2 = .09$]) (Figure 4B). There was a trend toward an overall effect of stimulus type ($F_{2,86} = 2.91$, $p = .060$, $\eta_p^2 = .063$) and a significant treatment × stimulus type interaction ($F_{2,86} = 5.36$, $p = .006$, $\eta_p^2 = .11$), with follow-up tests showing that the effect of FAAH inhibition on attenuation of negative affect was most robust for negative images ($p_{uncorrected} = .001$). There was no effect of stress on any other response to the affective images (Supplemental Results).

FAAH Inhibition Attenuates Autonomic, But Not Endocrine, Stress Responses

FAAH inhibition selectively attenuated the autonomic component of the stress response. We found significant main effects of session ($F_{1,40} = 34.8$, $p < .001$, $\eta_p^2 = .47$) and treatment ($F_{1,40} = 5.04$, $p = .030$, $\eta_p^2 = .11$) and a session × treatment interaction ($F_{1,40} = 4.71$, $p = .036$, $\eta_p^2 = .11$) on the frequency of skin conductance responses, such that FAAH inhibition attenuated skin conductance responses overall and in response to stress (effect of treatment at stress session: $p_{uncorrected} = .025$) (Figure 5A). As expected, the stress task was rated as more stressful than the control task (session [$F1,43 = 211$, $p < .001$, $\eta_p^2 = .83$]),

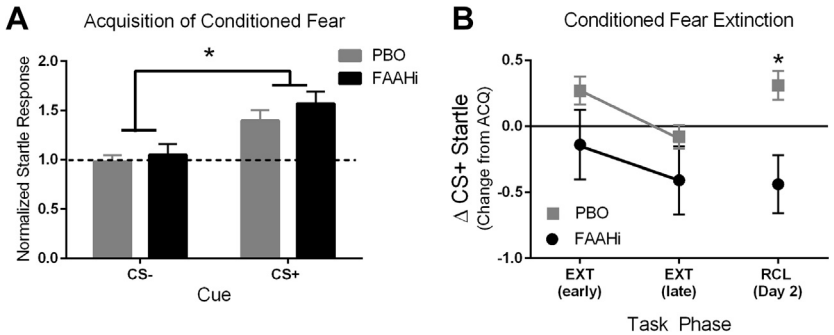

Figure 3. Fatty acid amide hydrolase (FAAH) inhibition promotes the recall of fear extinction memory (RCL). All participants acquired conditioned fear responding, as indicated by a greater conditioned stimulus (CS+) than CS− startle response; **(A)** this was not influenced by FAAH inhibition. **(B)** FAAH inhibition did not significantly facilitate within-session extinction (EXT), but markedly enhanced the RCL when tested 24 hours after EXT training. Bars and symbols represent means and error bars represent SEM. **(A)** Normalized startle response is calculated as [average startle response to CS]/[average startle response at rest] for each cue (CS+, CS−). Values >1 signify potentiation of fear; *$p < .05$ for main effect of cue. **(B)** The change in CS+ startle is quantified as [normalized startle at EXT] – [normalized startle at acquisition (ACQ)] for each phase individually, with values <1 signifying EXT of fear responding. For normalized startle responses at each task phase, see Supplemental Figure S3. *$p < .001$ for effect of treatment. FAAHi, fatty acid amide hydrolase inhibitor (PF-04457845); PBO, placebo.

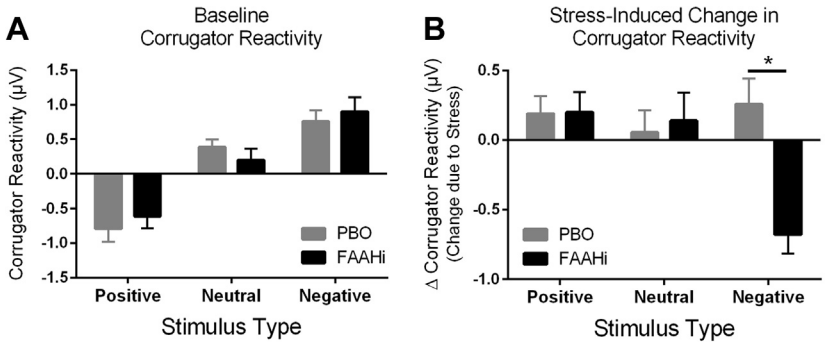

Figure 4. Fatty acid amide hydrolase (FAAH) inhibition attenuates the negative affective response to stress. **(A)** Ten days of FAAH inhibition did not influence baseline (i.e., prestress) affective reactions, measured via corrugator muscle reactivity. **(B)** However, following stress, FAAH inhibitor (FAAHi)–treated (PF-04457845) individuals demonstrated a reduction in negative affect (e.g., corrugator reactivity), particularly to negative stimuli. Note that panel **(B)** depicts the net change in corrugator reactivity due to stress, such that positive values indicate more corrugator activity than at baseline (prestress) [e.g., panel **(A)**] and negative values indicate less negative affect than that prior to stress. Bars and error bars represent mean and SEM, respectively; in panel **(B)**, values are the (post – pre) difference for the stress – control session. *$p < .05$ for effect of treatment. PBO, placebo.

and there was a nonsignificant trend toward a session × treatment interaction ($F_{1,43} = 3.99$, $p = .052$, $\eta_p^2 = .085$), suggesting that FAAH inhibition was associated with attenuated subjective stress ratings following stress exposure (effect of treatment at stress session: $p_{uncorrected} = .002$) (Figure 5B). There was no overall effect of treatment on subjective stress ($p = .22$).

We found a significant time × session interaction for cortisol ($F_{1,43} = 25.3$, $p < .001$, $\eta_p^2 = .37$) (Figure 5C), such that stress increased cortisol, but this did not differ between groups (Supplemental Results). There were no group differences in stress-induced heart rate or self-reported affect (Supplemental Results).

Stress-Induced Decrease in AEA Is Absent Following FAAH Inhibition

FAAH inhibition prevented stress-induced decreases in circulating AEA levels. Specifically, we found a significant effect of treatment on AEA (treatment [$F_{1,36} = 73.9$, $p < .001$, $\eta_p^2 = .67$], time [$F_{1,36} = 4.55$, $p = .040$, $\eta_p^2 = .11$], treatment × session × time interaction [$F_{1,36} = 4.31$, $p = .045$, $\eta_p^2 = .11$]) (Figure 5D). Post hoc tests revealed a significant effect of session (stress, control) in PBO-treated ($p_{uncorrected} = .009$) but not FAAHi-treated participants ($p = .65$). We also found between-groups differences in OEA and PEA, but not in 2-AG (Supplemental Results).

DISCUSSION

We provide preliminary evidence for beneficial effects of elevating AEA via FAAH inhibition on fear- and stress-related behavior, physiological responses, and biochemistry in humans. Ten days of FAAH inhibition produced marked increases in peripheral AEA levels. Individuals receiving the FAAH inhibitor demonstrated enhanced recall of fear extinction when tested 24 hours after extinction training. Moreover, FAAH inhibition attenuated specific components of the stress response and subsequently reduced negative affect objectively assessed via facial EMG. Treatment with the FAAH inhibitor did not influence self-reported mood, baseline affective responses (e.g., in the absence of stress), or the acquisition of fear. No serious adverse events occurred, while nonserious adverse events were few and did not differ between groups. Together, these data provide initial in-human evidence that FAAH inhibition has beneficial effects on fear extinction and stress reactivity. They strongly support the development of FAAH inhibitors as therapeutics for PTSD and other stress-related psychopathologies.

Converging evidence from preclinical models and human behavioral genetics suggests that elevated AEA via reduced FAAH activity can modulate the extinction of fear memories (8,14,18). Here, we find that FAAH inhibition resulted in greater recall of extinction when tested 24 hours after extinction training. We did not find a robust effect of FAAH inhibition on within-session extinction of fear, although a trend was present. These data show striking consistency with rodent findings, in which inhibition of FAAH does not influence within-session extinction but does augment the consolidation of extinction memory, resulting in an enhancement of fear suppression during extinction recall (8). These findings are also generally consistent with prior reports that synthetic Δ^9-tetrahydrocannabinol (dronabinol), an exogenous CB_1 receptor agonist, produces somewhat variable effects on extinction training per se, but more consistently enhances of extinction recall (36,37). Thus, driving eCB signaling via CB_1 receptor activation may be more effective in consolidating fear extinction memories than in facilitating fear extinction training itself (38–44).

The possibility that FAAH inhibition promotes consolidation of extinction learning has significant clinical implications. PE therapy, a common evidence-based treatment for PTSD, is based on the principles of extinction learning. We show that FAAH inhibition can enhance the recall of extinction learning, providing support for the use of FAAH inhibitors as adjuncts to PE therapy in patients with PTSD. Neuroimaging studies in healthy people suggest that individuals with elevated AEA show enhanced top-down regulation of the amygdala by the ventromedial prefrontal cortex (vmPFC)—neurocircuitry that is critical for extinction learning (14). Rodent work using optogenetics has similarly demonstrated that facilitation of neural activity in prefrontal inputs to the amygdala during extinction training has negligible effects on within-session fear extinction but augments extinction recall the following day (45). In addition, preclinical evidence suggests that AEA is critical for emotional memory consolidation, which relies on interactions between the amygdala and mPFC (46). Together, these findings suggest that elevated AEA signaling may enhance

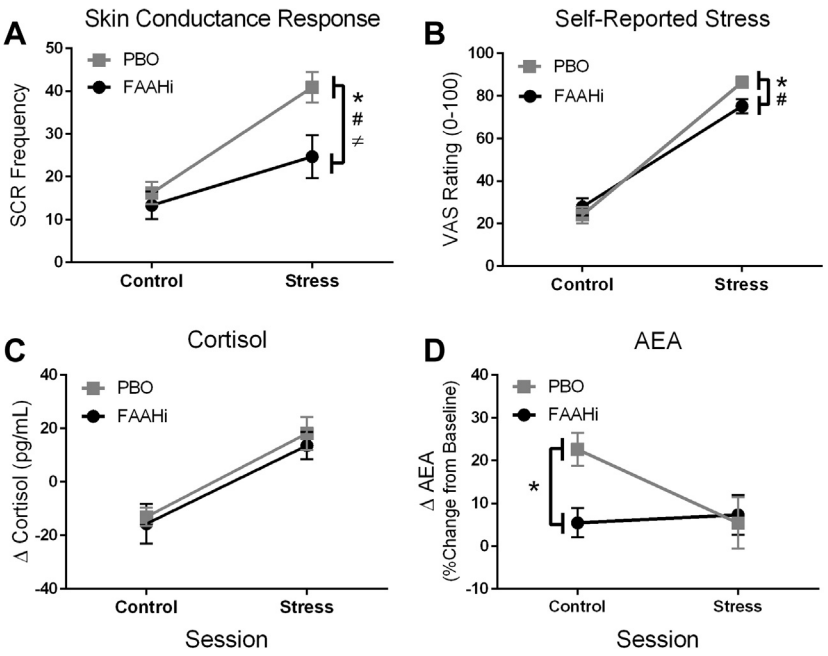

Figure 5. Fatty acid amide hydrolase (FAAH) inhibition attenuated **(A)** autonomic and **(B)** subjective stress reactivity but **(C)** did not influence neuroendocrine responses. **(D)** Moreover, stress was associated with a decrease in anandamide (AEA) levels in placebo (PBO)-treated individuals, but AEA levels were unaffected in FAAH inhibitor (FAAHi)–treated (PF-04457845) participants. Note: Values in panel **(D)** represent the percent change from baseline, such that values from both groups could be graphed on the same axis. However, absolute values of AEA are significantly higher at both the stress (5.66 ± 0.82 pmol/mL) and control (5.83 ± 0.82 pmol/mL) sessions in participants treated with the FAAHi compared with those treated with PBO (control session, 0.50 ± 0.04 pmol/mL; stress session, 0.49 ± 0.03 pmol/mL) (see Supplemental Table S5 for more details). Symbols and bars represent means and SEM, respectively; $^{*}p < .05$ for treatment × session interaction; $^{\#}p < .05$ for effect of treatment; $^{\neq}p < .05$ for effect of session.

top-down cortical control of amygdala activity to enhance the learning of fear extinction, as opposed to modulating fear expression. Conversely, PTSD is associated with amygdala hyperactivity, vmPFC hyporeactivity, and an uncoupling of these regions during extinction training (47–49). In patients with PTSD, enhancing AEA-mediated signaling via the CB_1 receptor could function to restore functional connectivity between the vmPFC and the amygdala, thus facilitating extinction learning and consolidation.

The eCB system has been proposed to more broadly buffer affective and behavioral consequences of stress (7,9,11–13,50,51). Accordingly, we find that FAAH inhibition resulted in advantageous emotion regulation following stress exposure. Participants treated with the FAAH inhibitor showed less corrugator ("frown") muscle activity after exposure to stress, reflecting attenuated negative affect. In contrast, stress produced increased negative affect in PBO-treated individuals, occurring at time when AEA levels were significantly lower compared with the control session. Thus, in the PBO-treated group, depletion of the putative emotional buffering capacity mediated by AEA coincided with an increase in negative affect. These effects are in accordance with our previous study, in which individuals homozygous for the loss-of-function *FAAH* mutation were also protected against stress-induced decreases in AEA and concomitant increases in negative affect (18). Our findings support a proposed model in which AEA signaling helps maintain emotional homeostasis (51). In this model, disruption of AEA signaling by stress, driven by an upregulation of FAAH-mediated AEA hydrolysis (52,53), facilitates an uncoupling of prefrontal-amygdala connectivity through alterations in synaptic transmission within both the vmPFC (54) and amygdala (51), promoting a neurobehavioral stress state. Genetic or pharmacological inhibition of FAAH activity maintains AEA signaling during periods of stress, favoring top-down cortical control of the amygdala and attenuating emotional changes produced by stress (51,55).

The role of the eCB system in emotion regulation is supported by neuroimaging studies exploiting functional genetic variation within the eCB system. The loss-of-function *FAAH* 385C→A mutation, which amplifies AEA signaling, is associated with enhanced emotion regulation (15) and with greater fear extinction (14,18), a form of implicit emotion regulation. Variation in the gene encoding the CB_1 receptor (*CNR1*) produces similar effects on emotion regulation following stress exposure, in the absence of differential neuroendocrine stress responses or baseline mood (56). Neurally, these effects manifest as enhanced prefrontal activity (56), reduced amygdala reactivity (17), and enhanced functional connectivity between emotion regulating prefrontal regions and subcortical emotion-generating regions (e.g., amygdala) (14–16,56). Conversely, pharmacological disruption of CB_1 receptor signaling promotes negative affective states in humans, such as depression and anxiety (57), promoting negatively valenced memory consolidation (58,59). Integrating these behavioral genetics studies with the current data supports the notion that AEA mediates an emotional buffer function, particularly during times of adversity (7,9,10,53).

Based on previous studies, it was unclear whether beneficial effects of FAAH inhibition would extend to the autonomic or neuroendocrine stress response. Exogenous CB_1 activation, e.g., by Δ^9-tetrahydrocannabinol, can produce either anxiolytic or anxiogenic responses to an acute stressor, depending on dosage (60). Here, we found that FAAH inhibition attenuated autonomic and subjective responses to stress. In contrast, FAAH inhibition had no effect on the peak neuroendocrine stress response, consistent with preclinical work indicating

limited effects of FAAH inhibition on the neuroendocrine arm of the stress response (61). This adds to previous work showing that elevated AEA conferred via the loss-of-function *FAAH* mutation is associated with a faster decline in stress-induced anxiety but with no difference in baseline or stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation in patients with comorbid PTSD and alcohol use disorder (21). However, it is still possible that AEA may impact later stages of HPA axis activity, such as HPA response termination and recovery (62). Thus, the effects of FAAH inhibition on acute stress responsivity appear to be independent of the HPA axis and may instead be attributed to modulation of frontolimbic neurocircuitry governing stress and emotion processing (55).

The ability of FAAH inhibition to selectively influence the autonomic, but not neuroendocrine, stress response is particularly interesting in the context of PTSD. Hyperarousal is a core feature of PTSD and contributes to a host of negative consequences, such as difficulties with sleep initiation and maintenance (63). Patients with comorbid PTSD and alcohol use disorder carrying the loss-of-function *FAAH* allele demonstrate a greater improvement in the PTSD symptom of hyperarousal. Moreover, in patients treated for cannabis use disorder, FAAH inhibition produced improved sleep quality and reduced anxiety during withdrawal (23). Thus, FAAH inhibition may mitigate hyperarousal symptoms and, as a result, provide additional therapeutic effects, such as improved sleep quality. The effect of FAAH inhibition on stress-related behaviors also indicates potential benefits in the treatment of other disorders characterized by elevated arousal, such as generalized anxiety disorder. The effect of on extinction recall would be beneficial for disorders that use an exposure-based therapeutic approach, such as specific phobias. However, it should be noted that other FAAH substrates, such as OEA (64), may have therapeutic potential. Future work may parse the individual and additive effects of these molecules, while studies in clinical populations will help to characterize the specific therapeutic profile of this mechanism.

Our study is a first of its kind and as such has several limitations. The size of the active treatment group was smaller than was originally planned owing to a procedural error. The results presented are of the per-protocol analysis of subjects who received active treatment, confirmed by biochemical analysis. This is appropriate in a mechanistic proof-of-principle study, but the decrease in sample size reduced our power. The effect sizes for most outcomes widely exceeded those used in the original power analysis, and we therefore believe that this limitation is less relevant. However, as a result of the reduced sample size, we were unable to examine possible sex differences, an important issue now left for future studies. Of note, the procedural error inadvertently generated an additional PBO group; this group did not differ from the planned PBO on any measure, supporting the robustness of the measures employed and the blinding. Second, and perhaps most important among the limitations, we demonstrated beneficial effects of FAAH-inhibition in healthy volunteers. It remains to be determined whether these effects will generalize to specific disease populations, most importantly to patients with PTSD. This appears plausible, as reduced AEA levels may be associated with PTSD (65) or, more specifically, with particular PTSD symptom clusters (66). Thus, elevating AEA via FAAH inhibition may be particularly beneficial in this population.

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EU Clinical Trials Register: Effects of FAAH inhibitor PF-04457845 on fear extinction in healthy volunteers; https://www.clinicaltrialsregister.eu/ctr-search/trial/2016-005013-47/SE; 2016-005013-47.

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