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Amygdala FAAH and anandamide: mediating protection and recovery from stress

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Abstract

A long-standing literature linking endocannabinoids (ECBs) to stress, fear, and anxiety has led to growing interest in developing novel anxiolytics targeting the ECB system. Following rapid on-demand biosynthesis and degradation upon neuronal activation, the ECB *N*-arachidonoylethanolamide (anandamide, AEA) is actively degraded by the serine hydrolase enzyme, fatty acid amide hydrolase (FAAH). Exposure to stress rapidly mobilizes FAAH to deplete the signaling pool of AEA and increase neuronal excitability in a key anxiety-mediating region – the basolateral amygdala (BLA). Gene deletion or pharmacological inhibition of FAAH prevents stress-induced reductions in AEA and associated increases in BLA dendritic hypertrophy and anxiety-like behavior. Additionally, inhibition of FAAH facilitates long-term fear extinction and rescues deficient fear extinction in rodent models by enhancing AEA–CB1 (cannabinoid type 1) receptor signaling and synaptic plasticity in the BLA. These preclinical findings propose restoring deficient BLA AEA levels by pharmacologically inhibiting FAAH as a mechanism to therapeutically mitigate the effects of traumatic stress.

Keywords

endocannabinoid; post-traumatic stress disorder; anxiety; fear; depression; 2-AG

Cannabis, endocannabinoids, and anxiety

Cannabis is one of the most widely used drugs in the world, with historical records dating use in Eastern cultures back millennia [1]. Cannabis and its derivatives have profound effects on a wide variety of behavioral and neural functions, ranging from feeding and metabolism to pain and cognition [2]. However, epidemiological studies have indicated that the most common self-reported reason for using cannabis is rooted in its ability to reduce feelings of stress, tension, and anxiety [3]. Significant numbers of people may be self-

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medicating with cannabis in an attempt to reduce excessive anxiety [4,5] (even though cannabis use can also cause paranoia and heightened anxiety in certain situations and predisposed individuals, depending on the dose [6]). Furthermore, studies in controlled clinical settings confirm that administration of synthetic variants of delta-9-tetrahydrocannabinol (THC), the psychoactive constituent of cannabis, can reduce anxiety in patients with anxiety disorders [7,8]. Finally, the anxiety-reducing properties of THC extend to preclinical rodent assays and models [9], demonstrating that the anxiolytic properties of cannabinoids are well conserved across species.

Many of the neural and behavioral effects of exogenously administered cannabinoids can be traced directly to activation of the `endocannabinoid' (ECB) system [10] – a set of neurochemicals and cognate receptors densely expressed throughout the brain [11]. The discovery of the ECB system raised the possibility that ECBs could be important modulators of anxiety, and might contribute to individual differences in anxious temperament and risk for anxiety disorders. It also led to the notion that targeting components of the ECB system could represent a novel therapeutic approach to developing effective anxiolytic medications devoid of the unwanted effects of cannabis (e.g., cognitive impairment, abuse liability) [4,12] (Box 1). That is, selective alleviation of symptoms could be produced by increasing levels of endogenously activated ECBs and avoiding widespread behavioral effects caused by exogenously applied, ubiquitously activating cannabinoid receptor type 1 (CB1R) agonists.

A substantial amount of research has been undertaken over the past decade to test these ideas. As a result, understanding the role of ECBs in controlling stress, fear, and anxiety has grown considerably in recent years, with some targets already having been advanced to preliminary clinical trials in patients. The broader literature on this topic has been covered in many excellent, comprehensive reviews [13] and will not be reiterated here. The focus of the current article is on one of the most rapidly moving developments in the field – the stress and anxiety-related role of a major modulatory component of the ECB system, known as fatty acid amide hydrolase (FAAH).

We first introduce the functional role of FAAH within the wider ECB system. Next, we discuss evidence from pharmacological and genetic approaches that loss/inhibition of FAAH function can both mitigate behavioral and neural sequelae of stress and promote learned reductions in fear via actions in the basolateral amygdala (BLA), a central node within the neural circuitry mediating stress and anxiety. We conclude with an outlook for the field going forward and pose some outstanding questions that remain to be addressed.

FAAH regulation of ECB signaling

ECBs are fatty acid amides and monoacylglyerols functioning as neuromodulator lipids that exhibit rapid (within seconds) on-demand biosynthesis in response to neuronal activation, and are subsequently degraded by specialized catabolic enzymes. There are two known receptors binding ECBs with high affinity – CB1R is the most densely expressed in the brain and is present at high levels in corticolimbic regions mediating anxiety, including the medial prefrontal cortex (mPFC) and hippocampus, as well as the BLA [11,14], whereas CB2R is

mainly found in the periphery but also in some microglia and neuronal populations in the central nervous system (CNS) [15,16]. Unlike most neurotransmitters, however, ECBs are not stored in readily releasable pools, but instead are rapidly synthesized `on-demand' upon depolarization-induced calcium increase. Such biosynthesis occurs tonically and, under strong neuronal activation, phasically. The ECB 2-arachidonoylglycerol (2-AG) is synthesized, postsynaptically, by diacylglycerol lipase, whereas the ECB anandamide (AEA) is predominantly synthesized by *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), also at postsynaptic sites in regions including the BLA, but also presynaptically at others (e.g., hippocampus) [17,18]. Following their synthesis, ECBs are retrogradely transported into the extracellular space to bind ECB receptors present on presynaptic terminals [19–21].

Stimulation of CB1R recruits various signaling pathways [22]. These pathways include CB1R coupling to $G_{i/o}$ proteins that reduces adenylyl cyclase activity and downregulates cyclic AMP/protein kinase A signaling [23], by $G_{\beta\gamma}$-induced phospholipase C-β-mediated increases in intracellular calcium influx, and by activation of mitogen-activated protein kinases [24,25]. In addition, CB1R negatively regulates N- and P/Q-type voltage-gated calcium channels [26] and positively regulates inwardly rectifying K^+ channels [26,27], as well as protein serine/threonine phosphatase 2B (calcineurin, PP2B) to change the phosphorylation state of various effector molecules [28,29]. At this time, the precise contribution of these various signaling pathways to ECB modulation of anxiety remains essentially unknown. Enriching the picture further, the actions of AEA are not restricted to CB1R, given that ECBs also act, sometimes in a non-retrograde manner, at CB2R [30], GPR55 [31], and TRPV1 (transient receptor potential vanilloid type 1) channels [32–34], as well as other G protein subtypes such as G_s and/or G_{q11} [35,36]. It should be noted that not all of these actions have been demonstrated in the BLA at the present time, and this may become an important consideration for future work given differences in CB1R signaling mechanisms across brain regions [37]. Indeed, recent evidence demonstrates that diverse effects are evident even within the extended amygdala [38], whereas other parts of the amygdala, notably central amygdala subnuclei, remain largely uncharacterized.

A key mechanism governing CB1R-mediated signaling is the active degradation of released ECBs. 2-AG is degraded presynaptically by monoacylglycerol lipase (MAGL). By contrast, the catabolic fate of a number of the *N*-acylethanolamine (NAE) group of ECBs, including *N*-palmitoyl ethanolamine (PEA) [39], *N*-oleoyl ethanolamine (OEA), and AEA [40], was suspected to be controlled by a common enzyme as early as 1984 [41]. This enzyme was later identified as the serine hydrolase enzyme, FAAH [42–44], which was then isolated and cloned and shown to be located postsynaptically [45]. In the rodent brain, the distribution of FAAH overlaps closely with CB1R in many but not all regions. Within amygdala subnuclei, FAAH is highly expressed on pyramidal neurons in the BLA, and to a significantly lesser extent in the central nucleus (CeA) [19,20,46,47]. Although activation of CB1R in the BLA causes a decrease in both glutamatergic and GABAergic transmission, there is typically a net reduction in neuronal excitability on application of CB1R agonists, probably due to CB1R-mediated presynaptic inhibition of glutamate release [48,49]. By contrast, CB1R signaling generated by increased AEA can also produce long-term depression of inhibitory

transmission in the BLA, a change in synaptic plasticity which can serve to promote excitability [50,51].

Collectively, these observations demonstrate that FAAH is functionally positioned to modulate the actions of AEA and other NAEs on BLA neurons and anxiety processes mediated by this region. By extension, manipulations that lead to alterations in FAAH activity, such as stress or administration of compounds targeting FAAH [52], would be predicted to functionally impact BLA functions, including anxiety. We consider emerging evidence in support of this hypothesis in the next section.

FAAH modulation of anxiety and stress

There is growing evidence that tonic ECB signaling in the BLA and elsewhere is mediated by AEA, whereas phasic ECB responses to robust neuronal activation are sub-served by 2-AG [53–56]. Interestingly, stress appears to produce divergent effects on AEA and 2-AG levels in the BLA – with an elevation in 2-AG levels [57], but a rapid induction of FAAH activity and a resultant decline in the pool of AEA [58–60] were reported following exposure to various types of stress. These differing directional and temporal responses probably indicate a difference in the mechanisms modulating the effects of these two ECBs and their catabolic enzymes in the BLA. These mechanisms are not currently understood, however, and it is also unclear exactly how the relative balance between changes in AEA and 2-AG impacts the response and recovery from stress.

Notwithstanding, several recent observations demonstrate that loss of AEA–CB1R signaling in the BLA can trigger behavioral and neuroendocrine responses to stress. First, presumably by removing AEA-mediated tonic inhibitory activity, CB1R antagonism increases BLA excitability [61–63], activates the hypothalamic–pituitary–adrenal (HPA) axis and increases anxiety-like behavior [58,64,65]. Second, increasing AEA by inhibiting FAAH (with URB597) reduces the HPA axis and anxiety-like response to stress [58,66]. These actions of FAAH inhibition have been localized to actions specifically within the BLA by the finding that only infusion of URB597 into the BLA, and not CeA or medial amygdala, is effective in producing these anti-stress effects [58]. Collectively, these findings support a model in which stress rapidly mobilizes FAAH, depletes the signaling pool of AEA, and increases BLA excitability to drive anxiety [67] (Figure 1).

Disruption of FAAH and AEA may be exacerbated under conditions of chronic stress. Rodents exposed to chronic stress show sustained enhancement of FAAH activity and prolonged reductions in tissue levels of BLA AEA that persist beyond exposure [59,68,69]. Additionally, multiple studies have found that chronic stress results in an increase in dendritic arborization and spinogenesis on BLA pyramidal neurons, increasing their intrinsic excitability and afferent stimulation in a manner that correlates with heightened anxiety-like behavior [70–72]. These morphological changes are absent in mutant mice lacking FAAH [68], whereas genetic loss or repeated, pre-stress pharmacological inhibition (JNJ5003) of FAAH prevents stress-induced reductions in AEA and increases in HPA activity and anxiety-like behavior [68,73,74]. Accordingly, inhibition of FAAH can potently ameliorate

multiple sequelae of chronic stress by enhancing AEA signaling and restoring BLA dysfunction (Figure 1).

The contribution of BLA 2-AG to stress responsivity and recovery remains, in contrast to FAAH–AEA signaling, much less certain at the present time. As noted above, stress increases 2-AG in the BLA, which could suggest a protective/restorative role to counter the effects of stress-induced AEA depletion. In this context, systemically administered MAGL inhibitors have been shown to acutely reduce anxiety-like behavior [75–78], and when administered chronically, to prevent chronic stress-induced increases in anxiety-like behavior and impairments in BLA synaptic plasticity [75]. Further studies will be needed to determine the role of MAGL–2-AG signaling in moderating stress and the manner in which this mechanism interacts with the FAAH–AEA system in the BLA. Indeed, these relationships may be further complicated by sex differences in the ECB system and ECB mediation of anxiety (Box 2).

FAAH mediation of fear and extinction

In addition to being a major target and modulator of stress, the BLA is a critical node within the neural circuitry subserving learned fear behaviors. The BLA is activated during the formation, expression, and extinction of conditioned fear memories, and damage to the BLA disrupts one or more of these processes [79,80]. Implicating BLA ECB signaling in fear conditioning, several studies have shown that injecting a CB1R agonist (WIN55212-2) into the BLA enhanced the consolidation of conditioned fear (but impaired fear reconsolidation [81]), whereas CB1R antagonism/inverse agonism (AM251) had the opposite effect, impairing fear memory formation [82]. These effects on fear are not restricted to the BLA but involve a pathway between the BLA and mPFC. Responses to fear cues in mPFC neurons receiving inputs from BLA were potentiated by the CB1R agonist WIN55212-2, and CB1R blockade/inverse agonism (with AM251) in these regions disrupts fear learning and learning-related synaptic plasticity (long-term potentiation) [83–85].

Extending these findings and demonstrating a role for ECBs in fear extinction, Marsicano and colleagues reported elevated AEA and 2-AG BLA levels after extinction training in mice [86], and produced impairments in extinction learning (but not fear conditioning) by CB1R KO or systemic antagonist (SR141716A) administration [28,86] (for studies reporting similar effects, see [87–89]). Subsequent studies showed that infusing SR141716A directly into the BLA (or mPFC [90]) was sufficient to impair a cued fear extinction in rats [91,92]. Some authors have posited that these effects on extinction reflect a more general role of ECBs in promoting the long-term adaptation to aversion situations [47,93,94]. The ability of ECBs to modulate fear extinction is bidirectional. Activating CB1R via systemic or intra-hippocampal or -mPFC administration of either the CB1R agonist, WIN55212-2, the ECB reuptake blocker, AM404, or AEA itself has been shown to facilitate rodent fear extinction, in most cases without affecting conditioned fear [89,95–98] (but see [90]). Initial work has found that similar effects can be produced in humans by administering THC [99].

The ability of ECBs to facilitate fear extinction is recapitulated by selective inhibition of FAAH. Rats infused with the FAAH inhibitor, URB597, into the mPFC prior to extinction

training show improved extinction retrieval when subsequently tested drug-free [90]. More recently, systemic administration of a novel, potent FAAH inhibitor, AM3506 [100], prior to extinction training augmented extinction-induced BLA AEA levels and reduced fear on extinction retrieval in a mouse model of impaired extinction [50]. Although this drug did not reduce fear during extinction training, reduced fear on extinction retrieval is not contingent upon observable facilitation of extinction learning [94,101]. The effects of AM3506 were attributed to the BLA by the finding that infusing the drug directly into the BLA promoted extinction, and the demonstration that intra-BLA infusion of SR141716A blocked the pro-extinction effect of systemically delivered AM3506 [50]. Thus, these data show that CEA–CB1R signalling in the BLA is both necessary and sufficient for FAAH inhibitors to facilitate fear extinction. Interestingly, there is preliminary evidence that analogous effects may result from genetically driven variation in FAAH in human subjects (Box 3).

The mechanisms underlying the pro-extinction effects of BLA–FAAH inhibition remain to be elucidated. One notable finding is that a CB1R-dependent form of synaptic plasticity in BLA [51,86], long-term depression of inhibitory GABAergic transmission (LTDi), is enhanced by FAAH KO [51] and by the FAAH inhibitor AM3506 at doses that promote extinction [50]. This suggests a scheme in which AEA released during extinction relieves a tonic inhibitory brake on BLA output neurons [50,51,86] necessary for the encoding of extinction memories [102] (Figure 2). However, it would be premature to discount the involvement of other mechanisms, including more direct effects on glutamatergic transmission, and this remains a key question for future work.

Another important question for future studies will be whether FAAH inhibitors work to normalize impairments in fear extinction that are known to be produced by environmental insults such as exposure to abused drugs and stress [97,103]. Indeed, several authors have posited utility of FAAH inhibitors for drug and alcohol addiction [104], and the effects related to stress are particularly pertinent given the protective effects of boosting ECBs (by inhibiting FAAH) for stress-induced anxiety-related behaviors discussed above. Interestingly in this context, deficient fear extinction caused by a restraint/swim stressor was recently shown to be rescued by intra-BLA (or hippocampal, but not mPFC) injection of a CB1R agonist (WIN55212-2) immediately after stress [105,106]. Demonstrating similar effects with selective FAAH inhibitors would support the utility of these compounds across a range of extinction-impaired settings.

Concluding remarks

Preclinical studies offer strong support for a major role of FAAH, via effects on BLA AEA–CB1R signaling, in modulating stress-induced anxiety and fear extinction. Moreover, recent clinical reports have demonstrated decreased peripheral levels of AEA and increases in brain CB1R-binding in patients with anxiety disorders, such as post-traumatic stress disorder [107]. Taken together, these findings encourage the development of novel anxiolytics based around restoring deficient AEA levels by pharmacologically inhibiting FAAH. Encouragingly, preliminary clinical trials with selective FAAH inhibitors, including PF04457845 and URB597, are either underway (www.clinicaltrials.gov/ct2/show/NCT01665573) or being planned. The `on-demand' nature of ECB release makes it a

particularly attractive target for drug development because FAAH inhibitors would selectively augment CB1R signaling in neural circuits where AEA was recruited [52]. This refined mechanism of action would avoid the widespread activity of THC or CB1R agonism and, as a result, is expected to produce fewer clinically unwanted side effects and be less liable to CB1R downregulation after repeated dosing. Although this prediction awaits thorough clinical investigation, and some important questions also remain to be addressed (Box 4), the field is at an exciting juncture and has genuine promise for advancing our understanding and treatment of anxiety disorders.

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Box 1. Developing selective targets for AEA and 2-AG

AEA and 2-AG have differential roles in ECB signaling, with AEA providing a tonic signal that offsets excess excitability and 2-AG producing more of the phasic ECB response [55]. AEA is a relatively low efficacy agonist, whereas 2-AG has much higher agonist efficacy and induces a robust CB1R G protein mediated response [55]. Drugs that act on the catabolic enzymes for each ECB (FAAH for AEA, MAGL for 2-AG) provide one excellent approach to selectively target each ECB for therapeutic applications, as biochemical studies support the feasibility of inhibiting FAAH without altering 2-AG levels and blocking MAGL without affecting AEA [55,108]. Reflecting the earlier discovery of FAAH as the hydrolytic enzyme for AEA, FAAH inhibitors have been well studied to date and appear to influence a more limited range of behaviors than MAGL blockers [52]. This could translate into a more favor side effect profile. Chronically elevating AEA with FAAH inhibitors may also be less prone to CB1R desensitization and the associated issue of behavioral tolerance that is likely with MAGL blockade induced increases in 2-AG [109,110]. These unique properties suggest that inhibiting FAAH is a particularly amenable approach to developing ECB medications for various therapeutic indications, including anxiety disorders.

Box 2. Sex differences in ECB and FAAH modulation of anxiety

There are known sex differences in the function of the ECB system that could impact how manipulation of CB1R and FAAH affect anxiety-related behaviors in rodents. There are some notable differences between males and females in CB1R-binding site density, including greater binding in the BLA of female rats [111] and across limbic regions in human female subjects [107]. Despite these differences, FAAH inhibitors retain anxiolytic- and antidepressant-like effects in ovariectomized female rats [112]. Interestingly, however, anxiolytic- and antidepressant-like effects produced by estradiol administration are attenuated by CB1R blockade [112], whereas estradiol administration increases AEA levels [113] or AEA signaling [114], possibly via downregulation of FAAH driven by an estrogen response element on the FAAH gene that suppresses FAAH transcription when bound by estrogen [115]. These observations suggest that FAAH–AEA signaling may be one important mechanism linking gonadal hormone status and account, at least in part, for sex differences in anxiety. Given the preponderance of diagnosed anxiety disorders in women, these emerging preclinical data will represent an important consideration of the potential clinical application of FAAH inhibitors for anxiety.

Box 3. FAAH gene variation and risk for anxiety disorders

Preclinical findings linking FAAH activity to stress and anxiety in rodents is supported by emerging evidence that gene variation in human *FAAH* may moderate risk for anxiety disorders by regulating amygdala processing of threat. A common (~25% in those of Caucasian ancestry [116]) single nucleotide polymorphism (C385A; rs324420) that results in the conversion of a conserved proline residue to threonine (P129T) in the amino acid sequence of the FAAH gene is associated with reduced expression of FAAH in lymphocytes and elevations in circulating levels of AEA [117,118]. Studies using BOLD functional magnetic resonance imaging have found that people with this gene variant had significantly less amygdala responses to threat, in the form of fearful faces, and trait anxiety levels [119] (but see [120]). Furthermore, in parallel with increased amygdala plasticity produced by FAAH inhibitors in mice [50], the same gene variant predicted more rapid habituation of amygdala responses to repeated threat [50]. These preliminary observations are in line with preclinical evidence that FAAH modulates amygdala responses to stress and fear extinction encoding, and lend support for a model in which increased FAAH works to mitigate the effects of stress.

Box 4. Outstanding questions

- What role does the FAAH–AEA–CB1R signaling system play in modulating the fear and anxiety-related functions of corticolimbic regions beyond the BLA, including the CeA, mPFC, and hippo-campus?
- How does 2-AG-related ECB signaling contribute to stress and extinction? And do 2-AG and AEA work in tandem or opposition to modulate these behaviors?
- The ECB system is perturbed by various environmental insults other than stress, including chronic alcohol exposure and traumatic brain injury. Does loss of FAAH function contribute to the fear- and anxiety-related abnormalities [103,121] associated with these insults?
- AEA regulates a wide range of behaviors in addition to stress and anxiety [2]. To what extent does amygdala FAAH contribute to behaviors, such as reward seeking, that are known to be subserved by the amygdala?
- What are the key upstream and downstream molecular mechanisms regulating FAAH activity? Elucidating these mechanisms could suggest new (e.g., pharmacological) approaches to modulating FAAH activity.
- Does treatment with FAAH inhibitors facilitate extinction in human subjects and/or normalize impaired extinction in patients with anxiety disorders?

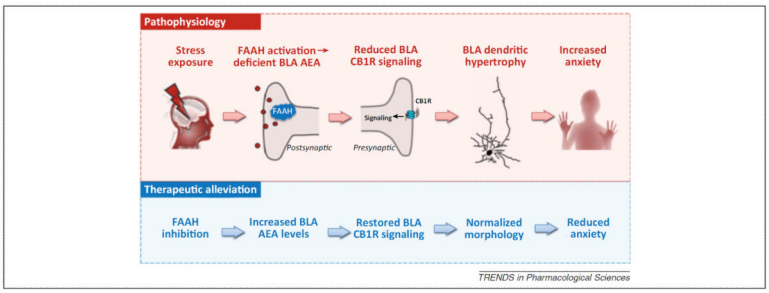

Figure 1.

Role of FAAH in the pathophysiology and therapeutic alleviation of stress-induced anxiety. Hypothesized pathophysiological scheme by which stress activates FAAH, leading to depletion of AEA in the BLA, a reduction in CB1R signaling and dendritic hypertrophy associated with anxiety. Therapeutic alleviation of stress-induced anxiety could be achieved by inhibiting FAAH to increase BLA AEA levels to restore CB1R signaling and possibly normalize dendritic abnormal morphology. Abbreviations: FAAH, fatty acid amide hydrolase; AEA, anandamide; BLA, basolateral amygdala; CB1R, cannabinoid receptor type 1.

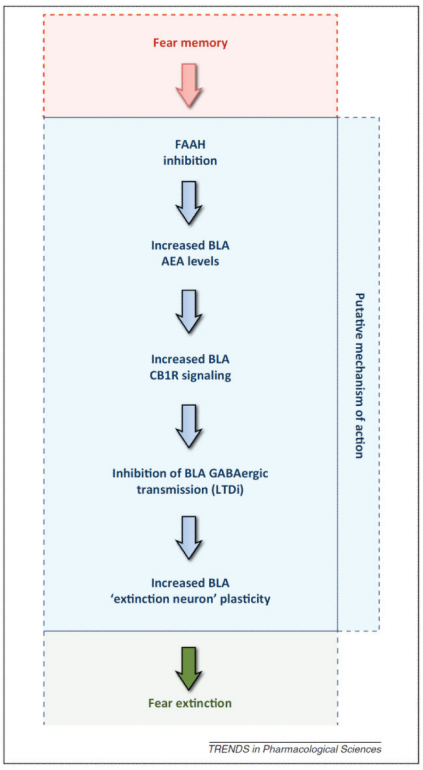

Figure 2.

Putative mechanism mediating fatty acid amide hydrolase (FAAH) inhibitor effects on fear extinction. Following formation of a fear memory, systemic administration of a FAAH inhibitor increases anandamide (AEA) levels in the basolateral amygdala (BLA) and increases cannabinoid receptor type 1 (CB1R) signaling to enhance attenuation of GABAergic transmission via long-term depression of inhibitory transmission (LTDi) [50]. Lesser inhibition may remove a brake on the activity/plasticity of BLA neurons [102] recruited to encode extinction, allowing FAAH to gate the formation of extinction memories.