Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Progress in Neuropsychopharmacology & Biological Psychiatry 105 (2021) 110116

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The endocannabinoid system in the amygdala and modulation of fear

Ozge Gunduz-Cinar*

Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcoholism and Alcohol Abuse, NIH, Bethesda, MD, USA

ARTICLE INFO

Keywords:
Anandamide
Fear extinction
Neurocircuit
FAAH
FAAH inhibitors
Translational study

ABSTRACT

Posttraumatic stress disorder (PTSD) is a persistent, trauma induced psychiatric condition characterized by lifelong complex cognitive, emotional and behavioral phenotype. Although many individuals that experience trauma are able to gradually diminish their emotional responding to trauma-related stimuli over time, known as extinction learning, individuals suffering from PTSD are impaired in this capacity. An inability to decline this initially normal and adaptive fear response, can be confronted with exposure-based therapies, often in combination with pharmacological treatments. Due to the complexity of PTSD, currently available pharmacotherapeutics are inadequate in treating the deficient extinction observed in many PTSD patients. To develop novel therapeutics, researchers have exploited the conserved nature of fear and stress-associated behavioral responses and neurocircuits across species in an attempt to translate knowledge gained from preclinical studies into the clinic. There is growing evidence on the endocannabinoid modulation of fear and stress due to their 'on demand' synthesis and degradation. Involvement of the endocannabinoids in fear extinction makes the endocannabinoid system very attractive for finding effective therapeutics for trauma and stress related disorders. In this review, a brief introduction on neuroanatomy and circuitry of fear extinction will be provided as a model to study PTSD. Then, the endocannabinoid system will be discussed as an important component of extinction modulation. In this regard, anandamide degrading enzyme, fatty acid amide hydrolase (FAAH) will be exemplified as a target identified and validated strongly from preclinical to clinical translational studies of enhancing extinction.

1. Introduction

Posttraumatic stress disorder (PTSD) is a chronic disease characterized by the abnormally persistent occurrence of fear memories and anxiety after trauma exposure. Within the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-V), PTSD is recognized apart from anxiety disorders as a trauma- and stressor -related disorder, where exposure to a traumatic event is a strict diagnostic criterion (American Psychiatric Association, 2013). Although, fear and anxiety are normal and adaptive responses to traumatic situations that involve threat and/or danger (McEwen, 1998), these emotional responses should gradually decline upon the reduction of threat and danger in a given situation. Known as 'extinction' learning, this phenomenon allows healthy individuals to return to a baseline level of emotional responding over time, even when re-exposed to stimuli that may trigger a traumatic memory. However, in individuals suffering from PTSD, due to certain underlying pathologies or exposure to threat/danger for an extended period, these adaptive responses can get out of control and not be extinguished properly (Quirk and Mueller, 2008). One major pathological feature of PTSD is the abnormal activity within brain regions critical to affective regulation which severely impairs the processing of extinguished cues and contexts (Milad and Quirk, 2012). Although treatments such as psychotherapy (cognitive, exposure, eye movement desensitization and reprocessing), pharmacotherapy (with selective serotonin reuptake inhibitors), and other complementary approaches exist, many individuals continue to suffer from debilitating, extinction resistant traumatic memories. Therefore, understanding the molecular and neural systems that contribute to fear extinction is vital to developing novel therapeutic approaches to treat PTSD. Because adaptative processes in trauma and stress related disorders, including fear and extinction learning, are common among different species, these processes can be studied in preclinical animal models and translated to humans. Convergent benefits of neuroanatomical homology between rodents and humans and integration of approaches (such as state of art techniques in neuroscience and transgenic mouse models) makes rodent to human translation easier. Using rodents provide access to brain tissues in different stages of the experiments that can be processed for molecular and anatomical findings that is not possible in human studies.

Fear and extinction learning are studied with classical Pavlovian learning experiments (Pavlov and Anrep, 1927). In fear conditioning, subjects are presented a previously neutral stimulus (also referred as

* Corresponding author at: 5625 Fishers Lane Rm 2N09, Rockville, MD 20852-9411, USA.
E-mail address: ozge.gunduzcinar@nih.gov.

https://doi.org/10.1016/j.pnpbp.2020.110116
Received 11 June 2020; Received in revised form 7 September 2020; Accepted 20 September 2020
Available online 23 September 2020
0278-5846/ Published by Elsevier Inc.

'conditioned stimulus, CS') which is usually a tone, light, image or a context that is often followed by a mild electric shock (also referred as 'unconditioned stimulus, US') so that an association between these stimuli is formed. Subject learns that CS is fearful and expresses fear response (also referred as 'conditioned response, CR'). In rodents these responses are measured by freezing (suppression of all movements except those required for breathing). In humans skin conductance response (where the perspiration induced electrical conductance or moisture level of the skin is measured on the extremities), electromyographic (where the electrical activity of skeletal muscles is measured on eyelid or forehead) or heart rate responses are measured. Apart from the psychophysiological measures human studies apply neuroimaging measures for brain activity via functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) (for more details please see (VanElzakker et al., 2014)).

In extinction learning sometime after the conditioning, subjects are presented the same CS multiple times in a different context and in the absence of shock. 'Acquisition of extinction' is referred to decrements of fear responses within the extinction training which is followed by 'consolidation phase'. The consolidation phase lasts few hours to have molecular events for strengthening and stabilizing the long-term memory of extinction and re-learning of the CS is no more fearful. This is often tested in 'retrieval phase' that consists of presenting the same CS that retrieves the extinction memory. In healthy subjects usually retrieval results with lower levels of measured fear responses. In fact, while a brief memory retrieval after fear conditioning enhances fear memory by 'reconsolidation', a longer re-exposure extinguishes this memory by weakening the retrieved fear memory (Kida, 2020). 'Renewal' of fear memories usually occurs when the CS is presented in a context other than the extinction context.

One promising group of molecules, the endocannabinoids, have been shown to play an important role in the adaptive response to stress, fear and extinction (see recent reviews: (Bedse et al., 2020, Sbarski and Akirav, 2020)). In this review, the neurocircuitry contributing to fear extinction will be introduced and discussed as a model to study PTSD, with a major focus on how the cannabinoid 1 receptor (CB1R) and the endocannabinoid anandamide (AEA) modulates extinction learning. Additionally, the therapeutic potential of enzymes involved in the synthesis and degradation of AEA, such as fatty acid amide hydrolase (FAAH) will be discussed (Fig. 1).

2. Neuroanatomy and neurocircuitry of fear extinction

Fear extinction is a form of inhibitory learning, whereby newly formed associations compete with fearful ones to inhibit fear formation and fear-related neural circuitry (Luchkina and Bolshakov, 2019). Human functional magnetic resonance imaging studies have shown that, during the retrieval of extinction memories, amygdala activity decreases while ventromedial prefrontal cortex, anterior cingulate cortex and hippocampus activity increases (Milad et al., 2007; Phelps et al., 2004). However, individuals suffering from trauma and anxiety related disorders display exaggerated amygdala activity and deficient prefrontal cortex activity as compared to healthy participants (Rauch et al., 2000; Shin et al., 2005).

To develop a detailed understanding of the molecular and circuit alterations contributing to trauma-related psychiatric conditions such as PTSD, animal models of fear and extinction learning have been employed. In particular, intensive study has been undertaken to understand the extinction of conditioned fear, as disturbances in this process are thought to be central to PTSD pathology. These studies have identified a network of highly conserved brain regions, including the prefrontal cortex, amygdala and hippocampus, as each being critical to distinct aspects of fear and extinction learning (Fig. 2).

The amygdala is bidirectionally connected to the ventral, infralimbic (IL) subregion of the rodent cortex (ventromedial prefrontal cortex 'vmPFC' in human). These connections are important for extinction learning: lesions, pharmacological or optogenetic inactivation of the IL impair the recall of extinction learning (Do-Monte et al., 2015; Fontanez-Nuin et al., 2011; Quirk et al., 2000; Santini et al., 2004; Santini et al., 2001). During extinction training optogenetic activation of the IL inputs into the amygdala (basolateral 'BLA' and basomedial 'BMA' subunits) selectively enhances extinction memory formation. Conversely, optogenetic or chemogenetic inhibition of these inputs impairs extinction learning (Adhikari et al., 2015; Bloodgood et al., 2018; Bukalo et al., 2015). Prefrontal cortex inputs into the amygdala are regulated by inhibitory interneurons. CB1Rs are expressed on local

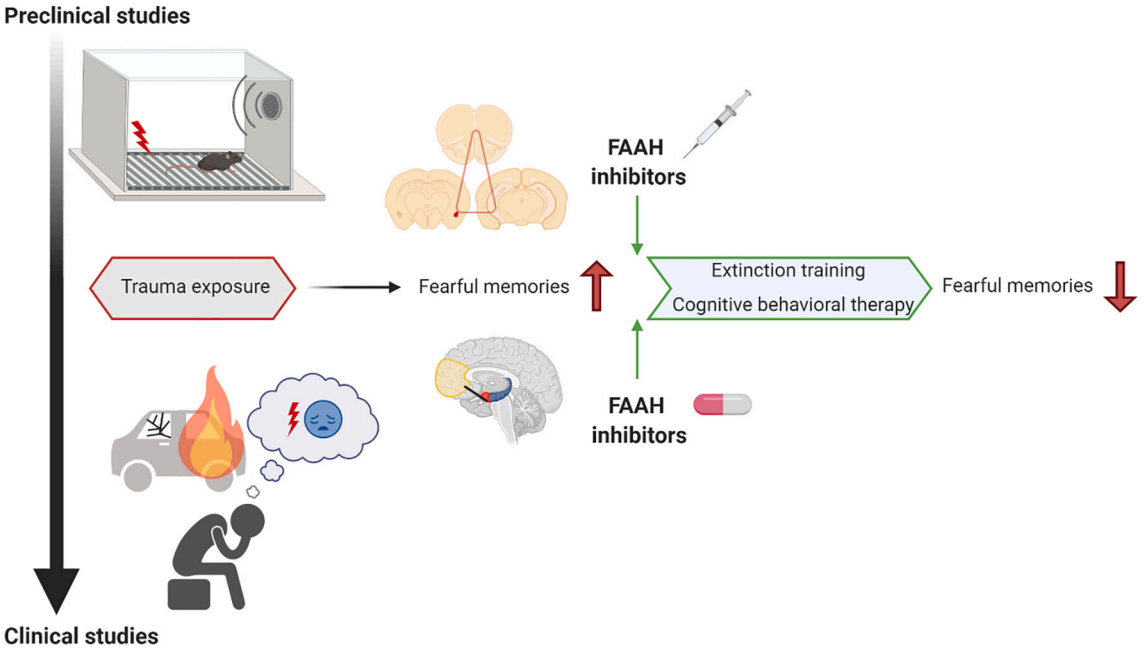

Fig. 1. Fear extinction from mice to human.
Trauma exposure leading to fear can be alleviated with FAAH inhibitors given adjunct to extinction/cognitive behavioral therapy. Both preclinical studies in rodents and clinical studies in healthy humans supports the therapeutic application of FAAH inhibitors.

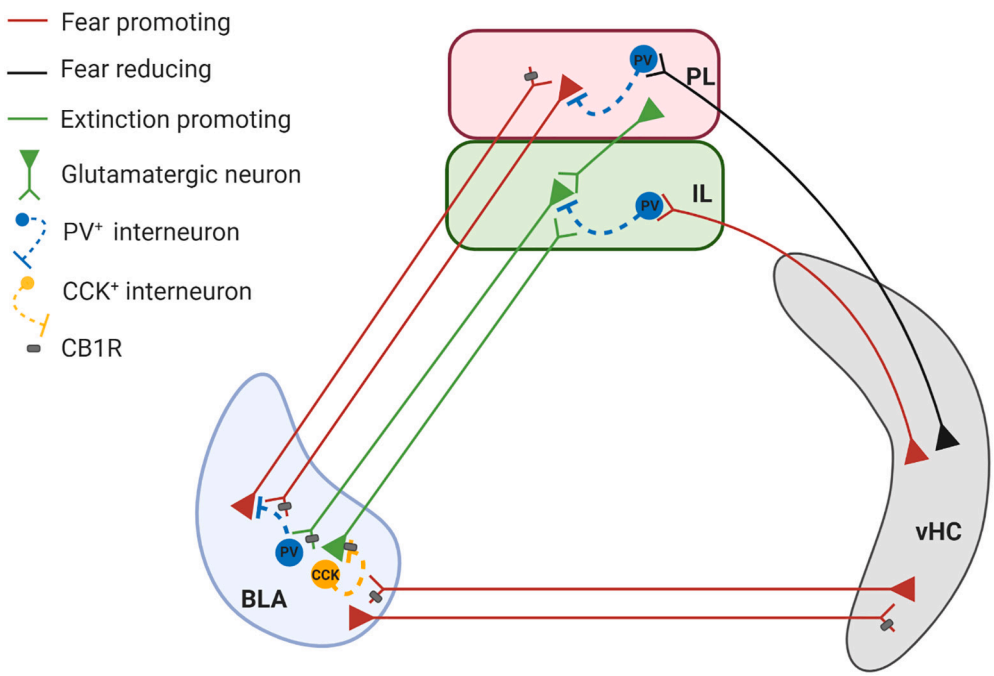

Fig. 2. Fear extinction circuitry.
Hypothetical model for the existence of CB1Rs in the known fear and extinction circuits. Connectivity from infralimbic (IL) subregion of the prefrontal cortex to the basolateral amygdala (BLA) is enhanced (extinction promoting glutamatergic neuron shown in green) with extinction training. Activation of prelimbic (PL) subregion of the prefrontal cortex to IL connection (extinction promoting glutamatergic neuron shown in green) increases fear extinction. Connectivity from BLA to the PL is enhanced (fear promoting glutamatergic neuron shown in red) with fear learning under the control of local parvalbumin interneurons (PV^+ IN) (shown in blue) in PL. Extinction training increases perisomatic connections of the local BLA interneurons (PV^+ IN) to the fear neurons. Extinction training increases perisomatic connections of CCK^+ interneurons (shown in orange) to fear neurons thus CB1R mediated inhibition of GABA release to reduce fear. The ventral subunit of hippocampus (vHC) connections to BLA increases fear (fear promoting glutamatergic neuron shown in red). vHC connections to PL (fear reducing glutamatergic neuron shown in black) decreases fear. vHC connections to IL (fear promoting glutamatergic neuron shown in red) increases fear and innervations onto local PV^+INs in IL control the feed forward inhibition thus the relapse of fear after extinction (renewal).

cholecystokinin (CCK) positive interneurons (INs) (also known as large somata CCK-INs). The large somata CCK-INs inhibit distinct subpopulations of principle neurons within the amygdala in cell type and pathway specific manner. For example, inputs from IL were shown to be modulated by the large somata CCK-INs (Vogel et al., 2016). Both fear and extinction learning change the plasticity of inhibitory neurons (Kasugai et al., 2019) thus modulate freezing behavior, for example extinction increases the parvalbumin positive interneurons (PV-IN) around the silent fear neurons while increasing the CB1Rs expressed on CCK-INs around active fear neurons (Trouche et al., 2013).

In contrast, the dorsal prefrontal cortex, prelimbic cortex (PL) (similar to dorsal anterior cingulate cortex 'dACC' in human) is important for the expression of conditioned fear and stress (Marcus et al., 2020; Padilla-Coreano et al., 2016; Sierra-Mercado et al., 2006; Sierra-Mercado et al., 2011). Activity within the PL is increased during high fear states, such as when extinction memory retrieval fails (Burgos-Robles et al., 2009). Local PV-INs that form perisomatic control over principle neurons contribute in regulating the freezing behavior as well, for example inhibition of the PV-INs was shown to change the activity of output neurons (such as PL➔BLA) thus increase the expression of fear (Courtin et al., 2014). Optogenetic silencing of the basolateral amygdala inputs into PL inhibits retrieval of fear memory and enhances extinction memory, conversely, silencing IL projections weakens extinction memory (Senn et al., 2014). In addition, a recently identified unidirectional PL to IL excitatory connection was also reported to drive fear extinction learning (Marek et al., 2018b). This was shown by optogenetic activation or inactivation of these excitatory projections in IL leading to facilitation or impairment of extinction, respectively, indeed very similar to direct stimulation of IL neurons. Collectively, suggesting that PL afferents drive fear via direct connection with BLA while being critical to extinction formation via intracortical communication with the IL (Marek et al., 2018b).

Generally, the hippocampus is thought to be important for the context dependence of fear and extinction learning. In particular, the ventral hippocampus (vHC) projects to both subunits of the prefrontal cortex and BLA to control the return of fearful memories in response to a previously extinguished conditioned stimulus (Marek et al., 2018a; Maren et al., 2013; Sotres-Bayon et al., 2012; Wang et al., 2016). Changes in stress responsivity is also dependent on the connectivity between vHC and BLA (Bluett et al., 2017). vHC projections to the IL are under inhibitory control by the local PV INs to provide a feed-forward inhibition for renewal of fear (Marek et al., 2018a). When vHC connections to inhibitory interneurons within PL are inactivated, fear is increased (Sotres-Bayon et al., 2012). In addition, vHC connections to the PL have been implicated in safety signaling as they inhibit responses to threats (Meyer et al., 2019).

Due to the complexity of connections within brain regions important for fear and extinction, manipulating behavior by selective targeting can be challenging. Therefore, there is a need to identify specific neuronal populations and sub-populations of neurons that are characterized by distinct cell types with unique expression of genes and proteins (see review for cell-type specific analysis of fear circuitry (McCullough et al., 2016)). In last years, the endocannabinoid system has emerged as a critical neuromodulator component of stress and fear regulation. Endocannabinoids are lipid molecules that mediate the balanced activity of the neuronal systems in brain regions highly important for fear and extinction. Unlike other neuromodulators that have vesicular storage and release properties, endocannabinoids have much rapid 'on demand' synthesis upon depolarization induced calcium increase, signaling tonically, via AEA, and upon robust neuronal activation, signaling phasically, via 2-arachidonoylglycerol (2-AG) (Azad et al., 2003; Hill et al., 2018; Katona et al., 2001; Marsicano et al., 2002; Yoshida et al., 2011). Another distinct feature is, they could be synthesized on the postsynaptic terminals and bind to CB1Rs mostly located on the presynaptic terminals thus signaling retrogradely endocannabinoids serve as synaptic circuit breakers (Katona and Freund, 2008). CB1Rs are one of the major G-protein coupled receptors in the brain (Fig. 3) (Herkenham et al., 1990), in addition to neurons, CB1Rs are also expressed in glial cells (astrocytes (see BOX 1), oligodendrocytes, microglia) (Han et al., 2012; Ilyasov et al., 2018; Lisboa et al., 2016; Molina-Holgado et al., 2002; Navarrete and Araque, 2008). In the next sections, each component of the endocannabinoid system will be reviewed focusing on how fear extinction is modulated. Then with a translational perspective from preclinical rodent studies to humans, targeting specific components of the endocannabinoid system will be discussed for their therapeutic potentials as treatments for fear and

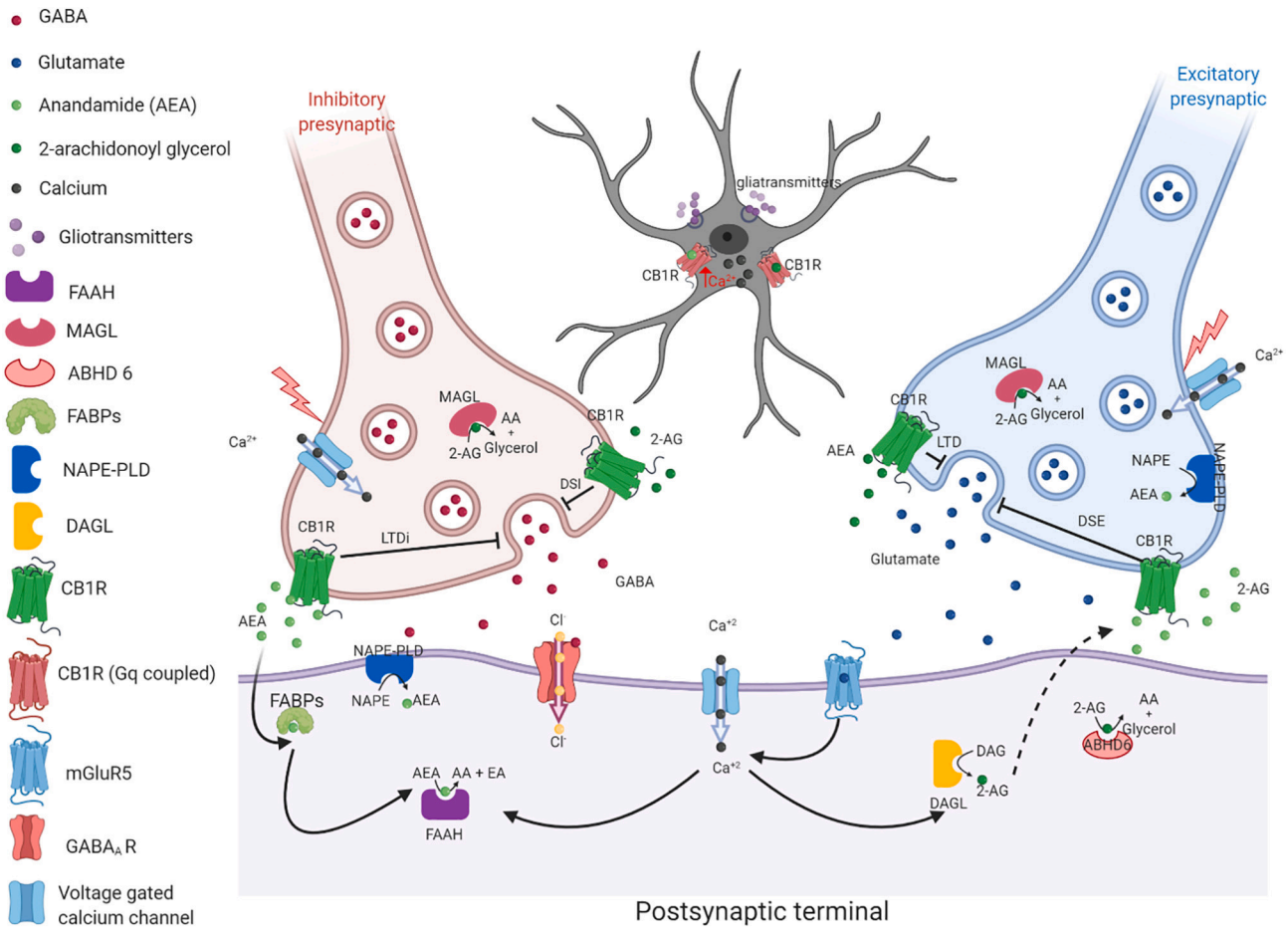

Fig. 3. Synaptic transmission with the endocannabinoid system.
Calcium influx via voltage gated calcium channels together with the depolarization induced glutamate release and binding to metabotropic glutamate receptor type 5 (mGluR5) increases endocannabinoid synthesis. Following retrograde diffusion from postsynaptic membrane and binding to presynaptic CB1Rs that are coupled to $G_{i/o}$ proteins that initiate signaling events: **1.** inhibition of adenylyl cyclase activity and downregulation of cyclic AMP/protein kinase A signaling (Azad et al., 2004; Howlett et al., 1986), **2.** negative regulation of N- and P/Q-type voltage-gated calcium channels and **3.** positive regulation of inwardly rectifying K^+ channels thus, inhibiting neurotransmitter release and consequently changing neuronal excitability (Azad et al., 2008; Childers and Deadwyler, 1996) and causing synaptic plasticity (eg. suppression of glutamate release at the excitatory synapses causing a synaptic plasticity, also known as depolarization induced suppression of excitation (DSE) or long-term depression (LTD), on the other hand suppression of GABA release at the inhibitory synapsis causing depolarization induced suppression of inhibition (DSI)). Other signaling effects includes through the release of $G_{\beta\gamma}$ subunits, phospholipase C-β-mediated increases in intracellular calcium influx, and through the induction of mitogen-activated protein kinases (Rhee et al., 1998) as well as protein serine/threonine phosphatase 2B, CB1Rs change the phosphorylation state of various effector molecules (Cannich et al., 2004; Heifets et al., 2008; Mato et al., 2008; Twitchell et al., 1997). With the possible involvement of fatty acid binding proteins (FABPs) endocannabinoid AEA and 2-AG are transferred to their catabolic enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) enzyme, respectively. Conversely, activation of the astrocytic CB1Rs that are coupled to G_{q11} proteins increases Ca^{2+} (Navarrete and Araque, 2008) and inducing release of 'gliatransmitters' that eventually shift the balance towards excitation.

anxiety disorders.

3. Fear extinction and the endocannabinoid system

3.1. Studying Cannabinoid receptor 1 for fear extinction

It has been almost two decades since the endocannabinoid system was first implicated in extinction learning (Marsicano et al., 2002). The involvement of CB1Rs in the retrieval of extinction memory was first shown with pharmacological antagonism and genetic mouse models. Global CB1R knockout (KO) mice demonstrated impaired extinction of aversive and spatial memory retrieval (Marsicano et al., 2002, Varvel et al., 2005).

Targeting the endocannabinoid system pharmacologically with infusions of CB1R agonists directly into IL and CA1 subregion of hippocampus facilitated the reconsolidation and extinction of fear (Lin et al., 2008; Lin et al., 2009; Santana et al., 2016). Intra-BLA infusion of CB1R agonists enhanced consolidation of conditioned fear (Lin et al., 2006) while intra-BLA infusions of CB1R antagonists attenuated short term extinction of fear (Roche et al., 2007).

These initial studies highlighted the complexity of CB1R mediated effects on fear behavior. One critical factor contributing to this complexity is the differential expression of CB1Rs in distinct neuron types. CB1Rs are mostly expressed on GABAergic INs that co-express the neuropeptide CCK in the amygdala (Katona et al., 2001; McDonald and Mascagni, 2001) and other brain regions (Bodor et al., 2005; Dudok et al., 2015; Katona et al., 1999). CB1R are also expressed on non-CCK GABAergic INs, as well as on glutamatergic, serotonergic, cholinergic and noradrenergic neurons (see distribution summarized in (Hu and Mackie, 2015)).

Although targeting CB1Rs genetically revealed information regarding the necessity of CB1Rs in extinction learning, they are inadequate for revealing the full complexity of the system. Fortunately, advances in genetic and viral approaches have provided researchers with the capability to target specific neuronal subpopulations that may be relevant to fear extinction. For example, the re-expression of CB1R in

dorsal telencephalic glutamatergic neurons of CB1R KO mice has been shown to rescue hippocampus dependent functions (although they were not sufficient in rescuing amygdala mediated functions such as fear extinction) (Ruehle et al., 2013). Interestingly, when the CB1R was re-expressed on forebrain GABAergic neurons, mice showed a slight improvement in fear extinction (Remmers et al., 2017).

In order to study the behavioral phenotypes of specific neuronal populations CB1Rs are expressed conditional mutant mice were also generated (Monory et al., 2006). Mice that lack the CB1R expression on cortical glutamatergic neurons (or on forebrain principle neurons (Marsicano et al., 2003)) displayed increased fear (Kamprath et al., 2009; Llorente-Berzal et al., 2015; Metna-Laurent et al., 2012) while mice lacking CB1R expression on cortical GABAergic neurons displayed decreased fear (Llorente-Berzal et al., 2015, Metna-Laurent et al., 2012). Indicating that these subpopulations of neurons have distinct roles in modulating fear behavior. CB1R on glutamatergic terminals facilitate fear rescue via phasic 2-AG signaling that restricts the glutamate release, therefore controlling fear responses. CB1Rs on GABAergic terminals controls fear expression via tonic AEA signaling that restores the excitatory balance, therefore promoting fear extinction.

CB1R expression on GABAergic interneurons may be a critical component of the molecular architecture allowing for extinction-related tuning of the amygdala activity. In support of this, computational and synaptic network simulation model analysis demonstrated that the endocannabinoid mediated inhibition of GABAergic transmission should strengthen extinction (Anastasio, 2013). Another model suggests that extinction activates the excitatory connections to the BLA extinction neurons while consecutively accumulating endocannabinoids that inhibit local CCK-INs, thus, ultimately dampening fear associated inputs to the extinction neurons (Bennett et al., 2019). While these are models that are useful, in vivo studies that would prove the validity are still awaited.

In an attempt to selectively manipulate CB1R expressing CCK-INs locally in the amygdala, we intercrossed a CCK-Cre line (to drive Cre recombinase in CCK interneurons) with a Dlx5/6Flpe line (to drive Flp recombinase expression in most GABAergic neurons) (Rovira-Esteban et al., 2019). The resulting CCK-Cre;Dlx5/6Flpe transgenic mice expressed both Cre and Flp recombinases in CCK interneurons. We then used an intersectional optogenetic strategy to target CCK-INs in the amygdala by injecting Cre_{on}/Flp_{on} INTronic Recombinase Sites Enabling Combinatorial Targeting (INTRSECT) viruses to visualize and control cells in a manner conditional on the presence of both Cre and Flp recombinases (Fenno et al., 2014; Taniguchi et al., 2011). Surprisingly, optogenetic activation of these amygdala CCK-INs that express CB1Rs during extinction training facilitated extinction retrieval. Electrophysiological and anatomical characterization further detailed the heterogeneous population of interneurons besides the CCK/CB1R expressing basket cells, that were targeted with this approach. However, our study revealed that a diverse group of CCK expressing interneurons contribute to extinction learning in a more complex manner (Rovira-Esteban et al., 2019).

While the IL-BLA pathway activation and synaptic strengthening in BLA reduces fear behavior possibly with an endocannabinoid mediated plasticity during extinction, BLA-PL pathway activation and synaptic strengthening promotes anxiety and fear behavior. Recently, Marcus et al. (2020) showed that stress exposure causes a decrease in 2-AG levels and enhances the activity of the glutamatergic BLA inputs in PL. Deletion of CB1Rs selectively on the BLA- > PL neurons enhanced anxiety like behaviors following stress exposure, and this was based on the altered 2-AG-CB1R signaling in this reciprocal circuit. It's intriguing to ask whether the same BLA-PL circuit affects fear learning, and whether the opposing reciprocal IL-BLA circuit changes strength to fine tune fear extinction via AEA-CB1R dependent mechanism. Interestingly, glutamatergic inputs from vHC to the BLA was shown to be highly sensitive to phasic 2-AG mediated suppression and characterized by a stress resilient phenotype in mice. Changes in this connectivity results in susceptibility to stress (Bluett et al., 2017). It's possible that CB1Rs located in the vHC to the BLA terminals are effective in reducing fear. Whether CB1R deletion on this pathway promotes extinction learning needs to be demonstrated.

Apart from the approaches mentioned above using inbred mouse strains are useful in identifying the genetic and biochemical fundamentals of fear and anxiety behaviors. We use an inbred mouse strain 129S1/SvImJ that has profound extinction and safety learning deficits across cued and contextual fear paradigms compared to normal extinguishing mouse strains (for example the common C57BL/6J) (Hefner et al., 2008). The extinction deficits can be rescued by pharmacological agents like FAAH inhibitors (see section 3.2a below) (Burghardt and Bauer, 2013; Camp et al., 2012; Gunduz-Cinar et al., 2013b; Karpova et al., 2011; Popova et al., 2014) and the prototypical selective serotonin reuptake inhibitor fluoxetine. Fluoxetine has off label use in PTSD and facilitates extinction when used chronically, contrary to the acute effects that was shown to enhance acquisition and expression of fear. Chronic fluoxetine enhances excitatory synaptic currents and long-term potentiation in amygdala altering plasticity-related proteins and perineuronal nets (Gogolla et al., 2009; Karpova et al., 2011). Our preclinical studies extended these previous findings and provided a link between the endocannabinoid signaling through CB1Rs and the extinction promoting effects of chronic fluoxetine. We demonstrated that chronic, but not acute fluoxetine, increased AEA levels in amygdala suppressing inhibitory transmission via tonic CB1R activation. This was verified by an increase in the GABAergic synaptic potentials (unmasking tonic inhibition) on amygdala slices treated with CB1R antagonist rimonabant in chronic fluoxetine treated mice compared to controls (Gunduz-Cinar et al., 2016).

Lastly, the existence of CB1Rs on astrocytes (Box 1) adds another mechanism to the endocannabinoid-mediated regulation of synaptic function in these complex networks.

Box1: Emerging new stars of fear extinction: Astrocytes

Our current understanding on fear learning and memory retrieval are extended with the involvement of gliotransmitters (such as ATP, D-serine, glutamate, dopamine and endocannabinoids) that are released by astrocytes, the star shaped glial cells, in support of synaptic function, a phenomenon known as 'tripartite synapse' (Araque et al., 1999; Kofuji and Araque, 2020). When the gliotransmitter release from the basolateral amygdala astrocytes is inhibited before the fear conditioning, fear memory consolidation is significantly interrupted in rats. This highlights the requirement of astrocytes in long term memory (Stehberg et al., 2012). In fact, moving forward from the tripartite synapse phenomenon Adamsky et al. (2018) showed that the activation of astrocytes in the CA1 subregion of the hippocampus sufficiently enhanced the retrieval of memory without affecting basal neuronal activity. With the availability of sophisticated tools, genetically engineered mouse lines, and cutting-edge techniques in neuroscience, the role of glial cells in circuit and cell specific communication will undoubtedly receive more recognition. Similar to neuronal CB1Rs, astrocytes respond to the endocannabinoids, which is thought to enhance gliotransmitter release and modulate synaptic plasticity at distal locations (Navarrete et al., 2014). Astrocytic CB1Rs regulate hippocampal long term depression and potentiation (Han et al., 2012; Navarrete and Araque, 2008, 2010; Smith et al., 2020) to control synaptic function (see for review (Oliveira da Cruz et al., 2016)). In the medial subunit of the central amygdala (CeM), astrocyte activation was shown to regulate excitatory synapses from the BLA and inhibitory synapses from the lateral subunit of central amygdala (CeL) via distinct mechanisms, thus in the same neuron affecting the balance and eventually reduce the fear expression (Martin-Fernandez et al., 2017).

3.2. Studying the endocannabinoid modulation

The ultimate goal of the preclinical studies highlighted above is to provide insights that may be used to alleviate the persistent fear that

characterizes trauma related disorders like PTSD. Although CB1Rs appear to be a tractable treatment target, directly targeting these receptors may not be favorable. First, CB1R are widely expressed in the central and peripheral nervous systems, which may increase the likelihood of detrimental side effects. Second, CB1Rs are prone to desensitize after repeated dosing, which may affect long-term treatment efficacy. Instead, targeting the endocannabinoid modulating enzymes (anabolic and catabolic) may be a better approach to fulfilling the physiological need while avoiding these unwanted effects.

The endocannabinoid anandamide is synthesized mainly by the membrane precursor N-arachidonoyl phosphatidylethanolamine with one step cleavage by phospholipase D (NAPE-PLD) or with a two-step cleavage by phospholipase C/phosphatase pathways (Di Marzo et al., 1994; Liu et al., 2006; Okamoto et al., 2004; Simon and Cravatt, 2006). NAPE-PLD is expressed presynaptically and contribute to anterograde transmission, in amygdala expression of NAPE-PLD differs in intensity, across subregions, with higher levels in the medial, as compared to the basal and lateral amygdala (Egertova et al., 2008). Postsynaptic expression and contribution into retrograde transmission was also reported (Puente et al., 2011). Biosynthesis of AEA can also be accomplished by αβ-hydrolase 4 (Abhd4), a presynaptically expressed enzyme without apparent brain region specific expression (Simon and Cravatt, 2006). AEA is catabolized into ethanolamine and arachidonic acid by a serine hydrolase enzyme named, fatty acid amide hydrolase (Cravatt et al., 1996). Expression of FAAH on postsynaptic terminals is higher in the lateral, basolateral and basomedial amygdala, compared to the central amygdala (Gulyas et al., 2004).

The endocannabinoid 2-arachidonoylglycerol is synthesized mainly by the enzyme diacylglycerol lipase (DAGL) in postsynaptic terminals and degraded by the enzyme monoacylglycerol lipase (MAGL) in presynaptic terminals. In addition to MAGL, two other enzymes αβ-hydrolase 12 and 6 (Abhd12 and Abhd6) have been reported to be involved in degradation of 2-AG in brain (Blankman et al., 2007; Marrs et al., 2010). Abhd12 is an integral membrane protein mostly expressed in microglia. Abhd6 is expressed on postsynaptic neurons side by side with the CB1s to regulate the amount of 2-AG synthesized, and expression also found in astrocytes. (Blankman et al., 2007; Savinainen et al., 2012). Endocannabinoids can also be metabolized by oxygenation which is mediated by cyclooxygenase-2 (COX-2), an enzyme responsible from prostaglandin synthesis from arachidonic acid (Kozak et al., 2000; Ueda et al., 1995). COX-2 expression is high in the lateral and basolateral amygdala (Breder et al., 1995).

Canonically, the synthesis of endocannabinoids is followed by retrograde diffusion to the synaptic cleft and binding to presynaptic receptors (see Fig. 3) (Fowler, 2013), which are then transferred to their catabolic enzymes by intracellular fatty-acid–binding proteins (FABPs) (Kaczocha et al., 2009). Among the numerous FABPs, FABP5 is secreted from neurons and astrocytes, and has been reported to have a role in the extracellular transfer of the endocannabinoids (Haj-Dahmane et al., 2018). KO mice for FABPs (FABP3, FABP5 and FABP7) displayed cognitive and emotional problems (Owada et al., 2006; Yabuki et al., 2018; Yu et al., 2014). Inhibitors of FABPs with ranging affinities towards FABP3, FABP5 and FABP7 were shown to elevate anandamide with in vivo antinociceptive effects (Kaczocha et al., 2014). Interestingly, two important phytocannabinoids (Δ^9-tetrahydrocannibinol (THC) and cannabidiol (CBD)) were shown to competitively displace AEA binding to FABPs, and augment AEA content without interacting with FAAH in vitro (Elmes et al., 2015). It is important to understand in more details how the differential expression of FABPs would contribute into pathophysiology of trauma and stress related disorders.

This wealth of molecular data has provided multiple avenues to bidirectionally target the endocannabinoid signaling, without directly affecting the CB1Rs. Based on this literature, multiple preclinical studies have demonstrated that endocannabinoid metabolism/uptake inhibitors and agonists can modulate short-term fear memory extinction (Chhatwal et al., 2005; Fidelman et al., 2018; Llorente-Berzal et al., 2015; Morena et al., 2018; Pamplona et al., 2008). In particular, augmenting the endocannabinoid activity by targeting the enzymes responsible for their synthesis or degradation has emerged as a promising therapeutic approach for trauma and anxiety related disorders.

a) Modulating AEA levels: FAAH as a potential target to enhance fear extinction

The ability of FAAH inhibitors to decrease anxiety by increasing AEA levels, was first shown by Kathuria et al. (Kathuria et al., 2003). Further studies have demonstrated that stress exposure reduces tonic AEA signaling through rapid activation of the FAAH enzyme (Hill et al., 2013b; Patel et al., 2005; Rademacher et al., 2008) (also see reviews (Gunduz-Cinar et al., 2013a, Lutz et al., 2015)). Knockout or pharmacological inhibition of FAAH in mice facilitates acquisition and extinction rates for spatial memory (Varvel et al., 2007). In line with this, low frequency stimulation in vitro in mice amygdala releases AEA that was shown to induce the long term depression of inhibitory GABAergic transmission (LTDi), thus, increasing synaptic excitability (Azad et al., 2004). Using our inbred, extinction deficit mouse we have shown that systemic or intra BLA administration of the FAAH inhibitor AM3506 before extinction training significantly increases AEA levels in the amygdala and facilitates the retrieval of extinction memory when tested 10 days later (Gunduz-Cinar et al., 2013b). Interestingly, FAAH inhibition with AM3506, which lasted upto 10 days, did not change fear within the extinction (acquisition) session, suggesting that augmenting AEA during extinction is necessary for consolidation of extinction memory, which occurs following the initial extinction memory formation. On synaptic level, AM3506 enhanced long-term depression of inhibition in the amygdala. Altogether, our data support previous findings on enhancing AEA tone in the amygdala with FAAH inhibitors that result in improved extinction learning, therefore suggesting a therepautic potential for FAAH inhibitors.

While AEA augmentation through inhibition of the catabolic FAAH enzyme improved extinction memory retrieval, studying the effects of AEA depletion on memory has proven challenging for two primary reasons. First, the absence of selective enzyme inhibitors to prevent synthesis of N-acylethanolamines (NAEs) like anandamide, that are also active in vivo, and second, other existing NAE substrates that may have interrupted synthesis by the major anabolic enzyme NAPE-PLD inhibition. In support of the latter, lipidomic profiling of three different NAPE-PLD knockout mouse strains showed that NAE expression changes in a brain region and fatty acid chain dependent manner (Leishman et al., 2016). In fact, both FAAH knock out mice (Cravatt et al., 2001) and FAAH inhibitors (Fegley et al., 2005) not only change the level of AEA, but also other NAEs (such as N-palmitoylethanolamide 'PEA', N-oleoyl ethanolamide 'OEA' and oleamide). Thus far multiple targets for these NAEs (such as peroxisome-proliferator-activated receptor-alpha and gamma (PPAR-α, PPAR-γ), TRPV1, GPR55 etc) have been described. Evidence exists that, like the endocannabinoids discussed here, some of these NAEs are involved in fear memory expression and extinction learning independent of CB1Rs (Kramar et al., 2017; Laricchiuta et al., 2013; Marsch et al., 2007; Provensi et al., 2017). Recent data from Mock et al. (2020) using the first centrally active NAPE-PLD inhibitor suggested that decreasing AEA may be a primary mechanism of affective changes following NAPE-PLD treatment. Systemic inhibition of NAPE-PLD with LEI-401 reduced multiple NAEs, including AEA, activated hypothalamus-pituitary axis, and impaired fear extinction. Critically, these effects were reversed by FAAH inhibitors (Mock et al., 2020). More detailed pharmacological and behavioral characterization of LEI-401 and other next generation NAPE-PLD inhibitors is still necessary to strengthen the hypothesis that the modulation of endogenous AEA tone is an important primary mechanism by which these compounds regulate anxiety and fear extinction.

Recently, Morena et al. elaborated on the tight control of the amygdala endocannabinoids by virally overexpressing the FAAH enzyme within BLA pyramidal neurons in rats. In line with the literature

discussed here, these researchers hypothesized that FAAH overexpression would potentiate fear related behaviors by decreasing AEA levels, diminishing the ability of AEA to inhibit GABA release at presynaptic terminals from GABAergic neurons. However, contrary to their expectations, FAAH overexpression prior to fear conditioning did not affect conditioning, extinction or retrieval and paradoxically resulted in reduced anxiety-like behavior. When the same manipulation was conducted after fear conditioning, fear acquisition was impaired, an effect opposite to what has previously been seen using systemically administered FAAH inhibitors (Morena et al., 2019). This study further underscores the complexity of the endocannabinoid system and suggests that FAAH overexpression in distinct neuronal subtypes may shift critical aspect of neuronal function such as inhibitory/excitatory balance, differentially affecting fear and extinction learning. Future studies will be important in understanding the compensatory mechanisms on different neuronal, or glial populations and involvement of other FAAH substrates.

b) Modulating 2-AG levels

Another example of the tight control of the endocannabinoid system is seen in the regulation of the endocannabinoid 2-AG. Acute stress exposure increased 2-AG levels as a compensatory mechanism to the elevated corticosterone that promotes anxiety. On the other hand, acute stress increases the level of FAAH enzyme therefore reducing AEA levels causing a disruption on the tonic AEA signaling in the amygdala. This has been shown to be prevented by augmenting the endocannabinoid 2-AG tone as a compensatory mechanism to reduce stress, through pharmacologically preventing it's degradation by inhibiting monoacylglycerol lipase enzyme (Bedse et al., 2017; Marcus et al., 2020). Chronic stress exposure decreases 2-AG levels, that can be reversed by chronic inhibition of MAGL enzyme (Hill et al., 2009; Patel et al., 2009; Sumislawski et al., 2011) (also see review (Bedse et al., 2020). The anxiety associated with the decreased levels of 2-AG can also be prevented by increasing AEA-CB1R signaling (Bedse et al., 2017) indicating the bidirectional compensatory mechanism for maintaining the tight balance.

Global knock out or pharmacological inhibition of DAGLα, the main enzyme for synthesis of 2-AG, increases fear learning and expression in addition to impairing extinction learning (Cavener et al., 2018; Jenniches et al., 2016). However, pharmacological blockage of MAGL increases fear expression and also impairs extinction learning (Hartley et al., 2016; Llorente-Berzal et al., 2015). The finding that bidirectional modulation of 2-AG levels via enzymatic modulation induces similar behavioral phenotypes (impairments in fear expression and extinction), may be due to differential effects of 2-AG acting on CB1Rs expressed on GABAergic or glutamatergic neurons, as well as possibly astrocytes. Altering the delicate balance of 2-AG within the amygdala may ultimately increase fear and impair extinction learning. To uncover this complex regulation of neural circuits by 2-AG in relation to CB1R signaling, more specific techniques that apply multiple recombinase expressions to define neuronal sub-populations, as in the INTRSECT approaches discussed above, will be required for future studies.

3.3. The endocannabinoid system in humans

Biomarkers from cells, tissues, or fluids (plasma and cerebrospinal fluid) can provide mechanistic evidence related to the etiology and pathophysiology of disorders, such as PTSD, and thus critical to translational studies. But determination and measurement of such samples must be standardized in an unbiased, accurate way that reliably controls for the confounding factors (i.e. age, sex, diet, smoking, time of sampling, and storage etc). Studies investigating the endocannabinoids in pathological conditions were summarized broadly before (Hillard, 2018). Here, changes after trauma and associated genetic variations are highlighted in relation to the translation of the existing preclinical and clinical findings.

3.3.1. Genetic variations

The preclinical evidence outlined here for the involvement of the endocannabinoid system in fear extinction parallels data from healthy human subjects implicating genetic variations within the human endocannabinoid system in anxiety and fear. Heitland et al. (Heitland et al., 2012) provided one of the first examples of the endocannabinoid system's involvement in human fear extinction, whereby a polymorphism in the promoter region of the CB1R gene (rs2180619) resulted in impaired fear extinction for homozygous AA allele carriers. Another well characterized single nucleotide polymorphism (SNP) exists on the FAAH gene. This SNP (rs324420) causes a mutation on proline at position 129, a highly conserved amino acid residue across different species, that results in conversion to a threonine residue (P129T). This mutation results in a functional, but easily degradable, FAAH variant (C385A) (Sipe et al., 2002). Carriers of this FAAH variant have lower FAAH binding (Boileau et al., 2015) and have elevated plasma AEA and NAEs (Sipe et al., 2010). We showed that people carrying this polymorphism had quicker habituation of amygdala reactivity to threat (Gunduz-Cinar et al., 2013b). Moreover, our FAAH SNP findings were reverse translated using a humanized mouse (knock-in) model where fear extinction and prefrontal cortex-amygdala resting state connectivity was found to be enhanced both in mice and humans (Dincheva et al., 2015; Mayo et al., 2018). Homozygous carriers of this FAAH variant were found to be protected from the stress induced changes in plasma AEA levels in humans, as well as in the prefrontal cortex and amygdala in humanized mice. In a different study homozygous carriers of FAAH variant showed decreased anxiety in stress challenge test and a faster decline of the hyperarousal as a PTSD symptom in comorbid alcohol use disorder/PTSD patients (Spagnolo et al., 2016). Altogether, these findings in humans strengthen the importance of FAAH as a potential therapeutic target, for the inhibitory control of fear extinction via increased AEA levels.

3.3.2. Translational studies

a) The endocannabinoid system in clinical populations

Besides genetic variances, identifying differences in the endocannabinoid system components in individuals with PTSD compared to healthy people are critical. For example, using radiotracers, the availability of CB1Rs were found to be significantly increased brain-wide in PTSD patients compared to trauma exposed controls (Neumeister et al., 2013). This upregulation of receptors was in accordance with low levels of AEA and cortisol found in PTSD patients, as compared to healthy controls. In the same study, another FAAH substrate N-oleoyl ethanolamide (OEA) levels were found to be decreased in trauma exposed controls as well as the PTSD group in comparison to the healthy controls (Neumeister et al., 2013). An opposite pattern of OEA changes in PTSD patients has also been reported (Schaefer et al., 2014). In another study, PTSD patients had no change in plasma NAEs, but a negative association were reported between AEA levels and the symptoms related with re-experiencing of the traumatic event (also known as intrusive symptoms). Interestingly, 2-AG levels were decreased in the same PTSD population and a positive correlation between cortisol levels and avoidance, which is another important symptom of PTSD denoting avoidance of stimuli associated with trauma, was reported (Hill et al., 2013a).

b) Pharmacological intervention of the endocannabinoid system in healthy populations

Initial studies to test the possibility of enhancing extinction learning by modulating the endocannabinoid system adjunct to cognitive behavioral therapy in humans, provided promising results. Acute administration of dronabinol, the synthetic analog of Δ^9-tetrahydrocannibinol (THC) the active ingredient of *Cannabis sativa* or *indica*, in healthy humans prior to extinction training increased ventromedial prefrontal cortex and hippocampus activation and attenuated the amygdala activity during the acute and long term recall of extinction memory (Hammoud et al., 2019; Rabinak et al., 2014; Rabinak

et al., 2013). In individuals suffering from PTSD, THC administration lowered the amygdala reactivity to threat and increased vmPFC activation during threat, while strengthening the functional connectivity between the ventromedial prefrontal cortex and amygdala compared to placebo controls (Rabinak et al., 2020). Recently, the therapeutic value of FAAH inhibition was validated in a double-blind, placebo-controlled study (Mayo et al., 2020) where healthy volunteers were randomized to receive an orally bioavailable FAAH inhibitor (PF-04457845, 4 mg once daily) or placebo for 10 days and were then assessed on a battery of psychophysiological tests like fear learning, stress reactivity and stress induced affect. FAAH inhibitor application for 10 days (whereas only acute single dose application in preclinical studies (Gunduz-Cinar et al., 2013b) produced nearly complete inhibition of the FAAH enzyme and significantly elevated AEA and OEA, while leaving levels of PEA and 2-AG unchanged. Similar to the preclinical studies FAAH inhibition had no effect on acquisition of fear, within session fear extinction, or fear renewal, but significantly enhanced the recall of extinction memory, consistent with the hypothesis that prolonging AEA availability during extinction consolidation is necessary for extinction facilitation. Stress induced changes in the negative affective responses were decreased by FAAH inhibition. Autonomic and subjective measures of stress reactivity were also decreased, although no changes in endocrine function were reported. Stress-induced depletion of AEA was not evident in the group that received FAAH inhibitor treatment as compared to placebo control, probably because increased AEA signaling would restore the balance in the amygdala, and the amygdala connectivity with other regions like the prefrontal cortex. Overall, these translational data corroborate the findings from preclinical animal studies. However, whether this compound has efficacy in a PTSD patient population remains to be seen.

3.3.3. Clinical studies

Translational studies following promising preclinical work, led the pharmaceutical industry to test various FAAH inhibitors in clinical trials (Hu and Mackie, 2015; Mallet et al., 2016). Phase 1 clinical studies to determine the clinical safety, tolerability, pharmacokinetics and pharmacodynamics of FAAH inhibitors in healthy volunteers were conducted for PF-04457845 of Pfizer (NCT00836082), V158866 of Vernalis (NCT01634529), and JNJ-42165279 of Johnson and Johnson (NCT03564379, NCT01650597, NCT01964651). PF-04457845 (NCT01665573, NCT02216097) reached to Phase 2 clinical trials where the efficacy and side effects were determined for fear and fear extinction, in people with PTSD. Even though FAAH inhibitors were tested in clinical trials for conditions other than PTSD (such as pain, insomnia, Tourette's Syndrome, anxiety, depression and alcohol dependence) they were all terminated due to portfolio prioritizations by various companies. This could be partly because of the unfortunate fiasco with the Phase 1 clinical trial conducted in France with the compound BIA 10–2474 of Bial Pharmaceuticals. The preclinical findings published after this incident suggest that, the BIA 10–2474 compound is not as selective as other clinically validated FAAH inhibitors (like PF-04457845) and alters lipid networks in the brain dramatically due to off-target effects (Bonifacio et al., 2020; van Esbroeck et al., 2017). It is still unclear how the off-target interactions went unnoticed in the initial toxicology studies (Hardisty et al., 2020). This tragic experience has led researchers to be more cautious in clinical testing of compounds prone to such off-target effects, and encouraged studies probing other aspects of the endocannabinoid system with the goal of developing a viable treatment for trauma related disorders such as PTSD.

3.4. Future perspective

At the time of writing, the world is grappling with new realities following the outbreak of the SARS-CoV-2 pandemic. When the pandemic ends, we will likely face a new era characterized by anxiety and anxiety-related disorders such as PTSD. It's imperative that novel psychopharmaceuticals be developed to combat this looming neuropsychiatric disease burden. Continued evaluation of FAAH inhibitors may provide some solutions, while other selective and efficacious modulators of the endocannabinoid system can be further optimized both pharmacokinetically and pharmacodynamically to mimic the tight physiological regulation of this system. Potential compounds include the small molecule irreversible (URB597, PF-04457845, PF-3845, JNJ-42165279) or reversible (SSR-411298, OL-135) FAAH inhibitors, as well as allosteric modulators of FAAH and hybrid FAAH inhibitors targeting multiple protein/receptor/enzymes (Dainese et al., 2020; Tripathi, 2020). Fine-tuning these and related compounds to optimize the next generation of endocannabinoid system modulators may revitalize the clinical relevance of this system.

Declaration of Competing Interest

The author has no conflict of interest.

Acknowledgements

OGC is supported by the NIH/NIAAA Intramural Research Program , USA. OGC would like to thank to Dr. Andrew Holmes and Holmes lab members (Dr. Olena Bukalo, Dr. Patrick Piantadosi, Laura I. Castillo) for critical reading and comments. OGC has no conflict of interest. Figures in this review were created by BioRender.com.

References

Adamsky, A., Kol, A., Kreisel, T., Doron, A., Ozeri-Engelhard, N., Melcer, T., et al., 2018. Astrocytic activation generates De novo neuronal potentiation and memory enhancement. Cell. 174, 59–71 e14.

Adhikari, A., Lerner, T.N., Finkelstein, J., Pak, S., Jennings, J.H., Davidson, T.J., et al., 2015. Basomedial amygdala mediates top-down control of anxiety and fear. Nature. 527, 179–185.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Arlington, VA, USA.

Anastasio, T.J., 2013. Computational search for hypotheses concerning the endocannabinoid contribution to the extinction of fear conditioning. Front. Comput. Neurosci. 7, 74.

Araque, A., Parpura, V., Sanzgiri, R.P., Haydon, P.G., 1999. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 22, 208–215.

Azad, S.C., Eder, M., Marsicano, G., Lutz, B., Zieglgansberger, W., Rammes, G., 2003. Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. Learn. Mem. 10, 116–128.

Azad, S.C., Monory, K., Marsicano, G., Cravatt, B.F., Lutz, B., Zieglgansberger, W., et al., 2004. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. J. Neurosci. 24, 9953–9961.

Azad, S.C., Kurz, J., Marsicano, G., Lutz, B., Zieglgansberger, W., Rammes, G., 2008. Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala. Learn. Mem. 15, 143–152.

Bedse, G., Hartley, N.D., Neale, E., Gaulden, A.D., Patrick, T.A., Kingsley, P.J., et al., 2017. Functional redundancy between canonical endocannabinoid signaling systems in the modulation of anxiety. Biol. Psychiatry 82, 488–499.

Bedse, G., Hill, M.N., Patel, S., 2020. 2-arachidonoylglycerol modulation of anxiety and stress adaptation: from grass roots to novel therapeutics. Biol. Psychiatry 88 (7), 520–530.

Bennett, M.R., Farnell, L., Gibson, W.G., Lagopoulos, J., 2019. A model of amygdala function following plastic changes at specific synapses during extinction. Neurobiol. Stress. 10, 100159.

Blankman, J.L., Simon, G.M., Cravatt, B.F., 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem. Biol. 14, 1347–1356.

Bloodgood, D.W., Sugam, J.A., Holmes, A., Kash, T.L., 2018. Fear extinction requires infralimbic cortex projections to the basolateral amygdala. Transl. Psychiatry 8, 60.

Bluett, R.J., Baldi, R., Haymer, A., Gaulden, A.D., Hartley, N.D., Parrish, W.P., et al., 2017. Endocannabinoid signaling modulates susceptibility to traumatic stress exposure. Nat. Commun. 8, 14782.

Bodor, A.L., Katona, I., Nyiri, G., Mackie, K., Ledent, C., Hajos, N., et al., 2005. Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. J. Neurosci. 25, 6845–6856.

Boileau, I., Tyndale, R.F., Williams, B., Mansouri, E., Westwood, D.J., Le Foll, B., et al., 2015. The fatty acid amide hydrolase C385A variant affects brain binding of the positron emission tomography tracer [11C]CURB. J. Cereb. Blood Flow Metab. 35, 1237–1240.

Bonifacio, M.J., Sousa, F., Aires, C., Loureiro, A.I., Fernandes-Lopes, C., Pires, N.M., et al., 2020. Preclinical pharmacological evaluation of the fatty acid amide hydrolase

inhibitor BIA 10–2474. Br. J. Pharmacol. 177 (9), 2123–2142.
Breder, C.D., Dewitt, D., Kraig, R.P., 1995. Characterization of inducible cyclooxygenase in rat brain. J. Comp. Neurol. 355, 296–315.
Bukalo, O., Pinard, C.R., Silverstein, S., Brehm, C., Hartley, N.D., Whittle, N., et al., 2015. Prefrontal inputs to the amygdala instruct fear extinction memory formation. Sci. Adv. 1.
Burghardt, N.S., Bauer, E.P., 2013. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: implications for underlying fear circuits. Neuroscience 247, 253–272.
Burgos-Robles, A., Vidal-Gonzalez, I., Quirk, G.J., 2009. Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. J. Neurosci. 29, 8474–8482.
Camp, M.C., Macpherson, K.P., Lederle, L., Graybeal, C., Gaburro, S., Debrouse, L.M., et al., 2012. Genetic strain differences in learned fear inhibition associated with variation in neuroendocrine, autonomic, and amygdala dendritic phenotypes. Neuropsychopharmacol. 37, 1534–1547.
Cannich, A., Wotjak, C.T., Kamprath, K., Hermann, H., Lutz, B., Marsicano, G., 2004. CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. Learn. Mem. 11, 625–632.
Cavener, V.S., Gaulden, A., Pennipede, D., Jagasia, P., Uddin, J., Marnett, L.J., et al., 2018. Inhibition of diacylglycerol lipase impairs fear extinction in mice. Front. Neurosci. 12, 479.
Chhatwal, J.P., Davis, M., Maguschak, K.A., Ressler, K.J., 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacol. 30, 516–524.
Childers, S.R., Deadwyler, S.A., 1996. Role of cyclic AMP in the actions of cannabinoid receptors. Biochem. Pharmacol. 52, 819–827.
Courtin, J., Chaudun, F., Rozeske, R.R., Karalis, N., Gonzalez-Campo, C., Wurtz, H., et al., 2014. Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature. 505, 92–96.
Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B., 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 384, 83–87.
Cravatt, B.F., Demarest, K., Patricelli, M.P., Bracey, M.H., Giang, D.K., Martin, B.R., et al., 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc. Natl. Acad. Sci. U. S. A. 98, 9371–9376.
Dainese, E., Oddi, S., Simonetti, M., Sabatucci, A., Angelucci, C.B., Ballone, A., et al., 2020. The endocannabinoid hydrolase FAAH is an allosteric enzyme. Sci. Rep. 10, 2292.
Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C., et al., 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature. 372, 686–691.
Dincheva, I., Drysdale, A.T., Hartley, C.A., Johnson, D.C., Jing, D., King, E.C., et al., 2015. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat. Commun. 6, 6395.
Do-Monte, F.H., Manzano-Nieves, G., Quinones-Laracuente, K., Ramos-Medina, L., Quirk, G.J., 2015. Revisiting the role of infralimbic cortex in fear extinction with optogenetics. J. Neurosci. 35, 3607–3615.
Dudok, B., Barna, L., Ledri, M., Szabo, S.I., Szabadits, E., Pinter, B., et al., 2015. Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. Nat. Neurosci. 18, 75–86.
Egertova, M., Simon, G.M., Cravatt, B.F., Elphick, M.R., 2008. Localization of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: a new perspective on N-acylethanolamines as neural signaling molecules. J. Comp. Neurol. 506, 604–615.
Elmes, M.W., Kaczocha, M., Berger, W.T., Leung, K., Ralph, B.P., Wang, L., et al., 2015. Fatty acid-binding proteins (FABPs) are intracellular carriers for Delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J. Biol. Chem. 290, 8711–8721.
Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarzia, G., et al., 2005. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. J. Pharmacol. Exp. Ther. 313, 352–358.
Fenno, L.E., Mattis, J., Ramakrishnan, C., Hyun, M., Lee, S.Y., He, M., et al., 2014. Targeting cells with single vectors using multiple-feature Boolean logic. Nat. Methods 11, 763–772.
Fidelman, S., Mizrachi Zer-Aviv, T., Lange, R., Hillard, C.J., Akirav, I., 2018. Chronic treatment with URB597 ameliorates post-stress symptoms in a rat model of PTSD. Eur. Neuropsychopharmacol. 28, 630–642.
Fontanez-Nuin, D.E., Santini, E., Quirk, G.J., Porter, J.T., 2011. Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. Cereb. Cortex 21, 727–735.
Fowler, C.J., 2013. Transport of endocannabinoids across the plasma membrane and within the cell. FEBS J. 280, 1895–1904.
Gogolla, N., Caroni, P., Luthi, A., Herry, C., 2009. Perineuronal nets protect fear memories from erasure. Science. 325, 1258–1261.
Gulyas, A.I., Cravatt, B.F., Bracey, M.H., Dinh, T.P., Piomelli, D., Boscia, F., et al., 2004. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur. J. Neurosci. 20, 441–458.
Gunduz-Cinar, O., Hill, M.N., McEwen, B.S., Holmes, A., 2013a. Amygdala FAAH and anandamide: mediating protection and recovery from stress. Trends Pharmacol. Sci. 34, 637–644.
Gunduz-Cinar, O., MacPherson, K.P., Cinar, R., Gamble-George, J., Sugden, K., Williams, B., et al., 2013b. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol. Psychiatry 18, 813–823.
Gunduz-Cinar, O., Flynn, S., Brockway, E., Kaugars, K., Baldi, R., Ramikie, T.S., et al., 2016. Fluoxetine facilitates fear extinction through amygdala endocannabinoids. Neuropsychopharmacol. 41, 1598–1609.
Haj-Dahmane, S., Shen, R.Y., Elmes, M.W., Studholme, K., Kanjiya, M.P., Bogdan, D., et al., 2018. Fatty-acid-binding protein 5 controls retrograde endocannabinoid signaling at central glutamate synapses. Proc. Natl. Acad. Sci. U. S. A. 115, 3482–3487.
Hammoud, M.Z., Peters, C., Hatfield, J.R.B., Gorka, S.M., Phan, K.L., Milad, M.R., et al., 2019. Influence of Delta9-tetrahydrocannabinol on long-term neural correlates of threat extinction memory retention in humans. Neuropsychopharmacol. 44, 1769–1777.
Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., et al., 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell. 148, 1039–1050.
Hardisty, J.F., Harris, S.B., Hayes, W.A., Weber, K., 2020. Oral repeated-dose toxicity studies of BIA 10–2474 in beagle dogs. Regul. Toxicol. Pharmacol. 111, 104555.
Hartley, N.D., Gunduz-Cinar, O., Halladay, L., Bukalo, O., Holmes, A., Patel, S., 2016. 2-arachidonoylglycerol signaling impairs short-term fear extinction. Transl. Psychiatry 6, e749.
Hefner, K., Whittle, N., Juhasz, J., Norcross, M., Karlsson, R.M., Saksida, L.M., et al., 2008. Impaired fear extinction learning and cortico-amygdala circuit abnormalities in a common genetic mouse strain. J. Neurosci. 28, 8074–8085.
Heifets, B.D., Chevaleyre, V., Castillo, P.E., 2008. Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin. Proc. Natl. Acad. Sci. U. S. A. 105, 10250–10255.
Heitland, I., Klumpers, F., Oosting, R.S., Evers, D.J., Leon Kenemans, J., Baas, J.M., 2012. Failure to extinguish fear and genetic variability in the human cannabinoid receptor 1. Transl. Psychiatry 2, e162.
Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., et al., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–1936.
Hill, M.N., McLaughlin, R.J., Morrish, A.C., Viau, V., Floresco, S.B., Hillard, C.J., et al., 2009. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacol. 34, 2733–2745.
Hill, M.N., Bierer, L.M., Makotkine, I., Golier, J.A., Galea, S., McEwen, B.S., et al., 2013a. Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the world trade center attacks. Psychoneuroendocrinology. 38, 2952–2961.
Hill, M.N., Kumar, S.A., Filipski, S.B., Iverson, M., Stuhr, K.L., Keith, J.M., et al., 2013b. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol. Psychiatry 18, 1125–1135.
Hill, M.N., Campolongo, P., Yehuda, R., Patel, S., 2018. Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. Neuropsychopharmacol. 43, 80–102.
Hillard, C.J., 2018. Circulating endocannabinoids: from whence do they come and where are they going? Neuropsychopharmacol. 43, 155–172.
Howlett, A.C., Qualy, J.M., Khachatrian, L.L., 1986. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. 29, 307–313.
Hu, S.S., Mackie, K., 2015. Distribution of the endocannabinoid system in the central nervous system. Handb. Exp. Pharmacol. 231, 59–93.
Ilyasov, A.A., Milligan, C.E., Pharr, E.P., Howlett, A.C., 2018. The endocannabinoid system and oligodendrocytes in health and disease. Front. Neurosci. 12, 733.
Jenniches, I., Ternes, S., Albayram, O., Otte, D.M., Bach, K., Bindila, L., et al., 2016. Anxiety, stress, and fear response in mice with reduced endocannabinoid levels. Biol. Psychiatry 79, 858–868.
Kaczocha, M., Glaser, S.T., Deutsch, D.G., 2009. Identification of intracellular carriers for the endocannabinoid anandamide. Proc. Natl. Acad. Sci. U. S. A. 106, 6375–6380.
Kaczocha, M., Rebecchi, M.J., Ralph, B.P., Teng, Y.H., Berger, W.T., Galbavy, W., et al., 2014. Inhibition of fatty acid binding proteins elevates brain anandamide levels and produces analgesia. PLoS One e94200, 9.
Kamprath, K., Plendl, W., Marsicano, G., Deussing, J.M., Wurst, W., Lutz, B., et al., 2009. Endocannabinoids mediate acute fear adaptation via glutamatergic neurons independently of corticotropin-releasing hormone signaling. Genes Brain Behav. 8, 203–211.
Karpova, N.N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Agustsdottir, A., et al., 2011. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science. 334, 1731–1734.
Kasugai, Y., Vogel, E., Hortnagl, H., Schonherr, S., Paradiso, E., Hauschild, M., et al., 2019. Structural and functional remodeling of amygdala GABAergic synapses in associative fear learning. Neuron. 104, 781–794 e4.
Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., et al., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. Nat. Med. 9, 76–81.
Katona, I., Freund, T.F., 2008. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat. Med. 14, 923–930.
Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., et al., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.
Katona, I., Rancz, E.A., Acsady, L., Ledent, C., Mackie, K., Hajos, N., et al., 2001. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J. Neurosci. 21, 9506–9518.
Kida, S., 2020. Function and mechanisms of memory destabilization and reconsolidation after retrieval. Proc. Jpn Acad. Ser. B Phys. Biol. Sci. 96, 95–106.
Kofuji, P., Araque, A., 2020. G-protein-coupled receptors in astrocyte-neuron communication. Neuroscience. https://doi.org/10.1016/j.neuroscience.2020.03.025. In

press.
Kozak, K.R., Rowlinson, S.W., Marnett, L.J., 2000. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J. Biol. Chem. 275, 33744–33749.
Kramar, C., Loureiro, M., Renard, J., Laviolette, S.R., 2017. Palmitoylethanolamide modulates GPR55 receptor signaling in the ventral hippocampus to regulate mesolimbic dopamine activity, social interaction, and memory processing. Cannabis Cannabinoid Res. 2, 8–20.
Laricchiuta, D., Centonze, D., Petrosini, L., 2013. Effects of endocannabinoid and endovanilloid systems on aversive memory extinction. Behav. Brain Res. 256, 101–107.
Leishman, E., Mackie, K., Luquet, S., Bradshaw, H.B., 2016. Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. Biochim. Biophys. Acta 1861, 491–500.
Lin, H.C., Mao, S.C., Gean, P.W., 2006. Effects of intra-amygdala infusion of CB1 receptor agonists on the reconsolidation of fear-potentiated startle. Learn. Mem. 13, 316–321.
Lin, H.C., Mao, S.C., Chen, P.S., Gean, P.W., 2008. Chronic cannabinoid administration in vivo compromises extinction of fear memory. Learn. Mem. 15, 876–884.
Lin, H.C., Mao, S.C., Su, C.L., Gean, P.W., 2009. The role of prefrontal cortex CB1 receptors in the modulation of fear memory. Cereb. Cortex 19, 165–175.
Lisboa, S.F., Gomes, F.V., Guimaraes, F.S., Campos, A.C., 2016. Microglial cells as a link between cannabinoids and the immune hypothesis of psychiatric disorders. Front. Neurol. 7, 5.
Liu, J., Wang, L., Harvey-White, J., Osei-Hyiaman, D., Razdan, R., Gong, Q., et al., 2006. A biosynthetic pathway for anandamide. Proc. Natl. Acad. Sci. U. S. A. 103, 13345–13350.
Llorente-Berzal, A., Terzian, A.L., di Marzo, V., Micale, V., Viveros, M.P., Wotjak, C.T., 2015. 2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons. Psychopharmacology 232, 2811–2825.
Luchkina, N.V., Bolshakov, V.Y., 2019. Mechanisms of fear learning and extinction: synaptic plasticity-fear memory connection. Psychopharmacology 236, 163–182.
Lutz, B., Marsicano, G., Maldonado, R., Hillard, C.J., 2015. The endocannabinoid system in guarding against fear, anxiety and stress. Nat. Rev. Neurosci. 16, 705–718.
Mallet, C., Dubray, C., Duale, C., 2016. FAAH inhibitors in the limelight, but regrettably. Int. J. Clin. Pharmacol. Ther. 54, 498–501.
Marcus, D.J., Bedse, G., Gaulden, A.D., Ryan, J.D., Kondev, V., Winters, N.D., et al., 2020. Endocannabinoid signaling collapse mediates stress-induced amygdalo-cortical strengthening. Neuron 105 (6), 1062–1076.
Marek, R., Jin, J., Goode, T.D., Giustino, T.F., Wang, Q., Acca, G.M., et al., 2018a. Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. Nat. Neurosci. 21, 384–392.
Marek, R., Xu, L., Sullivan, R.K.P., Sah, P., 2018b. Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. Nat. Neurosci. 21, 654–658.
Maren, S., Phan, K.L., Liberzon, I., 2013. The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat. Rev. Neurosci. 14, 417–428.
Marrs, W.R., Blankman, J.L., Horne, E.A., Thomazeau, A., Lin, Y.H., Coy, J., et al., 2010. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat. Neurosci. 13, 951–957.
Marsch, R., Foeller, E., Rammes, G., Bunck, M., Kossl, M., Holsboer, F., et al., 2007. Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J. Neurosci. 27, 832–839.
Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., et al., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 418, 530–534.
Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., et al., 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science. 302, 84–88.
Martin-Fernandez, M., Jamison, S., Robin, L.M., Zhao, Z., Martin, E.D., Aguilar, J., et al., 2017. Synapse-specific astrocyte gating of amygdala-related behavior. Nat. Neurosci. 20, 1540–1548.
Mato, S., Lafourcade, M., Robbe, D., Bakiri, Y., Manzoni, O.J., 2008. Role of the cyclic-AMP/PKA cascade and of P/Q-type Ca++ channels in endocannabinoid-mediated long-term depression in the nucleus accumbens. Neuropharmacology. 54, 87–94.
Mayo, L.M., Asratian, A., Linde, J., Holm, L., Natt, D., Augier, G., et al., 2018. Protective effects of elevated anandamide on stress and fear-related behaviors: translational evidence from humans and mice. In: Mol Psychiatry.
Mayo, L.M., Asratian, A., Linde, J., Morena, M., Haataja, R., Hammar, V., et al., 2020. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: a randomized, controlled experimental medicine trial. Biol. Psychiatry 87, 538–547.
McCullough, K.M., Morrison, F.G., Ressler, K.J., 2016. Bridging the gap: towards a cell-type specific understanding of neural circuits underlying fear behaviors. Neurobiol. Learn. Mem. 135, 27–39.
McDonald, A.J., Mascagni, F., 2001. Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. Neuroscience. 107, 641–652.
McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N. Engl. J. Med. 338, 171–179.
Metna-Laurent, M., Soria-Gomez, E., Verrier, D., Conforzi, M., Jego, P., Lafenetre, P., et al., 2012. Bimodal control of fear-coping strategies by CB(1) cannabinoid receptors. J. Neurosci. 32, 7109–7118.
Meyer, H.C., Odriozola, P., Cohodes, E.M., Mandell, J.D., Li, A., Yang, R., et al., 2019. Ventral hippocampus interacts with prelimbic cortex during inhibition of threat response via learned safety in both mice and humans. Proc. Natl. Acad. Sci. U. S. A. 116(52):26970-26979 116 (52), 26970–26979.
Milad, M.R., Quirk, G.J., 2012. Fear extinction as a model for translational neuroscience: ten years of progress. Annu. Rev. Psychol. 63, 129–151.
Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol. Psychiatry 62, 446–454.
Mock, E.D., Mustafa, M., Gunduz-Cinar, O., Cinar, R., Petrie, G.N., Kantae, V., et al., 2020. Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. Nat. Chem. Biol. 16(6):667-675 16 (6), 667–675.
Molina-Holgado, E., Vela, J.M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J., et al., 2002. Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. J. Neurosci. 22, 9742–9753.
Monory, K., Massa, F., Egertova, M., Eder, M., Blaudzun, H., Westenbroek, R., et al., 2006. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron. 51, 455–466.
Morena, M., Berardi, A., Colucci, P., Palmery, M., Trezza, V., Hill, M.N., et al., 2018. Enhancing endocannabinoid neurotransmission augments the efficacy of extinction training and ameliorates traumatic stress-induced behavioral alterations in rats. Neuropsychopharmacol. 43, 1284–1296.
Morena, M., Aukema, R.J., Leitl, K.D., Rashid, A.J., Vecchiarelli, H.A., Josselyn, S.A., et al., 2019. Upregulation of anandamide hydrolysis in the basolateral complex of amygdala reduces fear memory expression and indices of stress and anxiety. J. Neurosci. 39, 1275–1292.
Navarrete, M., Araque, A., 2008. Endocannabinoids mediate neuron-astrocyte communication. Neuron. 57, 883–893.
Navarrete, M., Araque, A., 2010. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron. 68, 113–126.
Navarrete, M., Diez, A., Araque, A., 2014. Astrocytes in endocannabinoid signaling. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 369, 20130599.
Neumeister, A., Normandin, M.D., Pietrzak, R.H., Piomelli, D., Zheng, M.Q., Gujarro-Anton, A., et al., 2013. Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. Mol. Psychiatry 18, 1034–1040.
Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., Ueda, N., 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. J. Biol. Chem. 279, 5298–5305.
Oliveira da Cruz, J.F., Robin, L.M., Drago, F., Marsicano, G., Metna-Laurent, M., 2016. Astroglial type-1 cannabinoid receptor (CB1): a new player in the tripartite synapse. Neuroscience 323, 35–42.
Owada, Y., Abdelwahab, S.A., Kitanaka, N., Sakagami, H., Takano, H., Sugitani, Y., et al., 2006. Altered emotional behavioral responses in mice lacking brain-type fatty acid-binding protein gene. Eur. J. Neurosci. 24, 175–187.
Padilla-Coreano, N., Bolkan, S.S., Pierce, G.M., Blackman, D.R., Hardin, W.D., Garcia-Garcia, A.L., et al., 2016. Direct ventral hippocampal-prefrontal input is required for anxiety-related neural activity and behavior. Neuron. 89, 857–866.
Pamplona, F.A., Bitencourt, R.M., Takahashi, R.N., 2008. Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. Neurobiol. Learn. Mem. 90, 290–293.
Patel, S., Roelke, C.T., Rademacher, D.J., Hillard, C.J., 2005. Inhibition of restraint stress-induced neural and behavioral activation by endogenous cannabinoid signaling. Eur. J. Neurosci. 21, 1057–1069.
Patel, S., Kingsley, P.J., Mackie, K., Marnett, L.J., Winder, D.G., 2009. Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. Neuropsychopharmacol. 34, 2699–2709.
Pavlov, I.P., Anrep, G.V., 1927. Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex. Oxford University Press, London.
Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E., 2004. Extinction learning in humans: role of the amygdala and vmPFC. Neuron. 43, 897–905.
Popova, D., Agustsdottir, A., Lindholm, J., Mazulis, U., Akamine, Y., Castren, E., et al., 2014. Combination of fluoxetine and extinction treatments forms a unique synaptic protein profile that correlates with long-term fear reduction in adult mice. Eur. Neuropsychopharmacol. 24, 1162–1174.
Provensi, G., Fabbri, R., Munari, L., Costa, A., Baldi, E., Bucherelli, C., et al., 2017. Histaminergic neurotransmission as a gateway for the cognitive effect of oleoylethanolamide in contextual fear conditioning. Int. J. Neuropsychopharmacol. 20, 392–399.
Puente, N., Cui, Y., Lassalle, O., Lafourcade, M., Georges, F., Venance, L., et al., 2011. Polymodal activation of the endocannabinoid system in the extended amygdala. Nat. Neurosci. 14, 1542–1547.
Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacol. 33, 56–72.
Quirk, G.J., Russo, G.K., Barron, J.L., Lebron, K., 2000. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. J. Neurosci. 20, 6225–6231.
Rabinak, C.A., Angstadt, M., Sripada, C.S., Abelson, J.L., Liberzon, I., Milad, M.R., et al., 2013. Cannabinoid facilitation of fear extinction memory recall in humans. Neuropharmacology. 64, 396–402.
Rabinak, C.A., Angstadt, M., Lyons, M., Mori, S., Milad, M.R., Liberzon, I., et al., 2014. Cannabinoid modulation of prefrontal-limbic activation during fear extinction learning and recall in humans. Neurobiol. Learn. Mem. 113, 125–134.
Rabinak, C.A., Blanchette, A., Zabik, N.L., Peters, C., Marusak, H.A., Iadipaolo, A., et al., 2020. Cannabinoid modulation of corticolimbic activation to threat in trauma-exposed adults: a preliminary study. Psychopharmacology 237, 1813–1826.
Rademacher, D.J., Meier, S.E., Shi, L., Ho, W.S., Jarrahian, A., Hillard, C.J., 2008. Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. Neuropharmacology. 54,

108–116.
Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., et al., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.
Remmers, F., Lange, M.D., Hamann, M., Ruehle, S., Pape, H.C., Lutz, B., 2017. Addressing sufficiency of the CB1 receptor for endocannabinoid-mediated functions through conditional genetic rescue in forebrain GABAergic neurons. Brain Struct. Funct. 222, 3431–3452.
Rhee, M.H., Bayewitch, M., Avidor-Reiss, T., Levy, R., Vogel, Z., 1998. Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. J. Neurochem. 71, 1525–1534.
Roche, M., O'Connor, E., Diskin, C., Finn, D.P., 2007. The effect of CB(1) receptor antagonism in the right basolateral amygdala on conditioned fear and associated analgesia in rats. Eur. J. Neurosci. 26, 2643–2653.
Rovira-Esteban, L., Gunduz-Cinar, O., Bukalo, O., Limoges, A., Brockway, E., Muller, K., et al., 2019. Excitation of diverse classes of cholecystokinin interneurons in the basal amygdala facilitates fear extinction. eNeuro. 6.
Ruehle, S., Remmers, F., Romo-Parra, H., Massa, F., Wickert, M., Wortge, S., et al., 2013. Cannabinoid CB1 receptor in dorsal telencephalic glutamatergic neurons: distinctive sufficiency for hippocampus-dependent and amygdala-dependent synaptic and behavioral functions. J. Neurosci. 33, 10264–10277.
Santana, F., Sierra, R.O., Haubrich, J., Crestani, A.P., Duran, J.M., de Freitas, Cassini L., et al., 2016. Involvement of the infralimbic cortex and CA1 hippocampal area in reconsolidation of a contextual fear memory through CB1 receptors: effects of CP55,940. Neurobiol. Learn. Mem. 127, 42–47.
Santini, E., Muller, R.U., Quirk, G.J., 2001. Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. J. Neurosci. 21, 9009–9017.
Santini, E., Ge, H., Ren, K., Pena de Ortiz, S., Quirk, G.J., 2004. Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. J. Neurosci. 24, 5704–5710.
Savinainen, J.R., Saario, S.M., Laitinen, J.T., 2012. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signaling through cannabinoid receptors. Acta Physiol (Oxford) 204, 267–276.
Sbarski, B., Akirav, I., 2020. Cannabinoids as therapeutics for PTSD. Pharmacol. Ther. 107551.
Schaefer, C., Enning, F., Mueller, J.K., Bumb, J.M., Rohleder, C., Odorfer, T.M., et al., 2014. Fatty acid ethanolamide levels are altered in borderline personality and complex posttraumatic stress disorders. Eur. Arch. Psychiatry Clin. Neurosci. 264, 459–463.
Senn, V., Wolff, S.B., Herry, C., Grenier, F., Ehrlich, I., Grundemann, J., et al., 2014. Long-range connectivity defines behavioral specificity of amygdala neurons. Neuron. 81, 428–437.
Shin, L.M., Wright, C.I., Cannistraro, P.A., Wedig, M.M., McMullin, K., Martis, B., et al., 2005. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch. Gen. Psychiatry 62, 273–281.
Sierra-Mercado, D., Corcoran, K.A., Lebron-Milad, K., Quirk, G.J., 2006. Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. Eur. J. Neurosci. 24, 1751–1758.
Sierra-Mercado, D., Padilla-Coreano, N., Quirk, G.J., 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacol. 36, 529–538.
Simon, G.M., Cravatt, B.F., 2006. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. J. Biol. Chem. 281, 26465–26472.
Sipe, J.C., Chiang, K., Gerber, A.L., Beutler, E., Cravatt, B.F., 2002. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc. Natl. Acad. Sci. U. S. A. 99, 8394–8399.
Sipe, J.C., Scott, T.M., Murray, S., Harismendy, O., Simon, G.M., Cravatt, B.F., et al., 2010. Biomarkers of endocannabinoid system activation in severe obesity. PLoS One 5, e8792.
Smith, N.A., Bekar, L.K., Nedergaard, M., 2020. Astrocytic endocannabinoids mediate hippocampal transient heterosynaptic depression. Neurochem. Res. 45, 100–108.
Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., Quirk, G.J., 2012. Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. Neuron. 76, 804–812.
Spagnolo, P.A., Ramchandani, V.A., Schwandt, M.L., Kwako, L.E., George, D.T., Mayo, L.M., et al., 2016. FAAH gene variation moderates stress response and symptom severity in patients with posttraumatic stress disorder and comorbid alcohol dependence. Alcohol. Clin. Exp. Res. 40, 2426–2434.
Stehberg, J., Moraga-Amaro, R., Salazar, C., Becerra, A., Echeverria, C., Orellana, J.A., et al., 2012. Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. FASEB J. 26, 3649–3657.
Sumislawski, J.J., Ramikie, T.S., Patel, S., 2011. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. Neuropsychopharmacol. 36, 2750–2761.
Taniguchi, H., He, M., Wu, P., Kim, S., Paik, R., Sugino, K., et al., 2011. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. Neuron. 71, 995–1013.
Tripathi, R.K.P., 2020. A perspective review on fatty acid amide hydrolase (FAAH) inhibitors as potential therapeutic agents. Eur. J. Med. Chem. 188, 111953.
Trouche, S., Sasaki, J.M., Tu, T., Reijmers, L.G., 2013. Fear extinction causes target-specific remodeling of perisomatic inhibitory synapses. Neuron. 80, 1054–1065.
Twitchell, W., Brown, S., Mackie, K., 1997. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J. Neurophysiol. 78, 43–50.
Ueda, N., Yamamoto, K., Yamamoto, S., Tokunaga, T., Shirakawa, E., Shinkai, H., et al., 1995. Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. Biochim. Biophys. Acta 1254, 127–134.
van Esbroeck, A.C.M., Janssen, A.P.A., Cognetta III, A.B., Ogasawara, D., Shpak, G., van der Kroeg, M., et al., 2017. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. Science. 356, 1084–1087.
VanElzakker, M.B., Dahlgren, M.K., Davis, F.C., Dubois, S., Shin, L.M., 2014. From Pavlov to PTSD: the extinction of conditioned fear in rodents, humans, and anxiety disorders. Neurobiol. Learn. Mem. 113, 3–18.
Varvel, S.A., Anum, E.A., Lichtman, A.H., 2005. Disruption of CB(1) receptor signaling impairs extinction of spatial memory in mice. Psychopharmacology 179, 863–872.
Varvel, S.A., Wise, L.E., Niyuhire, F., Cravatt, B.F., Lichtman, A.H., 2007. Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. Neuropsychopharmacol. 32, 1032–1041.
Vogel, E., Krabbe, S., Grundemann, J., Wamsteeker Cusulin, J.I., Luthi, A., 2016. Projection-specific dynamic regulation of inhibition in amygdala micro-circuits. Neuron. 91, 644–651.
Wang, Q., Jin, J., Maren, S., 2016. Renewal of extinguished fear activates ventral hippocampal neurons projecting to the prelimbic and infralimbic cortices in rats. Neurobiol. Learn. Mem. 134 Pt A, 38–43.
Yabuki, Y., Takahata, I., Matsuo, K., Owada, Y., Fukunaga, K., 2018. Ramelteon improves post-traumatic stress disorder-like behaviors exhibited by fatty acid-binding protein 3 null mice. Mol. Neurobiol. 55, 3577–3591.
Yoshida, T., Uchigashima, M., Yamasaki, M., Katona, I., Yamazaki, M., Sakimura, K., et al., 2011. Unique inhibitory synapse with particularly rich endocannabinoid signaling machinery on pyramidal neurons in basal amygdaloid nucleus. Proc. Natl. Acad. Sci. U. S. A. 108, 3059–3064.
Yu, S., Levi, L., Casadesus, G., Kunos, G., Noy, N., 2014. Fatty acid-binding protein 5 (FABP5) regulates cognitive function both by decreasing anandamide levels and by activating the nuclear receptor peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) in the brain. J. Biol. Chem. 289, 12748–12758.