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Cannabinoid interventions for PTSD: Where to next?

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Abstract

Cannabinoids are a promising method for pharmacological treatment of post-traumatic stress disorder (PTSD). Despite considerable research devoted to the effect of cannabinoid modulation on PTSD symptomology, there is not a currently agreed way by which the cannabinoid system should be targeted in humans. In this review, we present an overview of recent research identifying neurological pathways by which different cannabinoid-based treatments may exert their effects on PTSD symptomology. We evaluate the strengths and weaknesses of each of these different approaches, including recent challenges presented to favourable options such as fatty acid amide hydrolase (FAAH) inhibitors. This article makes the strengths and challenges of different potential cannabinoid treatments accessible to psychological researchers interested in cannabinoid therapeutics and aims to aid selection of appropriate tools for future clinical trials.

Keywords: endocannabinoids; posttraumatic stress disorder; translational medical research; cannabis; psychiatric disorders; traumatic stress; cannabinoids.
1. Introduction

1.1 Post-traumatic Stress Disorder

Post-traumatic Stress Disorder (PTSD) is a serious mental health condition that may develop following a traumatic experience. Symptoms include re-experiencing distressing aspects of the trauma, hyper-arousal, avoidance of trauma reminders, sleep disturbances and negative cognitions and mood (American Psychiatric Association, 2013). PTSD can be a chronic condition and is associated with significant distress and functional impairments. The gold-standard psychological treatment for PTSD is exposure therapy, where patients are encouraged to gradually confront reminders of their trauma, enabling them to learn to regulate their fear of the no-longer threatening situation (Graham, Callaghan, & Richardson, 2014). Using this technique, many PTSD patients experience a significant reduction in their symptoms. Unfortunately, approximately 40% of patients experience only minimal or partial response to exposure treatment (Bisson, Roberts, Andrew, Cooper, & Lewis, 2013), hence the focus of PTSD research is often to enhance the outcomes of exposure therapy. The use of pharmacological adjuncts to optimize exposure therapy and enhance treatment response has been a recent area of active research, though has yet to emerge with a promising pharmaceutical for this purpose (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015; Stein, Ipser, & Seedat, 2006).

1.1.1 PTSD Symptomology and Mechanisms

PTSD symptomology is believed to be largely maintained by a combination of dysregulated biological stress responding and maladaptive memory processes attributable to the extreme stress caused by a traumatic event (Pitman et al., 2012; Yehuda et al., 2015; Zuj, Palmer, Hsu, et al., 2016). At the time of trauma, sympathetic and central stress hormones (for example noradrenaline and cortisol) are released in excessive levels as part of the stress
response. Excessive release of these hormones assigns emotional salience to the events, which in turn leads to over-consolidation of the trauma and associated environment or context in which the trauma occurs (Pitman & Delahanty, 2005). This is achieved by a well-known mechanism by which emotional experiences are preferentially consolidated into long-term memory (McGaugh & Roozendaal, 2002; Roozendaal & McGaugh, 2011); however, in PTSD this occurs at an extreme and maladaptive level due in part to the excessive release of stress hormones (Pitman & Delahanty, 2005; Pitman et al., 2012). Over-consolidation of trauma memories leads to fractured and poorly contextualised memory traces, which may spontaneously arise post-trauma and are known as intrusive memories (Brewin, Gregory, Lipton, & Burgess, 2010; Pitman et al., 2012). Studies in humans have shown that interactions between noradrenaline and cortisol can predict the number of intrusive memories following exposure to emotional or traumatic images (Bryant, McGrath, & Felmingham, 2013; Chou, La Marca, Steptoe, & Brewin, 2014; Nicholson, Bryant, & Felmingham, 2014); however, it is clear that not all people exposed to a traumatic event will develop these symptoms. Therefore, PTSD researchers are often interested in understanding predisposing factors that determine whether someone will develop symptoms such as emotional or intrusive memories following trauma.

Following trauma, a significant proportion of persons who eventually develop PTSD show chronically decreased cortisol levels, which is due to increased negative feedback of the hypothalamic-pituitary-adrenal (HPA) axis (Galatzer-Levy, Ma, Statnikov, Yehuda, & Shalev, 2017; Yehuda, 2009). This means that PTSD subjects show decreased cortisol reactivity to stress (Griffin, Resick, & Yehuda, 2005; Stein, Yehuda, Koverola, & Hanna, 1997). Conversely, chronic noradrenergic hyperactivity has been reported in PTSD subjects and is believed, along with disruption to enhanced reduction in natural HPA reactivity, to contribute to hyperarousal features of the disorder (Pitman et al., 2012; Southwick et al., 1999).
Following trauma, there is also believed to be a deficient in what is termed “fear extinction” in the aetiology and maintenance of PTSD (Milad & Quirk, 2012; Zuj, Palmer, Lommen, & Felmingham, 2016). Fear extinction is part of a larger framework based on classical conditioning, whereby “fear” – whether it be purely physiological and unconscious or entirely conscious – is acquired during a stressful or traumatic experience. In the most common model of fear learning, fear of a stimulus or a situation is maintained via a process called associative learning, where an aversive experience in the past may provoke fearful responses to an otherwise benign stimulus or situation well into the future (LeDoux, 2014). In PTSD, fear learning occurs at the time of trauma and sustained fear of situations, stimuli and other reminders associated with the trauma contribute to the bulk of PTSD symptoms, including hyperarousal, situational avoidance and intrusive memories (LeDoux, 2014; Milad & Quirk, 2012; Shechner, Hong, Britton, Pine, & Fox, 2014). In contrast to fear learning, “fear extinction learning” describes the process by which a previously threatening or aversive situation or stimulus is learnt to be no longer threatening or aversive; this occurs through multiple experiences where the situation or stimulus is not paired with any aversive outcome. Fear extinction learning, then, describes the extinction of the fear memory and is the result of safety learning superseding the fear memory (Bouton, 2004; Milad & Quirk, 2012). Similarly, “fear extinction recall” refers to the ability to retain fear extinction learning over a prolonged period of time; often the fear memory will resurface (known as return of fear) and the extinction memory must be learnt again.

Critically, it is proposed that both fear extinction learning and fear extinction recall are inversely compromised in PTSD (Parsons & Hurd, 2015; Pitman et al., 2012; Zuj, Palmer, Lommen, et al., 2016). Firstly, standardised paradigms designed to test the efficacy of fear extinction processes have shown that PTSD subjects display poorer fear extinction learning and recall than controls (Milad et al., 2009; Norrholm et al., 2011; Shvil et al., 2014; Zuj,
Palmer, Hsu, et al., 2016). Secondly, efficacy of fear extinction prior to trauma exposure is correlated with the severity of PTSD symptoms post-trauma (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013). Finally, in the classical model of fear extinction learning, extinction memories are formed via a neural circuit involving the ventral medial prefrontal cortex (vmPFC), hippocampus and amygdala (LeDoux, 2014; LeDoux, Iwata, Cicchetti, & Reis, 1988; Phelps, Delgado, Nearing, & LeDoux, 2004). In this model, fear extinction is achieved by successful inhibitory signals from the higher-order vmPFC region attenuating fear signals from the amygdala. Similarly, contextual information of the fear memory stored in the hippocampus must also be over-ridden by extinction learning. Notably, it has been demonstrated using fMRI in humans that fear extinction is most successful with increased vmPFC volume and activation (Milad et al., 2009; Milad et al., 2005; Milad et al., 2007). Similarly, vmPFC and hippocampus activity is higher in healthy controls compared to PTSD patients during extinction recall (Milad, Pitman, et al., 2009). Moreover, cortisol reactivity is essential for fear extinction learning (Rodrigues, LeDoux, & Sapolsky, 2009), which is important given the lowered levels of cortisol production in PTSD subjects (Pitman et al., 2012; Yehuda, 2009).

1.2 The Endocannabinoid System

Recently, the endogenous cannabinoid (endocannabinoid) system has been recognised as a promising target for PTSD treatment (Hill, Campolongo, Yehuda, & Patel, 2018; Neumeister, Seidel, Ragen, & Pietrzak, 2015; Steenkamp, Blessing, Galatzer-Levy, Hollahan, & Anderson, 2017; Trezza & Campolongo, 2013; Zer-Aviv, Segev, & Akirav, 2016). The endocannabinoid system is comprised of distinct cannabinoid receptors (CB1 and CB2), as well as endogenous cannabinoid ligands, namely arachidonoyl ethanolamine (AEA; Devane, et al. 1992) and 2-arachidonoyl glycerol (2-AG; Mechoulam et al. 1995; Suiguiria et al., 1995).
AEA and 2-AG are derivatives of arachidonic acid, and are metabolised by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Howlett et al., 2002). Exogenous phytocannabinoids, such as delta9-tetrahydrocannabinol (THC) and cannabidiol (CBD) found in cannabis, are also effectors of the endocannabinoid system; though CBD is thought to exert its effects primarily through non-endocannabinoid pathways such as serotonin and adenosine receptors (McPartland, Duncan, Di Marzo, & Pertwee, 2015). Cannabinoid receptors are profusely located across both the central and peripheral nervous systems and are heavily involved in many neuro-modulatory functions through retrograde signalling (Howlett et al., 2002; Kano, Ohno-Shosaku, Hashimoto-dani, Uchigashima, & Watanabe, 2009). In addition to the cannabinoid receptors, cannabinoid ligands also show affinity for transient receptor potential vanilloid (TRPV) channels, G-protein coupled receptors 55, 18 and 119 (GPR55, GPR18, GPR119), glycine receptors and peroxisome proliferator-activated receptors, as well as other targets such as the opioid and serotonin systems (De Petrocellis & Di Marzo, 2010; Ligresti, De Petrocellis, & Di Marzo, 2016; Morales, Hurst, & Reggio, 2017). The endocannabinoid system is involved in a plethora of functions, including sleeping, eating, immune function, pleasure and emotional homeostasis (Kano et al., 2009; Mechoulam, & Parker, 2013).

1.2.1 Endocannabinoids and PTSD

Recently, the endocannabinoid system has also been recognised as central to stress responses, emotional memories and fear extinction (Hill et al., 2018; Morena, Patel, Bains, & Hill, 2016; Ney, Matthews, Bruno, & Felmingham, 2018), which represent important therapeutic targets. AEA levels tonically gate the stress response through the HPA axis, and rapidly decrease following acute stress via FAAH activation (Gray et al., 2015; Gunduz-Cinar et al., 2013; Hill et al., 2009; Morena et al., 2016; Natividad et al., 2017), allowing
activation of the HPA stress response. The resulting increase in glucocorticoids mobilizes 2-AG, which mediates glucocorticoid-driven negative feedback and termination of the stress response (Bedse et al., 2017; Di et al., 2016; Evanson, Tasker, Hill, Hillard, & Herman, 2010; Hill et al., 2011; Morena et al., 2016). Hence, the endocannabinoid system is a crucial mediator of the HPA response under stress. Consequently, symptoms in PTSD that are a product of maladaptive stress responding such as intrusive memories, hyperarousal and poor sleep quality also appear to be facilitated by decreased endocannabinoid signalling (Hill et al., 2018). Most importantly, preclinical models show that fear extinction – central to exposure therapy itself – is facilitated by enhanced endocannabinoid signalling and reduced by endocannabinoid inhibition (Aisenberg, Serova, Sabban, & Akirav, 2017; Bitencourt, Pamplona, & Takahashi, 2008; Do Monte, Souza, Bitencourt, Kroon, & Takahashi, 2013). Accordingly, PTSD has been associated with decreased endocannabinoid concentrations in war veterans (Wilker et al., 2016) and following the World Trade Centre attacks (Hill et al., 2013), implying that endocannabinoids are involved in trauma responses in humans.

Although the science concerning the contribution of endocannabinoids to PTSD is robust and well-accepted (reviewed in Hill, et al. 2017; Morena et al. 2016; Ney, et al. 2018), utilisation of this knowledge is still in its beginning stages. For instance, there is to date only one published double-blind, cross-over randomised controlled trial where ten PTSD patients were given nabilone to improve nightmare symptomology (Jetly, Heber, Fraser, & Boisvert, 2015). Similarly, only a few other studies have administered cannabinoids in open-label prospective (Roitman, Mechoulam, Cooper-Kazaz, & Shalev, 2014) or retrospective studies to PTSD patients (Cameron, Watson, & Robinson, 2014; Elms, Shannon, Hughes, & Lewis, 2018; Fraser, 2009), or in experimental studies to healthy participants (Das et al., 2013; Klumpers et al., 2012; Rabinak et al., 2014; Rabinak et al., 2013). Generally, the results of studies involving standardized extinction learning and recall tasks in healthy humans have
been promising. However, there is a current need for randomised controlled trials where PTSD patients’ symptomology is measured during and after cannabinoid therapy.

1.2.2 Challenges to Cannabinoid-Based PTSD Treatment

Aside from the tentative legality of cannabis-based products for medical purposes in most of the Western world, there are several barriers to testing therapeutic outcomes of cannabinoids for PTSD patients. Firstly, there are demonstrable adverse health risks associated with whole-plant cannabis use, with chronic recreational use associated with dependence and decreased mental health across a number of domains including cognition and risk of psychosis (Hall & Degenhardt, 2009; Moore et al., 2007). Similarly, chronic use of marijuana is associated with global cortical downregulation of CB1 receptor availability, as well as across regions such as the temporal lobe, nucleus accumbens and cingulate cortex (Ceccarini et al., 2015; Hirvonen et al., 2012). Downregulation of cannabinoid receptors results in reduced endogenous cannabinoid functioning, reflecting the tolerance and dependence effect chronic use on the endogenous system. Increased understanding of appropriate dosing levels must be further established before definitive clinical trials can begin, and this may increasingly reflect a move away from whole plant products due to difficulty in regulating levels of the active compounds in this form of the product. Further, the endocannabinoid system acts in different ways even in the same disease states (Di Marzo, 2008), meaning that for many conditions the direction in which the system should be modulated is unclear and upstream options for treatment should also be researched. There is potential that this problem might ultimately reflect endocannabinoids being a downstream focus for PTSD, as in schizophrenia where the traditional dopamine hypothesis as a treatment point is more recently being challenged by upstream targets such as glutamate (Howes & Kapur, 2009; Moghaddam & Javitt, 2012).
1.3 Focus of this Review

There is also indecision amongst researchers as to the best pharmacological avenue to pursue. Modulation of the endocannabinoid system can theoretically occur through multiple means, and each of these methods has been reviewed previously (Di Marzo, 2008; Micale, Di Marzo, Sukova, Wotjak, & Drago, 2013). For the remainder of this review, we will consider and compare each of the main ways in which pharmaceuticals may modulate the endocannabinoid system in a PTSD context, with reference to the strengths and weaknesses of each approach. These approaches will be limited to the primary methods used in human and animal research to achieve direct or indirect agonism of cannabinoid receptors and inhibition of degradation or reuptake of active cannabinoid ligands. Previous reviews have collated studies in the broader spectrum of anxiety research (Di Marzo, 2008; Micale et al., 2013) as well as in PTSD (Berardi, Schelling, & Campolongo, 2016), though there have been several important developments in recent years that may have changed the direction in which this field is going. This review is therefore not intended to be an exhaustive review of the studies conducted using each methodology, or by any means of the chemistry or pharmacological studies underlying each technique, but will provide essential information and updates on types of cannabinoid treatments under development specifically for PTSD. Interested readers should note that previous reviews (Berardi, et al. 2016; Micale, et al. 2013) outline a wider range of neurochemical interactions between the endocannabinoid and various other systems in PTSD mechanisms and symptoms; here we restrict our review of the literature to the most recent findings. Finally, there are many specialized and specific processes involved in memory in PTSD which have been comprehensively reviewed previously (eg. Brewin, 2011), but given that endocannabinoid research has largely focused
on fear extinction recall and emotional memory consolidation most of our review relates to these processes.

1.4 Method

Studies were selected for this review during several systematic searches of electronic databases Web of Science, PubMed and PsycINFO. Keywords searched were “cannabinoid”, “fear”, “FAAH”, “extinction”, “endocannabinoid”. “MAGL”, “cannabidiol”, “THC”, “cannabis”, tetrahydrocannabinol”, “trauma” or “PTSD” and searches were restricted to January 2016 until the present date. We only included papers that were printed in English. The reference lists of eligible papers were checked for additional relevant studies. Studies that did not address mechanisms directly relevant to PTSD were excluded.

2. Multiple Avenues for Endocannabinoid Modulation

2.1 THC and its recently identified effects on PTSD symptomology

The most obvious way to affect the endocannabinoid system is through direct agonism of cannabinoid receptors. THC is a partial agonist with high affinity to both CB1 and CB2 receptors, and, despite having multiple other molecular targets, is thought to exert most of its effects through these receptors (Ligresti et al., 2016). Similar to endogenous cannabinoid agonists, THC exerts its effect through CB1 receptors via presynaptic inhibition (Kano et al., 2009), though it is only one of the two phytocannabinoids that directly activates the cannabinoid receptors, with the other being cannabino1 (Ligresti et al., 2016; Showalter, Compton, Martin, & Abood, 1996). As a result, carriers of the polymorphism C385_A, implicated in lower levels of the FAAH enzyme and therefore higher levels of native cannabinoid ligands, are four times less likely to be dependent on THC due to the increased presence of endocannabinoids in these people (Tyndale, Payne, Gerber, & Sipe, 2007).
Several synthetic analogues of THC have been developed for therapeutic purposes, with dronabinol (Marinol, Solvay Pharmaceuticals) and nabilone (Cesamet, Valeant Pharmaceuticals) licensed for the treatment of nausea and vomiting caused by chemotherapy, and Sativex (GW Pharmaceuticals) for the treatment of late-stage cancer pain and spasticity in multiple sclerosis patients (Ligresti et al., 2016). While dronabinol and nabilone are synthetic THC analogues, Sativex is 1:1 THC:CBD.

THC has previously been shown to improve performance on fear extinction and analogue emotional memory tasks in animals, as well as reducing the stress response and promoting anti-anxiety behaviours at mild to moderate doses (Berardi et al., 2016; Micale et al., 2013). The most recent animal studies have continued to show this, with THC reducing anxiety produced by 2-AG synthesis inhibition (Bedse et al., 2017), by decreasing fearful behaviours to conditioned stimuli through medial prefrontal cortex (mPFC) CB1-dependent disruption of memory reconsolidation (Stern et al., 2015) and by reducing acquisition of fear learning (Ruhl, Zeymer, & von der Emde, 2017) (Table 1).

In humans, THC has been shown to improve fear extinction recall in healthy participants compared to placebo (Rabinak et al., 2013), possibly by bolstering the vmPFC and hippocampal responses during extinction recall as shown by fMRI (Rabinak et al., 2014). Marketed versions or synthetic analogues of THC have also improved PTSD symptoms, with nabilone and THC improving nightmares, sleep quality, hyperarousal and overall symptomology in PTSD patients (Cameron et al., 2014; Fraser, 2009; Jetly et al., 2015; Roitman et al., 2014). However, THC administered prior to memory retrieval was recently found to increase false recollection of emotional and non-emotional stimuli in healthy participants (Doss, Weafer, Gallo, & de Wit, 2018), suggesting that distortion, rather than reconsolidation, of memories may be an unwanted side-effect of THC treatment. Further, THC administration to rat nucleus accumbens increased salience of fear memories, which...
further suggests that THC may in some situations contribute to unwanted memory traces (Fitoussi, Zunder, Tan, & Laviolette, 2018). Interestingly, the effects of THC were dopamine-dependent, such that antagonism on nucleus accumbens dopamine receptors completely blocked this effect (Fitoussi et al., 2018). This suggests that future clinical studies should focus on discerning which aspects of symptomology with be improved or worsened given cannabinoid treatment. Randomised clinical trials using THC, dronabinol or nabilone for PTSD patients are currently being conducted (NCT03008005, NCT02759185, NCT02069366, NCT02517424 and NCT03251326).

2.1.1 Synthetic agonists and recently identified effects on PTSD symptomology

THC is a partial, non-selective agonist of CB1 and CB2 receptors, and has over time become less stigmatised as a tool for research purposes (Ligresti et al., 2016). Consequently, endocannabinoid research could not have possibly evolved without the development of potent agonists selective to CB1 and/or CB2 receptors (Pertwee, 2006). Many such agonists have been developed, with some of the most notable being WIN55,212-2 (Compton, Gold, Ward, Balster, & Martin, 1992), HU-210 (Mechoulam et al., 1987) and CP-55,940 (Howlett, Champion, Wilken, & Mechoulam, 1990), which are salient due to their continued and frequent use in preclinical research (Figure 1). The favourable effects of these potent agonists in animal models of traumatic stress have been previously reviewed (Berardi et al., 2016; Micale et al., 2013).

The most recent studies have continued to show that WIN55,212-2 and CP-55,940 produce anxiolytic effects in rodents (Bedse et al., 2017; Burstein, Shoshan, Doron, & Akirav,
These recent studies have contributed to our understanding of how cannabinoids might influence PTSD mechanisms, beyond what has been previously reported. Firstly, whereas mice susceptible to acute stress showed increased metabotropic glutamate receptor 5 levels in the hippocampus when acutely administered vehicle, acute dosing of WIN55,212-2 regulated these receptor densities, presumably reversing the stress-susceptible phenotype (Sun et al., 2017). Both WIN55,212-2 and CP-55,940 have also recently been shown to improve fear extinction learning (Burstein et al., 2018; Ghasemi, Abrari, Goudarzi, & Rashidy-Pour, 2017; Korem, Lange, Hillard, & Akirav, 2017; Lisboa et al., 2018; Sachser, Crestani, Quillfeldt, Souza, & de Oliveira Alvares, 2015; Shoshan & Akirav, 2017; Zubedat & Akirav, 2017), and to reduce fear memories more generally (Nasehi, Shahbazzadeh, Ebrahimi-Ghiri, & Zarrindast, 2018; Santana et al., 2016; Shoshan, Segev, Abush, Mizrachi Zer-Aviv, & Akirav, 2017). In accordance with past literature (Berardi et al., 2016), these effects were enhanced by glucocorticoid receptor antagonism and mediated by hippocampal cornu ammonis 1 (CA1) and basolateral amygdala (BLA) CB1 receptors (Santana et al., 2016; Shoshan & Akirav, 2017; Zubedat & Akirav, 2017), and may also involve BLA β1-adrenoceptors (Nasehi, Shahbazzadeh, et al., 2018). These memory effects may be caused by improved neuroplasticity, shown when WIN55,212-2 reversed the effects of acute stress on long-term potentiation pathways in the nucleus accumbens and CA1 (Segev & Akirav, 2016; Shoshan et al., 2017).

Interestingly, antagonism of 5-HT4 receptors reduced or enhanced the ameliorative effects of CB1 agonism on fear conditioning at higher or lower doses of 5-HT4 antagonism, respectively (Nasehi, Farrahizadeh, Ebrahimi-Ghiri, & Zarrindast, 2016). Further, WIN55,212-2 decreased hippocampal and PFC brain-derived neurotrophic factor (BDNF) concentrations, which were associated with PTSD symptomology in a rodent model of the
disorder (Burstein et al., 2018; Korem et al., 2017). Most recently, CB2 CA1 agonism using GP1a also had an impairing effect on passive avoidance memory consolidation in male mice, implicating a potential role for the peripheral cannabinoid system as well (Nasehi, Gerami-Majd, Khakpai, & Zarrindast, 2018). Finally, the beneficial effect of WIN55,212-2 on fear extinction and anxiety behaviour was associated with reduced interleukin 1β mRNA following repeated social defeat stress and contextual fear conditioning, suggesting that cannabinoids act on fear and stress partly by reducing neuroinflammation (Lisboa et al., 2018). Whilst these findings agree with the growing view of an expanded endocannabinoid system (Berardi et al., 2016; Hill et al., 2018; Morena et al., 2016; Ney et al., 2018), they also contribute valuable new insight into the molecular interactions between BDNF, endocannabinoid and serotonin systems during fear extinction. Combined with recent research identifying interactions between the dopamine and endocannabinoid (Fitoussi et al., 2018; Wenzel et al., 2018) systems during fear memory consolidation and extinction, understanding of endocannabinoid interactions highlights the importance of examining dopaminergic and similar classical memory and cognition circuits in the context of PTSD (Abraham, Neve, & Lattal, 2014; Lee, Wang, & Tsien, 2016).

2.1.2 Disadvantages and status of direct agonists of the endocannabinoid system

There are several disadvantages to directly stimulating cannabinoid receptors pharmacologically. Firstly, direct agonism of cannabinoid receptors displays marked biphasic effects. For instance, high doses of THC and other cannabinoid receptor agonists result in anxiogenic, rather than anxiolytic, effects in rodents (Fitoussi et al., 2018; Micale et al., 2013; Patel & Hillard, 2006; Rey, Purrio, Viveros, & Lutz, 2012; Sbarski & Akirav, 2018; Todd & Arnold, 2016), and in humans (7.5mg compared to 12.5mg oral THC; Childs, Lutz, & de Wit, 2017). Heavy use of whole plant marijuana in humans can also result in
psychiatric symptoms such as depression, cognitive impairment and psychosis (Hall & Degenhardt, 2009), as well as decreases in memory capacity compared to lower doses (Calabrese & Rubio-Casillas, 2018). Since synthetic cannabinoid agonists are considerably more potent than THC, these effects may potentially occur even at low doses in humans (Castaneto et al., 2014). Further, effective treatment of PTSD is likely to require ongoing medication until symptoms are fully alleviated, meaning that chronic dosing will likely be necessary. Synthetic cannabinoids that are recreationally available, such as “Spice”, have high efficiency as well as potency and are reported in numerous case studies and anecdotal reports to have far stronger physiological and psychological effects than THC (Spaderna, Addy, & D’Souza, 2013). There is also evidence suggesting that these enhanced effects are accompanied by higher rates of adverse events, such as increased rates of physical insults such as tachycardia, as well as increased risk of mental health problems such as psychotic features (Bush & Woodwell, 2013; Castaneto et al., 2014; Fattore, 2016; Forrester, Kleinschmidt, Schwarz, & Young, 2011; Hoyte et al., 2012; Spaderna et al., 2013).

Heavy cannabis use may also result in downregulation of endogenous cannabinoid signalling and decreased performance on fear extinction tasks in humans, which presumably occurs through sustained reliance on exogenous signalling (Hirvonen et al., 2012; McPartland, Guy, & Di Marzo, 2014; Papini et al., 2017). Importantly, recent naturalistic studies of cannabis use in PTSD have reported null (Johnson et al., 2016) or negative (Wilkinson, Stefanovics, & Rosenheck, 2015) associations between cannabis use and symptom severity, despite patients using the drug for temporary relief. Conversely, at therapeutic doses nabilone and dronabinol appear to be generally well tolerated with few severe side effects (U.S. Food and Drug Administration, 2004, 2006) though have not been thoroughly tested in humans with PTSD despite promising early trials in patient populations (Cameron et al., 2014; Jetly et al., 2015). These reports therefore highlight the importance of conducting safety...
trials for synthetic cannabinoid agonists and trialling cannabinoids in PTSD at controlled doses despite a discouraging literature surrounding the effects of whole-plant marijuana.

Perhaps the greatest problem with direct stimulation of cannabinoid receptors is the involvement of these receptors, particularly CB1, in producing unwanted intoxicant effects in areas that are not a therapeutic target. This problem is evident in animal studies where administration to one region, such as the mPFC with THC, can produce therapeutic effects such as reduced fear responding and anxiety, whereas administration to another region, such as the nucleus accumbens with THC, can produce negative effects such as potentiating fear reactivity (Fitoussi et al., 2018; Stern et al., 2015). Activation of cannabinoid receptors in the brain can produce intoxicant effects, which alter emotions, thought processes and perception (Pertwee, 2006). Intoxicant effects are sought by many people for recreational purposes, meaning that potent cannabinoid agonists have high potential for misuse (Ware, Martel, Jovey, Lynch, & Singer, 2018). For these reasons, there is a long-held hesitancy in advocating for chronic use of cannabinoid agonists for medical conditions, particularly psychiatric conditions and conditions comorbid with substance use disorders where patients are likely to seek intoxicant effects (Table 1). In the pharmacological community, attention towards treating PTSD—a psychiatric disorder marked by high rates of comorbid substance misuse—has instead turned to the development of indirect agonists that are unlikely to produce intoxicant effects, which will be reviewed next.
Table 1. Direct Agonists of the endocannabinoid system

<table>
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<tr>
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<th>Newly recognised mechanisms of action in PTSD processes</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Status</th>
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</thead>
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<tr>
<td>Delta9-tetrahydrocannabinol</td>
<td>Reduces nightmares</td>
<td>Role of NAc dopamine in modulating susceptibility to acute stress</td>
<td>Easy to manufacture</td>
<td>Unwanted effects (e.g., intoxicant effects)</td>
<td>Being tested in humans, some synthetic forms</td>
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<tr>
<td></td>
<td>Improves sleep</td>
<td>Improved fear extinction recall#</td>
<td>Effective in PTSD subjects</td>
<td>Tolerance and dependence due to downregulation of endocannabinoid system</td>
<td>Approved for some indications in some countries</td>
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<td></td>
<td>Quality</td>
<td>Stress</td>
<td>Some understanding of health effects</td>
<td></td>
<td></td>
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<tr>
<td>Synthetic cannabinoid agonists</td>
<td>Not tested in humans due to lack of safety studies</td>
<td>Decreasing HC and PFC BDNF</td>
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<td>Central side effects (off target)</td>
<td>Untested in humans</td>
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<td>WIN55,212-2; CP-55,940; HU-210;</td>
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<td>Biphase interaction with 5-HT4</td>
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<td>Interactions with BLA β1-adrenoceptors</td>
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<td>Via CB2 in CA1</td>
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Note: NAc = nucleus accumbens, HC = hippocampus, PFC = prefrontal cortex, BDNF = brain-derived neurotrophic factor, CB2 = cannabinoid receptor type 2, CA1 = cornu ammonis-1, BLA = basolateral amygdala

*Tested only in healthy humans in fear extinction studies at time of publication
2.2 Inhibition of Ligand Degradation – Enzyme and Transport Inhibition

Another way of enhancing endocannabinoid signalling is through inhibition of the catabolic enzymes FAAH and MAGL (Micale et al., 2013). MAGL is the primary metabolic enzyme for 2-AG and FAAH is primarily responsible for metabolism of AEA, though also plays a small part in 2-AG hydrolysis (Howlett et al., 2002). FAAH belongs to the amidase signature family, which are part of the serine hydrolase superfamily (McKinney & Cravatt, 2005). Similarly, MAGL is part of the serine hydrolase superfamily, though 2-AG is hydrolysed through multiple enzymes, with MAGL being the primary one (Murataeva, Straiker, & Mackie, 2014). Theoretically, inhibition of active ligand degradation will occur locally, which is in contrast to direct agonists of cannabinoid receptors which will tend to produce global effects (Di Marzo, 2009; Kano et al., 2009). This is because endogenous cannabinoids such as AEA and 2-AG are produced on demand and will only be synthesised at locations local to insult (Kano et al., 2009). FAAH inhibition can therefore theoretically target specific pathways and regions, and should not produce unwanted intoxicant and other like side-effects (Bisogno & Maccarrone, 2013).

Multiple FAAH inhibitors have been developed and these vary in FAAH enzyme selectivity and efficacy. URB597 is a potent and selective inhibitor of FAAH (Fegley et al., 2005; Kathuria et al., 2003) and has been the most popular choice for anxiety and PTSD research though has not progressed to research in humans. Three FAAH inhibitors (SSR-411298, JNJ-42165279 and PF-04457845) have progressed to Phase I human trials, and two (PF-04457845 and SSR-411298) to Phase II trials (Ahn et al., 2011; Johnson et al., 2011; Keith et al., 2015; Postnov et al., 2018). In the latter trials (PF-04457845 for knee pain and SSR-411298 for depression) there was no benefit in humans despite promising animal data (Huggins, Smart, Langman, Taylor, & Young, 2012; Sanofi, 2013). New inhibitors are being
continuously developed, including a dual MA GL/FAAH inhibitor (JZL195) (Long et al., 2009) which may act to increase local levels of both AEA and 2-AG.

Insert Figure 2 about here

2.2.1 Recently identified effects of catabolic inhibition of endocannabinoids on PTSD symptomology

Generally speaking, past reviews have found that FAAH inhibition favourably enhances fear extinction and other processes relevant to PTSD (Berardi et al., 2016; Micale et al., 2013). FAAH inhibitors, but not the MAGL inhibitor JZL184, have been found to decrease corticotrophin-releasing hormone mRNA in the hypothalamus and pituitary following stress (Bedse et al., 2014) as well as reducing stress and anxiety behaviours in rodents (Bedse et al., 2018; Danandeh et al., 2018; Griebel et al., 2018; Heinz, Genewsky, & Wotjak, 2017; Sartim, Moreira, & Joca, 2017), though it did not reduce corticosterone levels (Bedse et al., 2014; Griebel et al., 2018; Roberts, Stuhr, Hutz, Raff, & Hillard, 2014; Shoshan & Akirav, 2017). One recent study showed, however, that the FAAH inhibitor URB597 did reduce corticosterone responses to immobilisation stress in young rats, but only those who had been previously exposed to stress during the neonatal stage of life. This may suggest that FAAH inhibition may preferentially reduce stress responses in those who had been exposed to early life trauma and have a consequent developmentally-founded stress phenotype (McLaughlin, Verlezza, Gray, Hill, & Walker, 2016). Interestingly, the MAGL/FAAH inhibitor JZL195 did not decrease anxiety behaviour, whereas MAGL and FAAH inhibitors JZL184 and URB597 alone did, suggesting that the potential of dual FAAH/MAGL inhibitors for PTSD may be limited (Table 2) (Bedse et al., 2018).
Recent studies have also continued to show that URB597 promotes favourable outcomes in aversive conditioning paradigms (Aisenberg et al., 2017; Burstein et al., 2018; Fidelman, Mizrachi Zer-Aviv, Lange, Hillard, & Akirav, 2018; Morena et al., 2017; Segev et al., 2018; Zer-Aviv & Akirav, 2016), as do FAAH inhibitors AM3506 (Gunduz-Cinar, et al., 2013) and OL-135 (Bowers & Ressler, 2015; Burman et al., 2016). Recent studies have also identified that the effects of FAAH inhibition on extinction memory occur in the BLA, mPFC and CA1 through normalisation of CB1 receptors, as well as enhanced long-term potentiation and normalised BDNF levels, which is a similar mechanism to that observed in direct CB1 agonists (Aisenberg et al., 2017; Burstein et al., 2018; Fidelman et al., 2018; Sartim et al., 2017; Segev et al., 2018; Zer-Aviv & Akirav, 2016). The effect of FAAH inhibition on memory consolidation of stressful events more generally is inconsistent, with some studies finding FAAH inhibition enhances memory traces (Morena et al., 2014; Ratano, Palmery, Trezza, & Campolongo, 2017) and others finding it reduces memory (Aisenberg et al., 2017). One recent study found that URB597 improved impaired hippocampal long-term potentiation and performance on short-term memory tasks, and improved performance on overly expressive BLA-dependent memory tasks (Shoshan et al., 2017). At this stage, the role in memory consolidation of FAAH inhibition is ambiguous, as directionality appears highly sensitive to context, dosage, brain location and other factors (Morena & Campolongo, 2014).

2.2.2 Alternatives to FAAH inhibition

As FAAH degrades not just AEA but also other ethanolomides that have discrete molecular targets apart from the endocannabinoid system, FAAH inhibition has the potential to have off-target effects. For example, TRPV1 channels are activated by oleoyl ethanolamide – part of the endocannabinoid ligand family – and are recognised to produce anxiety when activated in rodents (Faraji et al., 2017). For this reason, TRPV1
channels are recognised to be one of the unwanted targets incidentally activated during FAAH inhibition using some inhibitors, such as URB597 (Bisogno et al., 1998; Micale et al., 2009). An approach to targeting the endocannabinoid system that counteracts this problem is by using N-arachidonoyl-serotonin (AA-5-HT), which is a dual inhibitor of FAAH and TRPV1 channels. AA-5-HT administered intraperitoneally, to the mPFC or to the BLA of rodents reduced anxiety (John & Currie, 2012; Micale et al., 2009) and stress responses (Sartim et al., 2017), and was more recently found to reduce fear memories when administered to the dorsal hippocampus (Gobira et al., 2017). Unfortunately, studies using this promising compound have been sparse, despite recent literature suggesting that low CB1:TRPV1 ratio potentiates fear learning (Back & Carobrez, 2018).

Recent studies have also begun examining the effect of MAGL inhibitors such as JZL184, and have generally found anxiolytic effects (Aliczki et al., 2013; Almeida-Santos, Moreira, Guimaraes, & Aguiar, 2017; Bedse et al., 2018; Bedse et al., 2017; Bluett et al., 2017; Bosch-Bouju, Larrieu, Linders, Manzoni, & Laye, 2016; Morena et al., 2017), but see (Heinz et al., 2017). Limited PTSD-oriented studies have found decreased stress responses following MAGL inhibition (Bluett et al., 2017; Roberts et al., 2014) along with decreased memory consolidation following stress (Morena et al., 2015), though possibly in anti-extinction learning effects as well (Hartley et al., 2016; Llorente-Berzal et al., 2015). MAGL inhibition is a rapidly growing area of research (Aghazadeh Tabrizi et al., 2018; Granchi, Caligiuri, Minutolo, Rizzolio, & Tuccinardi, 2017) and it will become increasingly clear over the next few years how 2-AG augmentation may benefit therapeutic outcomes, with the first Phase I clinical trials currently being conducted (NCT03447756).

2.2.3 Recently identified effects of reuptake inhibition of endocannabinoids on PTSD symptomology
Similar to catabolic enzyme inhibitors, previous research on the effect of cellular reuptake inhibitors with respect to PTSD and other psychiatric disorders have been reported (Berardi et al., 2016; Micale et al., 2013). As the mechanisms for cellular uptake of the endocannabinoids is poorly understood (Nicolussi & Gertsch, 2015), relatively little research has yet been conducted using reuptake inhibitors. Reuptake inhibitors of endocannabinoids share a similar mechanism to other drugs, such as selective serotonin reuptake inhibitors, whereby cellular transport of the active ligands after exerting their effects is inhibited, which delays reuptake. AM404 (Beltramo et al., 1997) is the most commonly used endocannabinoid reuptake inhibitor, though it primarily deactivates AEA transporters through poorly understood mechanisms (Nicolussi & Gertsch, 2015), and has off-target effects at TRPV1 as well as cyclooxygenase 1 and cyclooxygenase 2 for which activation can result in increased anxiety and reduced recovery from PTSD symptomology in rodents (Faraji et al., 2017; Gamble-George et al., 2016; Hogestatt et al., 2005; Wang et al., 2018). Other reuptake inhibitors, such as VDM11, AM1172 or the recently synthesized and highly selective AEA and 2-AG transport inhibitor WOBE437 (Reynoso-Moreno, Chicca, Flores-Soto, Viveros-Paredes, & Gertsch, 2018), are yet to garner attention in PTSD research. Regardless, the most recent studies using AM404 found that administration in rodent paradigms reduced the expression of fear (Llorente-Berzal et al., 2015) and potentiated learning of safety cues, an effect that was dependent on CB1 receptor integrity in the CA1 (Micale et al., 2017). Therefore, AM404 exerts anxiolytic effects through the cannabinoid system, though the off-target effects are also well documented. Importantly, recent developments have seen the synthesis of increasingly selective and potent endocannabinoid reuptake inhibitors, which may be a promising target for therapeutic modulation in the near future (Chicca et al., 2017).

2.2.4 Disadvantages and status of catabolic/reuptake inhibitors of endocannabinoids
Despite the fact that these studies agree with past reports on the potential efficacy of endocannabinoid hydrolysis inhibition for PTSD treatment (Berardi et al., 2016; Micale et al., 2013), recent developments have sparked concern in the safety of this approach (Table 2). Full assessment of the safety and efficacy of preclinical MAGL and FAAH inhibitors is difficult to achieve for a number of reasons. Firstly, the potential for off-target effects, due to the formulation of most of these compounds from existing serine hydrolase inhibitors, is problematic to establish due to the size of the serine hydrolase superfamily (Bisogno & Maccarrone, 2013; Di Marzo, 2008; Zhang et al., 2007). For instance, URB597, which is most commonly utilised in animal research, displayed off-target effects for triacylglycerol hydrolase of at least the same potency as FAAH (Lichtman et al., 2004), making it unsuitable for use in humans. Further, FAAH in humans and animals displays structural differences (Di Venere et al., 2012), which may explain why some highly efficacious inhibitors developed for animals are ineffective in humans (Bisogno & Maccarrone, 2013). In a sparse and growing literature, one recent study found the MAGL inhibition resulted in negative consequences for memory and learning in rodents, suggesting that the safety profile of such compounds may be poor (Griebel et al., 2015).

Unfortunately, a recent Phase I clinical trial using the FAAH inhibitor BIA 10-2474 reported that five out of their six participants in a fifth cohort of the study had experienced serious adverse events, including one death (Kerbrat et al., 2016). Indeed, a subsequent study found that BIA 10-2474 targeted multiple serine hydrolases in addition to FAAH; many of which were lipophilic and therefore possible contributors to the neurological damage induced in participants (van Esbroeck et al., 2017). Participants of this particular trial tolerated BIA 10-2474 well at 20mg or 100mg acutely, but not 50mg over six days (Kerbrat et al., 2016). This event represents a setback in the development of FAAH inhibitors for clinical purposes, as most current clinical trials using any related compounds have been halted, despite the US
Food and Drug Administration ruling that FAAH inhibitors aside from BIA 10-2474 are likely to be safe (U.S. Food and Drug Administration, 2016). Such a serious adverse event advocates for more rigorous safety profiling prior to bringing new drugs to clinical trial, which will need to be increasingly rigorous for FAAH inhibitors given how easy it is to miss off-target effects (Bisogno & Maccarrone, 2013; Piscitelli & Di Marzo, 2012).

Further to this, a recent human study examined the frequency of the low-FAAH expressing allele of the single nucleotide polymorphism rs324420 in relation to adult depression and anxiety following childhood adversity (Lazary, Eszlari, Juhasz, & Bagdy, 2016). It was found in this study that higher adult anxiety and depression was associated with genetically reduced FAAH expression only if participants reported childhood adversity. Therefore, despite other reports suggesting stress-resilient phenotypes in low-FAAH expressing rs324420 carriers (Dincheva et al., 2015; Spagnolo et al., 2016), more research is needed to understand under which conditions and for what populations FAAH inhibition will be efficacious.
<table>
<thead>
<tr>
<th>Table 2. Degradation/reuptake inhibitors of endocannabinoid ligands</th>
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<tbody>
<tr>
<td>Effects in humans</td>
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<tr>
<td>FAAH inhibitors</td>
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<tr>
<td>URB597, SSRI-41</td>
</tr>
<tr>
<td>411298, JNJ-4265297</td>
</tr>
<tr>
<td>04457845</td>
</tr>
<tr>
<td>AM3506, OL-135, BIA 10-2474</td>
</tr>
<tr>
<td>Dual FAAH/TRPV1 inhibitors</td>
</tr>
<tr>
<td>AA-5-HT, OMDM198</td>
</tr>
<tr>
<td>MAGL inhibitors</td>
</tr>
<tr>
<td>JZL-184</td>
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<tr>
<td>Inhibitors</td>
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<tr>
<td>------------</td>
</tr>
<tr>
<td>Dual FAAH/MAGL inhibitors</td>
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<tr>
<td>JZL-195</td>
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<tr>
<td>Reuptake inhibitors</td>
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<tr>
<td>AM404, VDM11, AM1172, WOBE437</td>
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Note: ^indicates FAAH inhibitors that have progressed to clinical trial but have not been used in PTSD animal research.

AEA = arachidonoyl ethanolamine, 2-AG = 2-arachidonoyl glycerol, NAc = nucleus accumbens, HC = hippocampus, PFC = prefrontal cortex, BDNF = brain-derived neurotrophic factor, CB1 = cannabinoid receptor type 1, CA1 = cornu ammonis-1, BLA = basolateral amygdala, LTP = long-term potentiation, CRH = corticotropin-releasing hormone, eCB = endocannabinoids
2.3 Cannabidiol

The endocannabinoid system may also be stimulated indirectly. Cannabidiol (CBD) is one way of doing this (Figure 3). CBD is the primary non-intoxicant phytocannabinoid native to the *Cannabis Sativa* plant. CBD has very low affinity for both CB1 and CB2 receptors, and exerts effects at moderate levels at multiple targets in both the central and peripheral nervous systems (Ligresti et al., 2016; McPartland et al., 2015). Many of CBD’s effects rely on the integrity of cannabinoid receptors (Bitencourt et al., 2008; Campos, Ortega, et al., 2013; Capasso et al., 2008), and CBD may possibly exert some of its effects through inhibition of the endocannabinoid catabolic enzymes (Bisogno et al., 2001; McPartland et al., 2015). Therefore, CBD is believed to primarily activate the cannabinoid receptors indirectly and work through the endocannabinoid system, though there is evidence of affinity for multiple other molecular targets where this may be achieved (Ligresti et al., 2016; Mechoulam, Peters, Murillo-Rodriguez, & Hanus, 2007). Firstly, it was established relatively early that CBD is an inverse agonist of both CB1 and CB2 receptors (Mechoulam et al., 2007), as it was shown to antagonise cannabinoid receptor agonists in vivo (Pertwee, Ross, Craib, & Thomas, 2002). However, a recent meta-analysis recognised that CBD also enhances the effects of cannabinoid receptor agonists in some situations (McPartland et al., 2015; Todd & Arnold, 2016). CBD also binds to and desensitises TRPV1 channels (Iannotti et al., 2014), which explains its anxiolytic action to some extent (Campos & Guimarães, 2009; Ligresti et al., 2016). More likely, since CBD has some small affinity for the 5-HT1A receptor (Campos & Guimaraes, 2008; McPartland et al., 2015; Russo, Burnett, Hall, & Parker, 2005), activity through serotonergic pathways may explain part of its anxiolytic (Campos, Moreira, Gomes, Del Bel, & Guimaraes, 2012; Lee, Bertoglio, Guimaraes, & Stevenson, 2017) and antidepressant effects (Sales, Crestani, Guimaraes, & Joca, 2018). CBD also exhibits anti-cancer, anti-epileptic and immune boosting properties, which are
probably mediated by activity at GPR18, GPR55, α3 glycine and peroxisome proliferator-activated receptor gamma receptors, as well as inhibition of adenosine reuptake (Carrier, Auchampach, & Hillard, 2006; Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009; Ligresti et al., 2016; McPartland et al., 2015). Therefore, as an inverse agonist with low affinity for cannabinoid receptors CBD has some direct influence on the endocannabinoid system, though is reported to primarily work as indirect agent on endocannabinoid functioning via independent paths.

Notably, CBD is recognised as having a relatively safe profile in doses up to 1500mg (Zuardi, Morais, Guimaraes, & Mechoulam, 1995), and only minor adverse effects are noted in studies of chronic dosing of between 400-800mg/day in humans (Bergamaschi, Queiroz, Crippa, & Zuardi, 2011; Iffland & Grotenhermen, 2017; World Health Organisation, 2017). A recent World Health Organisation report concluded that there is no evidence of potential for abuse or dependence in humans, and no reported recreational profile or public health concerns relating to CBD use (World Health Organisation, 2017). CBD appears to have low toxicity and is well tolerated in most recipients (World Health Organisation, 2017). CBD is reported to exhibit some metabolic drug interactions through the CYP450 enzyme family that may affect its use in combination with some common medications, particularly anti-epileptics. Further, in vitro studies have demonstrated that CBD may show some cytotoxicity, though moderate or major adverse effects have only been observed at extreme doses in a large range of clinical trials (Bergamaschi et al., 2011). Importantly, CBD is known to counteract many of the central intoxicant effects of THC when administered simultaneously as a potential inverse agonist (World Health Organisation, 2017). This means that adverse effects associated with direct cannabinoid receptor activation in the central nervous system are avoided, though CBD may also potentiate these effects in some cases in the peripheral system (McPartland et al., 2015). In a minority of studies CBD has displayed biphasic effects, with
smallest doses (.5mg/kg in zebrafish or 100mg in humans) being ineffective and large doses (10mg/kg in zebrafish or 900mg in humans) being anxiogenic rather than anxiolytic (Nazario et al., 2015; Zuardi et al., 2017), however most animal and human studies report anxiolytic effects exclusively (Blessing, Steenkamp, Manzanares, & Marmar, 2015). Overall, CBD has been shown to have potent antioxidant, antipsychotic, anxiolytic, antiemetic and anti-inflammatory properties, among others (Izzo et al., 2009; Ligresti et al., 2016; Zuardi, 2008).

2.3.1 Recently identified effects of cannabidiol on PTSD symptomology

Preclinical research also shows great promise for CBD as an enhancer of fear extinction and therapeutic consolidation of emotional memories (Table 3, previously reviewed in Blessing, Steenkamp, Manzanares, & Marmar, 2015; Campos et al., 2012; Lee et al., 2017). CBD exerts anti-stress effects following both acute and chronic stress (Campos, Ferreira, & Guimaraes, 2012; Campos, Ortega, et al., 2013; Granjeiro, Gomes, Guimaraes, Correa, & Resstel, 2011), which, similar to its anxiolytic effects, appears to recruit a serotonergic mechanism through the 5-HT1A receptors in addition to the endocannabinoid system (Campos, Ferreira, et al., 2012; Lee et al., 2017; Resstel et al., 2009). With respect to the existing endocannabinoid-mediated stress model (Balsevich, Petrie, & Hill, 2017; Morena et al., 2016), however, the exact mechanism of CBD on stress responses is incompletely understood, as few studies have provided evidence of endocannabinoid mechanisms following CBD and during stress (Campos, Ortega, et al., 2013; Stern, de Carvalho, Bertoglio, & Takahashi, 2018). CBD has in most studies enhanced fear extinction learning and retention (Blessing et al., 2015; Campos, Moreira, et al., 2012; Lee et al., 2017). Recent studies have supported this effect by showing impaired fear memory consolidation through
the mPFC which was associated with decreased dopamine release (Rossignoli et al., 2017), the mesolimbic serotonergic system (Gomes et al., 2012; Norris et al., 2016) and hippocampal CB1 and CB2 receptors (Stern et al., 2017). Fear extinction is enhanced by CBD only if cannabinoid receptors are not antagonised (Bitencourt et al., 2008; Do Monte et al., 2013), though may interact with the strength of fear conditioning with weak and strong conditioning resulting in heightened and decreased fear expression, respectively (Song, Stevenson, Guimaraes, & Lee, 2016). Further, recent research has shown that CBD administered prior to fear conditioning can increase generalised fear and impair subsequent extinction through increased hippocampal spine density growth, implying that human trials involving experimental fear conditioning and extinction will require careful consideration of timing of administration of CBD doses (Uhernik, Montoya, Balkissoon, & Smith, 2018).

There is very little published research on the effect of CBD on memory consolidation and retrieval relevant to intrusive memories in PTSD, which may be a topic of future investigation due to CBD’s strong anxiolytic and anti-stress effects. This is likely to be due to the multi-target nature of CBD; whereas the memory literature is well defined at specific molecular targets. Research on memory processes has found that CBD exerts biphasic effects on memory. Larger doses have been shown to impair memory (Nazario et al., 2015) and lower doses have been shown to rescue memory impaired due to inflammation (Cassol-Jr et al., 2010; Fagherazzi et al., 2012) and THC use (Englund et al., 2013; Wright, Vandewater, & Taffe, 2013). More specifically to PTSD, limited research suggests that CBD administration may improve performance on inhibitory avoidance tasks (Campos, de Paula Soares, et al., 2013; Soares et al., 2010), which is likely to simulate memory and learning in rodents (Gold, 1986). However, these studies were not specifically targeted towards stress-related emotional memory performance, so whether CBD may provide a therapeutic avenue in that respect is uncertain.
Despite its excellent safety profile and promising preclinical background, there have been very few human PTSD or stress studies utilising CBD. Elms et al. (2018) recently reported significant reductions in symptomology in ten out of eleven PTSD patients prescribed 25-100mg of CBD per day over an eight week trial. Experimental research has shown that CBD inhaled compared to placebo improved performance on a standard fear conditioning and extinction task (Das et al., 2013). Further, in a case study of a young girl with PTSD, CBD oil led to reduced anxiety and improved sleep (Shannon & Opila-Lehman, 2016). Improving impoverished sleep is considered to be critical to improving waking PTSD symptoms, especially intrusive memories (Germain, 2013; Pace-Schott, Germain, & Milad, 2015). In a recent series of 72 case control studies involving patients with anxiety or sleep problems, four-fifths of participants experienced a reduction in anxiety symptoms and two-thirds showed improvements in sleep quality following sustained CBD treatment (Shannon, Lewis, Lee, & Hughes, 2019). Importantly, CBD (Chagas et al., 2014; Shannon et al., 2019), as well as CBD compared to placebo (Carlini & Cunha, 1981), has been shown to improve sleep quality and duration in participants with insomnia and other sleep disorders, though this is probably only likely to occur in higher doses with 15mg of CBD promoting alertness (see Nicholson, Turner, Stone, & Robson, 2004). Similarly, biphasic effects of CBD dosage were recently observed during a stressful public speaking test, with 300mg, but not 100mg or 900mg, reducing subjective anxiety post-speech (Zuardi et al., 2017). There are currently three clinical trials being conducted for CBD in PTSD (NCT03518801, NCT02517424 and NCT03248167), however initial trials have been promising (Elms et al., 2018).

2.3.3 Disadvantages and status of Cannabidiol

Whereas there are previous incidences of serious adverse effects of synthetic FAAH inhibition (Kerbrat et al., 2016), CBD appears to impair endocannabinoid ligand metabolism
to some extent without the side effects seen in other FAAH inhibitors. However, there are several disadvantages to using CBD. Firstly, the mechanisms for how CBD exerts its effects are largely unknown. CBD is thought to act through multiple different pathways, including, but not limited to, 5-HT$_{1A}$ receptors, adenosine reuptake, GPR receptors and TRPV channels (Ligresti et al., 2016; McPartland et al., 2015; Morales, Hurst, et al., 2017). Another current challenge is how much CBD needed to be an effective dose in humans. As an oral dose, large amounts of cannabidiol in either capsules or in oil is required for beneficial effects, due to low bioavailability of CBD on ingestion, with preliminary clinical figures ranging from 6% to a more optimistic 19% oral bioavailability following first pass metabolism (Hawksworth & McArdle, 2004; Scuderi et al., 2009). Due to studies generally showing that large amounts of CBD (between 150-600mg per day) are required for effective treatment of anxiety and other similar disorders (World Health Organisation, 2017; Zhornitsky & Potvin, 2012), there are some concerns that CBD may not be currently cost effective for consumers (Breuer et al., 2016). It is reported that inhaled CBD has higher bioavailability, with an average of 31% in cannabis users (Consroe, Kennedy, & Schram, 1991). However, due to the relatively brief duration of action of smoked CBD (Huestis, 2007) and the general discommendation of inhalation of medicinal products, slow-release oral medicinal products will most likely be preferable for the treatment of chronic ailments.

Enhancing the efficacy of CBD for medical purposes is a current topic of pharmacological research. There are seven known CBD-type molecules aside from CBD found in *cannabis sativa*, some of which have begun to show therapeutic potential for epilepsy and cancer (Morales, Reggio, & Jagerovic, 2017). Recent synthetic CBD derivatives have also shown promise for enhancing the potency and overall efficacy of the drug, though have not necessarily been targeted towards psychiatric illness (Morales, Reggio, et al., 2017). For instance, recent alterations on the alkyl chain resulted in KLS-13019, which
has a far higher potency and safety rating than pure CBD, with capacity for improved bioavailability (Kinney et al., 2016). Other approaches include hydroxyl and other alkyl chain (dimethylheptyl) substitutions (Hanus et al., 2005; Mechoulam, Kogan, Gallily, & Breuer, 2008; Takeda, Usami, Yamamoto, & Watanabe, 2009), though these have had mixed success in terms of improved potency or safety. Another promising recent approach has been the addition of fluorine atoms to CBD (Breuer et al., 2016). Many pharmaceutical drugs are fluorinated to enhance their stability, potency and bioavailability, which is thought to occur through improved receptor binding and selectivity with the addition of fluorine atoms to drug compounds (Muller, Faeh, & Diederich, 2007). Recently, a joint Israeli-Brazilian initiative synthesized several fluorinated CBD compounds, of which HUF-101 exhibited enhanced efficacy for decreasing anxiety behaviours (Breuer et al., 2016) and increased antinociception in rats compared to CBD (Silva et al., 2017). In both of these studies, HUF-101 administration produced the desired effect at lower doses than CBD, suggesting that it may be a potential therapeutic option (Breuer et al., 2016; Silva et al., 2017). However, fluorination can also affect the pharmacological profile and targets of compounds, and the toxicity profile of a fluorinated compound must be firmly established before human use (Liang, Neumann, & Ritter, 2013). To date, trials using several brands of fluorinated synthetic cannabinoid drugs have reported kidney toxicity in humans (Banister et al., 2015). Therefore, further refinement of CBD and CBD derivatives may be necessary before these products are ready for the consumer market.
Table 3. Cannabidiol modulation of the endocannabinoid system

<table>
<thead>
<tr>
<th>Cannabidiol</th>
<th>Recognised clinical effects</th>
<th>Newly recognised mechanisms of action in PTSD processes</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabidiol</td>
<td>Improves sleep quality</td>
<td>Interacts with dopamine and serotonin systems</td>
<td>Efficacious in healthy and clinical subjects</td>
<td>Difficult and expensive to manufacture</td>
<td>Being tested in humans, emerging medical recognition for some indications in some countries</td>
</tr>
<tr>
<td></td>
<td>Improved fear extinction recall</td>
<td>Reduced anxiety, PTSD symptoms</td>
<td>Excellent safety profile</td>
<td>Poorly understood mechanisms of action</td>
<td></td>
</tr>
<tr>
<td>Halogenated</td>
<td>Not tested in humans</td>
<td>CB2 dependent</td>
<td>More potent</td>
<td>Needs further investigation</td>
<td>Untested in humans, needs further investigation</td>
</tr>
<tr>
<td>derivatives</td>
<td></td>
<td></td>
<td>Cheaper due to potency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUF-101</td>
<td>Not tested in humans</td>
<td>Not tested</td>
<td>More potent</td>
<td>No follow-up studies, needs further</td>
<td></td>
</tr>
<tr>
<td>C40-Alkyl</td>
<td>Not tested in humans</td>
<td></td>
<td>More potent</td>
<td>Needs further</td>
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<tr>
<td>Chain</td>
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<td>Cheaper due to potency</td>
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<td>derivatives</td>
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<tr>
<td>KLS-13019</td>
<td></td>
<td></td>
<td>Improved safety profile</td>
<td></td>
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</tbody>
</table>

Note: Other cannabidiol derivatives are described in Morales, Reggio, et al. (2017), though have not been described in PTSD animal research

HC = hippocampus, CB1 = cannabinoid receptor type 1, CB2 = cannabinoid receptor type 2
3. Summary/Conclusions

Mounting preclinical evidence shows that downregulation of the endocannabinoid system is pivotal to the development and maintenance of PTSD, as well as other anxiety disorders (Hill et al., 2018; Morena et al., 2016; Ney et al., 2018; Patel, Hill, Cheer, Wotjak, & Holmes, 2017). The most recent research has continued to show that cannabinoids act through CB1 receptors in the classical memory systems most relevant to fear circuitry, such as the CA1 of the hippocampus, the BLA and the mPFC. Studies have also begun to show that the endocannabinoid system interacts with multiple signalling systems across different brain regions to influence PTSD aetiology and maintenance. For instance, recent research has shown that cannabinoid modulation of fear and extinction memory is dependent on the dopamine and serotonin systems (Campos, Ferreira, et al., 2012; Fitoussi et al., 2018; Nasehi et al., 2016; Rossignoli et al., 2017; Wenzel et al., 2018), which is congruent with the increasing recognition in the PTSD literature of importance of mesolimbic reward system in PTSD maintenance (Abraham et al., 2014; Kalebasi et al., 2015; Lee et al., 2016), as well as the expanded endocannabinoid system (De Petrocellis & Di Marzo, 2010; Ligresti et al., 2016; Ney et al., 2018). Further, although classically assumed that CB1 was the primary receptor involved in modulating these processes, some research has begun to identify a role for CB2 receptors in the hippocampus (Nasehi, Gerami-Majd, et al., 2018; Stern et al., 2017). Finally, cannabinoid modulation may exert effects on memory processes through alteration of BDNF concentrations in the hippocampus and the BLA, as well as altering long-term potentiation in hippocampal neurons (Burstein et al., 2018; Korem et al., 2017; Segev & Akirav, 2016; Segev et al., 2018; Shoshan et al., 2017). Development of new chemicals that combine aspects of FAAH inhibition with MAGL and TRPV1 inhibition have been mildly successful (Bedse et al., 2018; Llorente-Berzal et al., 2015), but are still in their infancy.
Despite early clinical evidence showing improvements in PTSD symptomology with regular THC and CBD treatment (Cameron et al., 2014; Elms et al., 2018; Fraser, 2009; Jetly et al., 2015; Passie, Emrich, Karst, Brandt, & Halpern, 2012; Shannon & Opila-Lehman, 2016), chronic THC administration is problematic for multiple reasons including off-target effects, endocannabinoid system downregulation, difficulties regulating dosing and mental health complications (Ceccarini et al., 2015; Hall & Degenhardt, 2009; Hirvonen et al., 2012; Johnson et al., 2016; Moreira, Grieb, & Lutz, 2009; Ware et al., 2018; Wilkinson et al., 2015). Therefore, the future of cannabinoid-based treatments for PTSD and related disorders is through inhibition of catabolic enzymes or through cannabidiol, which is the non-intoxicant compound found in cannabis. Unfortunately, there are currently significant setbacks in the advancements of these treatments as well. For instance, a clinical trial using FAAH inhibitor BIA 10-2474 resulted in serious adverse events including death (Kerbrat et al., 2016), and other FAAH inhibitors have not shown the same efficacy in humans as compared to animals (Huggins et al., 2012; Sanofi, 2013). Further, cannabidiol is currently expensive to manufacture and requires large amounts when orally ingested to be a feasible treatment option.

3.1 Future Directions

Recent manipulation of endocannabinoids in animal models of anxiety and PTSD have not only informed us about the strengths and differences of alternative pharmacological techniques, but have also increased our understanding about how the endocannabinoid system interactions with neighbouring systems to modulate mood, memory and symptomology. Future research will benefit from focusing on the role of the endocannabinoid system in other systems relevant to PTSD, such as the dopaminergic and serotonergic systems (Abraham et al., 2014; Lee et al., 2016). Given the recent move towards understanding shared
mechanisms between psychiatric fields, such as between PTSD and the psychotic illnesses (Duncan et al., 2018), there is increasing need for molecular mediators between these illnesses that explains the similarities. The endocannabinoid system is a likely system where shared mechanisms may be identified; thus exploration of its varied molecular interactions in anxiety and PTSD may benefit other fields. Clinical trials are desperately needed around the world for cannabinoid products, especially for cannabidiol and FAAH inhibitors, once safety trials have been successfully completed. This is increasingly easy to achieve in many countries as cannabinoids become legalised for medicinal purposes. However, given the weaknesses of current approaches, development, validation and safety testing of new pharmacological agents should also be a priority moving forward. Promising molecules such as HUF-101, KLS-13019 and novel FAAH inhibitors need to be extensively tested in animals, and anxiety and PTSD preclinical laboratories should consider using options such as these in their research.

Finally, there are important differences in endocannabinoid mobilisation and cannabinoid metabolism between the sexes that need to be considered before cannabinoids can be pursued as therapies for anxiety and PTSD, which are both at least twice as prevalent in women compared to men (Kessler et al., 2017; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995; Ney et al., 2018). For instance, recent animal studies have begun to demonstrate that the endocannabinoid system is mobilised to a greater degree in males compared to females in response to stress (Wyrofsky, Reyes, Yu, Kirby, & Van Bockstaele, 2018; Xing et al., 2011; Zer-Aviv & Akirav, 2016). Similarly, men have been reported to have higher hair and plasma concentrations of endocannabinoids, especially 2-AG, than women (Fanelli et al., 2012; Mwanza et al., 2016), and men display stronger physiological effects to cannabis than women (Haney, 2007; Leatherdale, Hammond, Kaiserman, & Ahmed, 2007; Penetar et al., 2005). Therefore, future clinical trials will need to incorporate the
increasingly evident modulatory role of sex hormones and sex differences on the effects of cannabinoids in humans (Ney, et al. 2018).

3.2 Conclusion

In conclusion, the endocannabinoid system is a promising novel target for reduction in anxiety and PTSD symptomology, or as an adjunct to enhance the effect of behaviourally-based psychological treatments (Berardi et al., 2016; Hill et al., 2018; Morena et al., 2016; Patel et al., 2017). However, it is inconclusive what types of pharmacological agents may positively modulate the endocannabinoid system for reducing PTSD symptoms without aversive side effects. In this review, we have summarised recent studies using several of these methods whilst highlighting the challenges remaining for these approaches. Future research will need to be geared towards improving safety of FAAH inhibitors, as well as improving the potency of cannabidiol analogues and understanding both the therapeutic potential and mechanisms of actions of different drugs. Future research will also benefit from attending to the growing recognition of molecular interactions in both PTSD and the endocannabinoid system; here we have recommended several areas where this understanding may be beneficially improved.

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**Figure Captions**

*Figure 1.* Chemical structures of cannabinoid receptor agonists

*Figure 2.* Chemical structures of endocannabinoid catabolic and reuptake enzyme inhibitors

*Figure 3.* Chemical structures of cannabidiol and notable cannabidiol derivatives
Highlights: Cannabinoid interventions for PTSD: Where to next?

- Cannabinoids are efficacious for PTSD in animal models of the disorder.
- Cannabinoid agents work through multiple pathways, such as serotonin and BDNF, to modulate PTSD symptomology.
- Different cannabinoid agents are reviewed, with strengths, weaknesses and current challenges of each approach summarised and compared.
- Clinical trials for PTSD should be constructed based on current directions in pharmacological development of cannabinoids.
Figure 1
Figure 3

- CBD
- KLS-13019
- HUF-101