Inhibition of fatty acid amide hydrolase in the central amygdala alleviates co-morbid expression of innate anxiety and excessive alcohol intake

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ABSTRACT

Fatty acid amide hydrolase (FAAH) is an enzyme that prominently degrades the major endocannabinoid N-arachidonoylethanolamine (anandamide). Inhibition of this enzyme leads to increased anandamide levels in brain regions that modulate stress and anxiety. Recently, we found that genetically selected Marchigian Sardinian alcohol-preferring (msP) rats display hyperactive FAAH in amygdalar regions that was associated with increased stress sensitivity and a hyper-anxious phenotype. Our previous work has also demonstrated that msPs display an innate preference for and excessive consumption of alcohol, potentially reflecting a form of self-medication to gain relief from hyper-anxious states. Here, we expand on our previous work by microinjecting the selective FAAH inhibitor URB597 (vehicle, 0.03, 0.1 and 1.0 μg per rat) into the central amygdala (CeA) and basolateral amygdala in msP versus non-selected Wistar rats to evaluate the effects of localized FAAH inhibition on operant alcohol self-administration and restraint-induced anxiety using the elevated plus maze. Intra-CeA URB597 significantly reduced alcohol self-administration in msP but not in Wistar rats. Intra-basolateral amygdala URB597 also attenuated alcohol drinking in msPs, although the effect was less pronounced relative to CeA treatment. In contrast, control experiments administering URB597 into the ventral tegmental area produced no genotypic differences in drinking. We also found that URB597 treatment in the CeA significantly reduced the anxiogenic effects of restraint stress in msPs, although no effects were detected in Wistars. Dysregulation of FAAH regulated systems in the major output region of the amygdala may drive the propensity for co-morbid expression of anxiety and excessive alcohol use.

Keywords abuse, addiction, alcoholism, ethanol, stress.

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INTRODUCTION

Alcohol abuse is often co-diagnosed with major psychiatric illnesses that may contribute to the development of alcohol dependence. For instance, alcohol use disorders are co-morbid with pathological anxiety in humans (Kushner, Abrams, & Borchartd 2000; Goodwin & Stein 2004; Smith & Randall 2012) and have led to the ‘self-medication hypothesis’, in which alcohol drinking may serve to alleviate distress caused by negative affective states (Kushner et al. 2000). Under these circumstances, alcohol consumption is progressively escalated and alcohol use disorders develop more perniciously relative to circumstances void of pre-existing mood disorders. Amygdaloid structures, particularly in the central nucleus division (CeA), may represent a major integrative hub for the intersection of stress signaling systems and cortico-limbic processes that augment the expression of co-morbidity (Koob 2008, 2009; Gilpin, Herman, & Roberto 2015). In this regard, the amygdala plays a critical role in driving alcohol intake to offset the negative affective symptoms of withdrawal in alcohol-dependent individuals (Wang et al. 2003; Kushner et al. 2005; Lovinger 2012). In addition, evidence of the direct role of this area in the regulation of alcohol drinking has emerged in earlier pre-clinical work (Hyytia & Koob 1995).
Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats display co-morbid symptoms of anxiety and excessive alcohol drinking. Our prior work has shown that a major contributor of elevated intake in this rodent model relates to the attenuation of anxiety-like and depressive-like behaviors, consistent with the predictions of the ‘self-medication’ model in alcohol-dependent individuals (Ciccocioppo et al. 1999; Ciccocioppo et al. 2006; Hansson et al. 2006; Hansson et al. 2007). Of importance, we have shown that msPs display pronounced aberrations in brain stress signaling that are commensurate with anxiety-like predispositions. Notably, msPs display an upregulation of corticotropin-releasing factor 1 (CRF1) receptor systems in the amygdala linked to a single-nucleotide polymorphism in the promoter region of the gene (Hansson et al. 2006; Hansson et al. 2007; Ayanwuyi et al. 2013; Gray et al. 2015). MsPs also display evidence of dysregulated antistress systems in the CeA, as in the example of the neuropeptide nociceptin that normally suppresses the effects of stress and anxiety (Ciccocioppo et al. 2014). More recently, our studies involving the endogenous cannabinoid (eCB) system offer further insight into the role of dysfunctional antistress mechanisms in the CeA of msP rats (Natividad et al. 2017). The discovery of dysregulated eCB signaling in msPs raises the possibility that the eCB system may critically underlie the co-morbid expression of behavioral anxiety and excessive alcohol drinking (Giulfrida, Beltramo, & Piomelli 2001; Huggins et al. 2012).

The major eCB, N-arachidonoylethanolamine (anandamide; AEA), is synthetized on demand in postsynaptic neurons and retroactively acts on cannabinoid type 1 (CB1) receptors in presynaptic terminals to suppress neurotransmitter release (Marsicano & Lutz 1999; Marsicano et al. 2002; Diana & Marty 2004). In response to stressors, there is a rapid induction of AEA clearance mediated by the serine hydrolase fatty acid amide hydrolase (FAAH) in the amygdala, thus counteracting the anxiolytic properties of AEA (Gorzalka & Hill 2009; Hill et al. 2009). Recent observations demonstrate that the loss of AEA-CB1 receptor signaling in the basolateral amygdala (BLA) is critical in modulating the behavioral and neuroendocrine responses to stress (Newsom et al. 2012; Ramikie & Patel 2012). For example, the removal of AEA-mediated inhibitory control is shown to increase BLA excitability (Patel, Cravatt, & Hillard 2005), activate the hypothalamic–pituitary–adrenal axis and facilitate anxiety-like behavior (Ganon-Elazar & Akirav 2009; Hill et al. 2009). In agreement with these findings, intrabLA treatment with a FAAH inhibitor produces anxiolytic effects that are blocked with a CB1 receptor antagonist (Hill et al. 2009; Hill & Patel 2013; Gunduz-Cinar et al. 2013b). Using msP rats, these mechanisms were recently extended into the CeA region, where an innate hyperfunction of the CRF1 receptor system was linked to upregulated amygdalar FAAH activity, resulting in diminished basal AEA tone and hypereexcitability of stress-reactive circuits in the CeA (Natividad et al. 2017).

Taken together, these studies suggest the possibility that innate increases in AEA degradation in the CeA may critically underlie the co-morbid expression of anxiety-like behavior and alcohol drinking in msPs. On the basis of this hypothesis, we predict that selective FAAH inhibition in the amygdala would likely reduce symptoms of co-morbidity in msP rats. To test this hypothesis, we delivered the selective FAAH inhibitor URB597 into the CeA and BLA regions and evaluated subsequent responses on operant alcohol self-administration and stress-induced anxiety-like behavior. Control experiments were carried out by administering URB597 into the ventral tegmental area (VTA), a midbrain region where we might not expect to observe genotypic differences in co-morbidity.

**MATERIALS AND METHODS**

**Animals**

The studies were conducted in male Wistar (Charles River, Calco, Italy) and msP rats bred at the School of Pharmacy (University of Camerino). Rats weighed approximately 200–225 g at the beginning of each study and were housed in common cages contained within a temperature-controlled and humidity-controlled vivarium (20–22°C; 45–55%) under a reverse 12:12 hour light/dark cycle (lights off at 9:00 AM). During the experiments, animals were given *ad libitum* access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). All experimental sessions were conducted during the rats’ dark phase. Animals were treated in accordance with the guidelines of the European Community Council Directive for Care and Use of Laboratory Animals.

**Drug administration**

The selective FAAH inhibitor, URB597, was purchased from Sigma SRL (Milano, Italy) and dissolved in 20% (v/v) solution of Dimethyl-sulfoxide (DMSO) diluted in sterile isotonic saline.

**Intracranial surgery**

Animals were anesthetized by intramuscular injection (100–150 μl) of a solution containing tiletaminechlorohydrate (58.17 mg/ml) and zolazepamchlorohydrate (7.5 mg/ml). For drug injections, guide cannulas (0.65 mm outside diameter) were stereotaxically implanted bilaterally and cemented onto the skull. We used the following stereotaxic coordinates...
relative to bregma (in mm): CeA, anteroposterior (AP) − 1.6, lateral (L) ± 5.0, and ventral (V) 7.0; BLA, (AP) − 1.4, (L) ± 5.8, and (V) 8.0; and VTA, (AP), −6.0; (L), ±2.2; (V), −7.4; angle 12°. All coordinates were based on the Paxinos and Watson (1998) atlas and were adjusted for the body weight of the animals. Surgery was followed by a 7-day recovery period; during that time, rats were left undisturbed in their home cages.

At the completion of the experiments, we injected 0.5 µl per site of black India ink into the CeA, BLA or VTA. Rats were then immediately euthanized and histologically examined for cannula placement. All other drug or vehicle solutions were injected bilaterally at a volume of 0.3 µl per site through a stainless-steel injector that was 1.5 mm longer than the guide cannula, allowing for the tip of the injector to protrude into the area under investigation.

Acute restraint stress
To elicit acute stress, msP and Wistar rats were restrained for 1 hour in a cylindrical tube made of clear Plexiglas® and measuring 21.5 cm long with an internal diameter of 6.3 cm. The sliding plugs allowed for adjustments in length based on the animals’ size.

Rats from both genotypes were divided in two groups (restrained and non-restrained animals). The resulting four groups were then divided into subgroups (n = 6–7 group) receiving intracranial injections of URB597 (1.0 µg per rat) or vehicle (0.0 µg per rat). Solutions were administered 15 minutes prior to the incidence of restraint procedures. After 1-hour restraint, rats were returned to their home cages, where they remained for 15 minutes before undergoing evaluation of anxiety-like behavior in the elevated plus maze (EPM) test.

Elevated plus maze
The EPM apparatus was located in a sound-attenuated room illuminated by red light (30 lux). It consisted of two black wooden open arms and two enclosed arms (40-cm-high walls), arranged so that the similar arms were opposite of each other. Each 5-minute trial began when the animal was placed in the center of the maze, facing a closed arm. A rat was considered to be on the central platform when at least two of its paws were placed inside these dimensions. An entry was defined as the presence of all four paws inside the respective arm. The number of open-arm and closed-arm entries and the time spent in each arm were recorded. The percentage of time spent in the open arms [% OAT = (time in open arm/time in ‘open’ arm + time in ‘closed’ arm) × 100] and the percentage of open-arm entries [% OAE = (number of open-arm entries/number of ‘open’ + ‘closed’ arm entries) × 100] are considered to be a reliable index of anxiety-like behavior, whereas the number of total arm entries was used as an index of generalized locomotor activity (Pellow et al. 1985; Cippitelli et al. 2011; Domi et al. 2016).

Operant alcohol self-administration
Operant training and testing were performed in self-administration chambers (Med Associates) equipped with a drinking reservoir (volume capacity 0.30 ml) and two retractable levers. Visual stimuli were presented via a light located on the front panel. Rats were trained to self-administer 10% alcohol (v/v) in 30-minute daily sessions on a fixed-ratio 1 schedule of reinforcement. To facilitate self-administration training, we adapted a saccharin-fading procedure from Weiss et al. (1993). Briefly, during the first 5 days of training, active lever presses were reinforced by the delivery of 0.2% (w/v) saccharin solution into the drinking receptacle. After the acquisition of the saccharin-reinforced response, alcohol was added to the solution and progressively increased to the final concentration of 10% (v/v) ethanol. Saccharin was progressively faded out. The delivery of the solutions was followed by a 5-second time-out period; during that time, the reinforced lever remained inactive. Each response resulted in the delivery of 0.1 ml of fluid. The number of operant responses of both the active and inactive levers and the number of reinforcers received were recorded.

Statistical analysis
Data were analyzed using within-factor or between-factor analyses of variance (ANOVs) or within-group paired t-tests. ANOVAs were followed by Newman–Keuls post hoc tests where appropriate. Statistical significance was set at P < 0.05. Animals with incorrect cannula placements were excluded from further analysis (n = 3 for the VTA, n = 4 for the CeA and n = 1 for the BLA).

RESULTS
Experiment 1: effect of URB597 microinjection into the CeA on alcohol self-administration in msP and Wistar rats
We administered URB597 or vehicle treatment following the acquisition of a stable baseline of alcohol self-administration. Treatments were administered 5 minutes before the beginning of the self-administration session, with rats receiving intra-CeA injections of URB597 (0.03, 0.1 and 1.0 µg per rat) or vehicle in a counterbalanced manner. Drug treatment was performed every fourth day of testing. The day after drug testing, rats remained in their home cages, whereas for the
following 2 days, baseline alcohol self-administration was reestablished prior to the subsequent drug test. A one-way, within-subject ANOVA in msP rats (n = 10) revealed a main effect of treatment [F(3, 27) = 5.41, P < 0.01]. As shown in Fig. 1a, Newman–Keuls post hoc tests confirmed a significant difference between vehicle controls and rats that were administered URB597 at the 0.1 μg (P < 0.01) and 1.0 μg per rat doses (P < 0.05). Specifically, URB597 treatment markedly reduced operant responses on the alcohol-reinforced lever (Fig. 1a), whereas responses on the inactive lever remained negligible and unaltered by URB597 treatment [F(3, 27) = 2.06, P = ns]. Similar analyses in Wistar rats (n = 9) revealed no overall effect of URB597 treatment on alcohol self-administration [F(3, 26) = 1.8, P = ns] or inactive lever responding [F(3, 26) = 0.7, P = ns] (Fig. 1b).

**Experiment 2: effect of URB597 microinjection into the BLA on alcohol self-administration in msP and Wistar rats**

We followed similar treatment strategies as mentioned earlier in separate cohorts of msP rats (n = 7) receiving intra-BLA injections of URB597 (0.03, 0.1 and 1.0 μg per rat) or vehicle. A one-way, within-subject ANOVA revealed an overall effect of treatment [F(3, 6) = 5.0, P < 0.05]. As shown in Fig. 2a, Newman–Keuls post hoc tests confirmed the efficacy of URB597 treatment at the highest dose (1.0 μg per rat) that reduced alcohol self-administration as compared with vehicle controls (Fig. 2a). Responding on the inactive lever was once again negligible and unaltered by drug treatment [F(3, 6) = 0.91, P = ns]. Similar analyses in Wistar rats (n = 10) revealed no overall effect of URB597 treatment on alcohol self-administration [F(3, 9) = 2.5, P = ns] or inactive lever responding [F(3, 9) = 0.34, P = ns] (Fig. 2b).

**Figure 1** Effect of intra-central amygdala (CeA) injections of URB597 on alcohol self-administration in (a) Marchigian Sardinian alcohol-prefering (msP) rats and (b) Wistar rats. URB597 at the doses of 0.1 and 1.0 μg per rat decreased alcohol-reinforced active lever responding in msP but not in Wistar rats. Values represent the mean ± (SEM) of number of rewards obtained by active lever responding (top panel) and responses on the inactive lever (bottom panel). Significant differences from vehicle-treated rats: *P < 0.05, **P < 0.01.

**Experiment 3: effect of URB597 microinjection into the VTA on alcohol self-administration in msP rats**

Control experiments were conducted on an additional group of msP rats (n = 9) receiving intra-VTA injections of URB597 (1.0 μg per rat) or vehicle. We followed similar strategies as mentioned earlier in msPs only, given that intra-amygdalar URB597 treatment had no behavioral consequences in Wistars. Moreover, we evaluated operant responding at the dose that produced maximal effects in msPs. A one-way, within-subject ANOVA revealed no overall effect of URB597 treatment on alcohol self-administration [t(8) = 0.4967, P = ns] or inactive lever responding [t(8) = 1.244, P = ns] (Fig. 3).

**Experiment 4: effect of URB597 microinjection into the CeA on restraint-induced anxiety-like behavior in msP and Wistar rats**

A two-way, between-subject ANOVA of the percentage of time spent in the open arms in msP rats (n = 6/7 group) revealed a main effect of intra-CeA URB597 treatment [F(1, 22) = 14.99; P < 0.001], as well as a treatment by stress interaction [F(1, 22) = 9.28; P < 0.01]. As shown in Fig. 4a, Newman–Keuls post hoc tests revealed that URB597 treatment (1.0 μg per rat) significantly increased the percentage of time spent in the open arms of the EPM in restrained rats as compared with vehicle controls (P < 0.001). The anxiolytic effect produced by URB597 treatment was observed only in animals that were subjected to restraint procedures (P < 0.01).

Similar analyses of the percentage of open-arm entries revealed an overall effect of treatment [F(1, 22) = 22.93; P < 0.001], stress [F(1, 22) = 6.65; P < 0.05] and treatment by stress interaction [F(1, 22) = 12.29; P < 0.01]. As shown in Fig. 4b, Newman–Keuls post hoc tests revealed that URB597 treatment (1.0 μg per rat)
significantly increased the percentage of open-arm entries in restrained rats as compared with vehicle controls ($P < 0.001$). Consistent with our analyses of open-arm time, URB597 treatment was effective only in animals that were subjected to restraint procedures ($P < 0.001$).

Similar analyses of the percentage of closed-arm entries revealed an overall effect of treatment [$F(1, 24) = 1.54; P = ns$], stress [$F(1, 22) = 0.39; P = ns$] or interaction of these variables [$F(1, 22) = 1.89; P = ns$] (Fig. 4d).

A two-way, between-subject ANOVA of the percentage of time spent in the open arms in Wistar rats ($n = 7$ group) revealed an overall effect of stress [$F(1, 24) = 5.9; P < 0.05$], although there was no main effect of treatment [$F(1, 24) = 0.0002; P = ns$] or interaction of these variables [$F(1, 24) = 0.08; P = ns$]. As shown in Fig. 5a, restraint procedures induced a significant decrease in the percentage of time spent in the open arms but did not appear to be influenced by URB597 treatment.

Similar analyses of the percentage of open-arm entries revealed no main effects of drug treatment [$F(1, 24) = 0.7; P = ns$], stress [$F(1, 24) = 1.5; P = ns$] or interaction of these variables [$F(1, 24) = 0.02; P = ns$] (Fig. 5b). We also observed null findings for subsequent analyses of the percentage of closed-arm entries [treatment: $F(1, 24) = 0.7; P = ns$, stress: $F(1, 24) = 1.6; P = ns$, treatment by stress: $F(1, 24) = 0.02; P = ns$, (Fig. 5c)] and total arm entries [treatment: $F(1, 24) = 0.2; P = ns$, stress: $F(1, 24) = 3.7; P = ns$, treatment by stress: $F(1, 24) = 0.5; P = ns$, (Fig. 5d)]

**DISCUSSION**

In summary, our studies revealed that blockade of FAAH-mediated hydrolysis in the amygdala significantly reduces
Figure 4  Effect of intra-central amygdala (CeA) injections of URB597 (1.0 μg per rat) in Marchigian Sardinian alcohol-preferring (msP) rats on (a) percent time spent in open arms, (b) percent number of open-arm entries, (c) percent number of closed-arm entries and (d) total arm entries. URB597 exerted an anxiolytic-like effect in rats subjected to restraint stress as indicated by a higher percentage of time spent in the open arms. The effect of URB597 on open and closed-arm entries are consistent with evidence of increased time spent exploring the open arms. Data are represented as the mean ± SEM. ###P < 0.001 statistical differences versus V-STR (vehicle, restrained animals); **P < 0.01; ***P < 0.001 statistical differences versus V-V (vehicle, non-restrained animals).

Figure 5  Effect of intra-central amygdala (CeA) injections of URB597 (1.0 μg per rat) in Wistar rats on (a) percent time spent in open arms, (b) percent number of open-arm entries, (c) percent number of closed-arm entries and (d) total arm entries. As opposed to the data collected from Marchigian Sardinian alcohol-preferring (msP) rats, URB597 did not differentially alter elevated plus maze behaviors in Wistars. Data are represented as the mean ± SEM.
co-morbid expression of pathological anxiety and excessive alcohol drinking. Specifically, we report that intra-CeA, more so than intra-BLA URB597 treatment, attenuated operant alcohol self-administration in msPs showing innate preference for and spontaneous intake of alcohol. Additional work revealed that intra-CeA FAAH inhibition alleviated stress-induced anxiety-like behavior in msPs but not Wistars. The selective response in the CeA extends our previous work examining the role of antistress mechanisms in modulating amygdalar output and delineates a critical role of eCB signaling in driving co-morbid symptoms of behavioral anxiety and excessive alcohol intake. The findings complement our recent work showing that the msP anxious phenotype is related in part to dysfunctional AEA signaling elements in the CeA (Natividad et al. 2017). Specifically, increased amygdalar FAAH activity in msPs is accompanied by reductions in dialysate AEA levels in the CeA and is constitutively linked to the overexpression of CRF1 receptor signaling in the amygdala, depending on the presence of two SNPs located in proximity to the promoter region encoding for this receptor (Hansson et al. 2006; Hansson et al. 2007; Ayanwuyi et al. 2013; Cippitelli et al. 2015). Here, we provide behavioral evidence of site-specific amelioration of co-morbid behaviors, consistent with the restoration of dysfunctional AEA signaling in the CeA. Our findings are consistent with emerging evidence supporting the role of the eCB system in modulating stress and anxiety (Hill et al. 2013; Gunduz-Cinar et al. 2013a; Bluett et al. 2014; Gray et al. 2015; Morena et al. 2016) and extend this work to include elements of pathological anxiety known to facilitate alcohol consumption.

Intra-amygdalar URB597 attenuated alcohol self-administration, although we observed regional distinctions in CeA versus BLA treatment. Of note, URB597 reduced alcohol self-administration at lower doses in the CeA versus BLA. The finding suggests that the effect of URB597 on alcohol intake may be strongly influenced by AEA signaling in the CeA, whereas the smaller effect observed in the BLA may have resulted from drug diffusion, given the close proximity of these amygdalar regions. While the involvement of the BLA region cannot be fully excluded, we observed that intra-VTA treatment failed to alter alcohol intake. The finding is significant in ruling out the possibility that URB597 may have acted on distal midbrain regions via drug diffusion into the brain parenchyma.

In contrast to the present results, our previous work in msPs showed that peripheral injections of URB597 did not influence alcohol drinking (Cippitelli et al. 2008). In other work conducted in a different alcohol-prefering line (i.e. alko, alcohol rats), it was reported that alko, alcohol rats display decreased FAAH mRNA transcripts and enzyme activity in the prefrontal cortex (PFC), leading to locally overactive eCB transmission. Moreover, intra-PFC URB597 treatment was observed to enhance alcohol self-administration in non-selected Wistars (Hansson et al. 2007). An attractive explanation to reconcile these findings is that eCB mechanisms in the PFC and CeA may play opposing roles in the regulation of alcohol drinking. In this regard, enhanced eCB transmission may facilitate or inhibit alcohol drinking, depending on the brain region that is primarily affected (i.e. the PFC or CeA, respectively). As a result of these site-specific differences, peripherally injected URB597 would likely increase eCB tone in both regions, resulting in a null effect on alcohol drinking.

A broad set of studies has shown that administration of URB597 exerts anxiolytic effects and attenuates stress responses, presumably by bolstering AEA signaling (Gaetani, Cuomo, & Piomelli 2003; Hill et al. 2010; Bedse et al. 2014). Noteworthy, msP rats are sensitized to stressors, show excessive anxiety and alcohol intake and are motivated to consume alcohol to ameliorate innate symptoms of negative affect (Ciccocioppo et al. 2006). On the basis of these findings, we predicted that the inhibitory effect of URB597 on alcohol self-administration, that is, specific for msP rats, was dependent on the ability of the drug to attenuate anxiety following CeA injection. To test this hypothesis, we evaluated anxiety-like behavior following intra-CeA URB597 at the same dose that produced maximal effects on alcohol self-administration. As expected, FAAH inhibition reduced anxiety-like effects in msPs but not Wistars and was specific to the alleviation of stress-induced anxiety via restraint. Given the unique propensity for pathological anxiety, it is not surprising that msPs exhibited a floor effect in the EPM study, wherein reductions in open-arm time were likely occluded by their innate anxious demeanor (Ciccocioppo et al. 2006). Interestingly, Wistars demonstrated an anxiogenic response to restraint stress that was unaltered by URB597 treatment.

Our findings also underscore the significance of CeA endocannabinoid signaling, particularly in the correction of aberrant stress reactivity. In this regard, our previous work demonstrated that msPs exhibit heightened spontaneous excitatory signaling and increased basal glutamatergic levels in the CeA that are sensitized to stressors like restraint immobilization. In contrast, CeA glutamatergic transmission was not differentially altered by restraint in Wistar rats (Natividad et al. 2017). These findings prompt the hypothesis that local enhancement of endocannabinoid levels in the CeA may reduce problematic behaviors influenced by neuroadaptations in stress-reactive circuits in the amygdala. This is consistent with our recent data showing that FAAH inhibition attenuates stress-induced increases in CeA glutamatergic transmission and delineates a critical role of eCB signaling in driving antistress mechanisms in modulating amygdalar output.
transmission (Natividad et al. 2017), presumably by bolstering deficient AEA tone in msPs. Noteworthy, FAAH co-localizes with CBR1 receptors that are primarily expressed on glutamatergic terminals in CeA. Endocannabinoids and via retrograde negative feedback mechanisms, exert inhibitory control of glutamatergic signalling (Egertova, Cravatt, & Elphick 2003; Egertova et al. 1998).

In conclusion, our results demonstrate that selective FAAH inhibition alleviates co-morbid symptoms of anxiety and excessive alcohol drinking, which we propose are driven in part by chronic dysregulation of stress signalling in the major output region of the amygdala. Moreover, innate dysregulation of CeA endocannabinoid signaling may exacerbate the underlying pathology of co-morbidity. Although it is anticipated that the therapeutic benefits of FAAH inhibitors are due to the amplification of AEA-CB1 signaling, it is worth mentioning that FAAH contributes to the degradation of other N-acylethanolamines such as oleoyl ethanolamide and palmitoylethanolamide. These molecules have been shown to exert anxiolytic effects and even reduce alcohol drinking through activation of PPARα and PPARγ receptors (Stopponi et al. 2011; Stopponi et al. 2013; Bilbao et al. 2016; Domi et al. 2016). Future studies should focus on systematically exploring the significance of these pathways in mediating the therapeutic benefits of FAAH inhibitors.

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Author Contributions

All of the authors contributed to the experimental design and data analyses. Additionally, SS, LN, YF, AD, and AAB conducted the experiments and wrote the manuscript. NC, RC, and MR designed, supervised the study, and contributed to prepare and write the manuscript.

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