



Review

Cannabinoids, interoception, and anxiety

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ABSTRACT

The use of cannabis is rapidly gaining legal status across North America. Such dramatic legislative shifts have prompted an urgency in elucidating the stimulus effects of cannabis consumption. Cannabis use, though relatively safe compared to other drugs of abuse, has been associated with greater risk of mental health disorders, possibly via its primary psychoactive constituent, Δ -9-tetrahydrocannabinol (THC). In this review, we discuss endocannabinoid activation and cannabis constituents from the perspective of subjective interoceptive (internally-perceived) states and how that relates to anxiety. Human studies have examined these subjective effects through use of self-report questionnaires. However, non-human studies use proxy methods of assessing anxiety states, such as elevated plus maze and fear conditioning paradigms. So far, this body of research has demonstrated that both endogenous and exogenous cannabinoid activation generally elicits biphasic effects on expression of the subjective state, with lower doses appearing to have anxiolytic properties and higher doses perceived as anxiogenic. Unfortunately, research with these compounds has been historically limited due to excessively tight regulatory control. Therefore, much work remains regarding the investigation of interactions between cannabinoid receptor activity and cannabis constituents on anxiety. Ongoing changes in legal status will hopefully mitigate the challenges faced by researchers attempting to access cannabis and THC that is inherently built in by federal and international classifications.

1. Shifting views on cannabis

The rapidly-changing sociopolitical shifts in attitude toward cannabis in recent years are in many ways reflective of accounts of cannabis use across history. Policy changes including decriminalization and legality are, in part, an acknowledgement that the recreational value of cannabis to society may outweigh its costs (Nutt et al., 2007). Furthermore, such policy changes also indicate an acceptance of the value of research investigating the interactions between exogenous and endogenous cannabinoids, the endocannabinoid (eCB) system, and the behavioral outcomes evoked by the subjective experience of the drug. Additionally, these modifications to policies help facilitate and encourage research in this field, which has been particularly challenging given federal and international classification schemes (National Academies of Sciences, Engineering, and Medicine, 2017a; Covey et al., 2015; Ware, 2018; Nutt et al., 2013). In light of the historical background, the purpose of the current review is to discuss what is known about the mechanisms mediating the effects of specific endogenous and exogenous cannabinoids, the resulting subjective experience, and how this relates to anxiety.

Known today primarily for its ability to induce a relaxed, euphoric

state, cannabis has an extensive history. It is thought to have begun during the rise of the Chinese empire in which it was cultivated primarily for hemp fibres, and the fruits and seeds were used for medicinal purposes including treating rheumatic pain, constipation, and malaria as well as anesthesia during surgery (Zuardi, 2006). As the use of cannabis spread throughout Asia, the Middle-East, and Europe, its psychotropic qualities perpetuated its recreational use. It was also at times an entheogen paired with religious rituals, and in India was considered sacred as it was a “source of happiness, donator of joy, and bringer of freedom” (Zuardi, 2006; Macrae, 1998). By the 1800s, cannabis was being traded throughout the world including North America. However, at the beginning of the 1900s, attitudes toward the plant began to change as it was increasingly treated as a drug among others, including alcohol and opiates (Zuardi, 2006; Hudak, 2016). Further, its impending illegality included deep motivations of institutionalized racism that contributed to the U.S. Federal Bureau of Narcotics labelling cannabis as an addictive substance and the plant becoming federally restricted around the time of the 1930s (Hudak, 2016; Bridgeman and Abazia, 2017). By the 1960s, the international community followed suit, with the United Nations creating the International Narcotics Control Board, which, in turn, classed cannabis as a Schedule I

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substance (Hudak, 2016). This classification legally declared cannabis as having the purportedly highest level of abuse potential with no medicinal use, and it resulted in excessive undue burden on scientific investigation that is a continuing problem today (National Academies of Sciences, Engineering, and Medicine, 2017a; Covey et al., 2015; Ware, 2018; Nutt et al., 2013).

Gradually, clinicians, caregivers, and individuals struggling with debilitating illnesses such as AIDS and cancer began raising awareness about the feasibility of medicinal cannabis. What began as an underground, grassroots movement with little credibility slowly and steadily accelerated until the pressure reached a tipping point. Finally, the first state to criminalize cannabis, California, also became the first state to permit medical cannabis prescriptions through the enactment of the *Compassionate Use Act* which ultimately paved the way for surrounding states to do the same (Hudak, 2016; Bridgeman and Abazia, 2017). Since then, the push for the legalization of cannabis for both medicinal and recreational purposes has spread throughout North America, including Canada where complete legalization has recently been enacted in spite of the violations of international law (Hudak, 2016; Cox, 2018; Habibi and Hoffman, 2018). There has been a parallel rise in research related to the impact of cannabis on various diseases (Svíženská et al., 2008; Mücke et al., 2018; National Academies of Sciences, Engineering, and Medicine, 2017b). Cannabis use regulates nausea and vomiting in patients receiving cancer treatment, ameliorates the impact of epilepsy by reducing the frequency of seizures, and for people living with HIV/AIDS, smoking cannabis is a common method of treating various symptoms including stress, loss of appetite, weight loss, nausea, pain, and vomiting (Devinsky et al., 2014; Sharkey et al., 2014; Grotenhermen and Müller-Vahl, 2012). Despite the growing body of evidence revealing the ability of cannabis to target specific brain pathways to relieve symptoms of certain illnesses, its influence on non-specific pathways and the potential to promote adverse side effects is largely unknown and requires more attention (Sharkey et al., 2014; Barrus et al., 2018).

Beyond the medical benefits, accelerating legality of recreational cannabis has sparked important conversations regarding the risks associated with its use. Such risks include increased rates of cannabis use disorder, potential impacts on cognition and neural development, increased abuse by adolescents and subsequent impact on academic performance and lifetime achievement, interactions between cannabis consumption and severe mental illness (e.g. schizophrenia), greater involvement in motor-vehicle accidents, and more broad effects on health and safety (National Academies of Sciences, Engineering, and Medicine, 2017b; Volkow et al., 2014; Lake and Kerr, 2016; Rehm et al., 2016; Hajizadeh, 2016; Bowles et al., 2017; Bloomfield et al., 2018; Sarvet et al., 2018a). There is also some intriguing conflicting speculation that cannabis legalization itself may play a key role in the current opioid crisis. For example, one study found that between 2011 and 2016, medical and recreational cannabis laws were associated with lower opioid prescribing rates (Wen and Hockenberry, 2018). In contrast, cannabis use has also been associated with the initiation or increase in illicit opioid use (Olsson et al., 2018). Therefore, more work on the factors that may contribute to the ‘substitutability’ of cannabis for opioids is clearly warranted (Rogeberg et al., 2018). Finally, the subjective biphasic nature of cannabis effects has also gained increased attention recently in light of policy changes. A high dose of the primary psychoactive constituent of cannabis, Δ -9-tetrahydrocannabinol (THC) (Mechoulam et al., 1970), is associated with anxiogenic responses, whereas a lower dose of THC is anxiolytic (Childs et al., 2017), exemplifying the need for research that emphasizes and compares dose-dependent effects of THC in cannabis. Indeed, depending on the individual, variations in dose or method of consumption may provoke an unexpected response that can result in unpleasant, or potentially more serious, mental health risks such as anxiety disorders or psychosis (Moreira and Wotjak, 2010; Shah et al., 2017). With the recent legalization of recreational cannabis across Canada and the steady state-by-

state legalization in the United States, the need to understand how the effects of cannabis has become far more urgent (Hudak, 2016; Rehm et al., 2016; Crépault et al., 2016). Unfortunately, access to cannabis for research is still quite limited given its technical federal illegality in the USA and its classification by the UN, though appeals for increased research are becoming more prevalent (National Academies of Sciences, Engineering, and Medicine, 2017a; Covey et al., 2015; Ware, 2018; Nutt et al., 2013).

2. Endocannabinoid system

Despite an extensive history of use, rigorous cannabis research only began fairly recently during the 1960s with investigation of the mechanisms underlying its reported effects. Constituents of cannabis were isolated, including THC, which was identified as the primary psychoactive component of the plant (Mechoulam et al., 1970). THC was then used to help identify receptors, receptor localization and subtypes, and endogenous ligands (endocannabinoids; eCBs) (Devane et al., 1988, 1992). In this review, we discuss most specifically the mechanisms underlying the role of the eCBs in anxiety. Though extensive description of the eCB system as a whole is outside the scope of the current paper, we refer the reader to any of the following reviews on the subject from a range of perspectives including development, drug discovery, addiction, reward, stress, and mental health (Covey et al., 2015; Svíženská et al., 2008; Murray and Bevins, 2010; Mechoulam and Parker, 2013; Howlett and Abood, 2017; Lutz et al., 2015; Lu and Mackie, 2016; Di Marzo and Piscitelli, 2015; Di Marzo, 2018; Sloan et al., 2018; Micale and Drago, 2018; Manzanares et al., 2018; Hill et al., 2018; Worley et al., 2018; Morena et al., 2016a; Tan et al., 2014; Laviolette and Grace, 2006; Fernández-Ruiz et al., 2000; Zahm et al., 1999; Demuth and Molleman, 2006; Lafenêtre et al., 2007; Onaivi et al., 2012).

2.1. Receptors

CB receptors are among the most abundant G-protein coupled receptors (GPCRs) in humans, and to date, they are typically divided into two main groups (Howlett and Abood, 2017). The first are cannabinoid 1 (CB₁) receptors which are primarily distributed throughout the central nervous system (CNS), spanning across various regions including the cortex, basal ganglia, hippocampus, and the cerebellum. Receptor localization is of particular importance for understanding receptor function in this system. Specifically, CB₁ receptors are primarily localized presynaptically on GABAergic neurons (Katona et al., 1999), indicating a neuromodulatory role of eCBs. The release of eCBs is triggered as a result of postsynaptic excitation (Adermark and Lovinger, 2007) and this retrograde signalling activates these presynaptic CB₁ receptors, inhibiting subsequent neurotransmitter release from the presynaptic terminal (Hashimoto et al., 2008; Lisboa et al., 2018). CB₁ receptor activation has been shown to inhibit adenylyl cyclase (Demuth and Molleman, 2006; Howlett and Fleming, 1984), ultimately resulting in decreased cAMP production. Thus, activation of these receptors can have different effects depending on localization and have been implicated in anxiety, emotion-related behaviors, and the neuroendocrine response (Herkenham et al., 1991; Haller et al., 2004; Rey et al., 2012). Indeed, there are several studies indicating a bidirectional role of the eCB system in the anxiety response dependent on CB₁ receptor localization (Rey et al., 2012; Moreira et al., 2009; Lafenêtre et al., 2009).

The second primary eCB receptor, cannabinoid 2 (CB₂) receptors, are expressed at lower levels relative to CB₁ receptors and have been implicated in peripheral and central immune system maintenance (Galiegue et al., 1995; Ashton and Glass, 2007). Like CB₁ receptors, CB₂ receptors are also activated by endogenous and exogenous ligands and function by inhibiting the activity of adenylyl cyclase (Demuth and Molleman, 2006; Shoemaker et al., 2005). Recent evidence shows that CB₂ receptors are also implicated in anxiety-related behaviors (Ortega-

Alvaro et al., 2011; Robertson et al., 2017; Ishiguro et al., 2018; García-Gutiérrez and Manzanares, 2011). Furthermore, dysfunction of CB₂ receptors has been related to other psychiatric disorders including schizophrenia, depression, and addiction (Mechoulam and Parker, 2013; Onaivi et al., 2012; Ishiguro et al., 2010; Ishiguro et al., 2007; Onaivi et al., 2008). These studies indicate that CB₂ receptors indeed play a significant role in neuropsychiatric disorders and highlight the need to further consider how CB₂ impacts CNS function.

There is also evidence of CB receptors distinct from CB₁ and CB₂ (Overton et al., 2006; McHugh et al., 2008; Járαι et al., 1999; Mackie and Stella, 2006; Brown, 2009; Rodriguez de Fonseca and Schneider, 2008). G-protein coupled receptor 55 (GPR55) and G-protein coupled receptor 119 (GPR119) are two examples of GPCRs that have been suggested as potential novel, atypical CB receptors (Brown, 2009). GPR119 is activated by anandamide (*N*-arachidonylethanolamine, AEA) and the fatty acid oleoylethanolamide (OEA) and its activation has been implicated in energy maintenance and body weight (Overton et al., 2006; Brown, 2009). GPR55 is also activated by eCBs as well as several different types of synthetic CBs (Brown, 2009; Sawzdargo et al., 1999). Though GPR55 utilizes distinct signalling systems from that known for CB₁ and CB₂ receptors, recent evidence of heteromerization with CB₁ and CB₂ receptors and its distribution throughout the CNS makes it the leading candidate for becoming the third official CB receptor (Lehmann et al., 2016). Furthermore, whereas most of the evidence points to GPR55 as a potential target to treat inflammation, there is also recent evidence for its role in modulating anxiety-related behavior in animal models (Rahimi et al., 2015; Shi et al., 2017).

2.2. Endogenous and exogenous ligands

The two most prevalent eCBs that have been studied the most to date include AEA and 2-arachnidonoyl-glycerol (2-AG), however there are several other more recently-discovered eCBs including noladin ether (2-arachidonyl glyceryl ether; 2-AGE) (McInnes et al., 2000), virodhamine (*O*-arachidonyl ethanolamine; OAE) (Porter, 2002), and *N*-arachidonyldopamine (NADA) (Bisogno et al., 2000). Further, lysophosphatidylinositol is a primary ligand to the GPR55 receptor, suggesting that it may qualify as yet another eCB (Piñeiro and Falasca, 2012). The distinct roles of these ligands in the eCB system are still unclear and yet to be fully elucidated. Given the prevalence of cannabis consumption, CB compounds found in cannabis (phytocannabinoids) are frequently investigated for their interaction with eCBs. For example, cannabigerol (CBG) is found in cannabis in moderate concentrations and is non-psychoactive (Elshohly et al., 2016). While it has a low affinity for CB receptors itself, CBG impacts eCB system by inhibiting AEA uptake (Morales et al., 2017). Importantly, CBG has been investigated for the treatment of various health conditions including inflammation (Couch et al., 2018; Borrelli et al., 2013), neurodegenerative diseases (Granja et al., 2012; Valdeolivas et al., 2015), and cancer (Borrelli et al., 2014; Śledziński et al., 2018; Soderstrom et al., 2017). CBG also has antidepressant-like effects in rodents, is an α₂ adrenoceptor agonist and 5-HT_{1A} receptor antagonist, making it a potential candidate to further explore its role in mental health (Cascio et al., 2010; Russo, 2016).

In addition to phytocannabinoids and eCBs, CB receptor agonists have been developed as analogs of THC (in most cases), making them synthetic CBs (Tambaro and Bortolato, 2012; Cannaert et al., 2016). Synthetic CBs have structural similarities to THC, but are generally more potent given their greater affinity and full agonist effects on the CB₁ receptor (Davidson et al., 2017). Though their development is aimed at facilitating exploration of potential medical uses targeting the eCB system and its receptors, these synthetic CBs are being abused increasingly by adolescents who are under the impression that these drugs are comparable to cannabis, in that they provide safe highs (Tait et al., 2016; Warren et al., 2017). In reality, mounting evidence reveals that synthetic CBs have the potential to elicit adverse physiological effects including acute kidney injury, tachycardia, seizures, nausea and

vomiting, and in some cases mortality. The adverse psychological effects of synthetic CBs extend to psychiatric presentations including psychosis, hallucinations and paranoia, panic attacks, and anxiety (Tait et al., 2016).

3. Interoception, insula, and cannabinoids

Both the adverse and appealing effects elicited by cannabis and CB consumption involve the explicit or implicit perception of the internal state. Interoception includes physiological (e.g., hunger) and emotional or affective (e.g., stress) states (Craig, 2003; Paulus and Stewart, 2014; Craig, 2002). Interoceptive signals evoke emotional responses that serve to motivate behavior. Homeostatic mechanisms shift from being initially implicit (subconscious), but can later transition to explicit (conscious) regulation when maintaining homeostasis requires a behavioral response (LeDoux and Pine, 2016; LeDoux, 2000; Critchley et al., 2004; Lau and Rosenthal, 2011). Increases in internal temperatures, for example, can lead to a physiological reaction such as perspiration as an attempt to regulate temperature changes. Such an implicit homeostatic response can lead to awareness of this change in interoceptive state (i.e., awareness of increased temperature) and elicit motivated behavior such as lowering the thermostat or removing one's sweater. The neural basis of this dual processing of homeostatic regulation of temperature has been identified in rodent models. Rats with lesions of the hypothalamus – a region responsible for autonomic homeostatic maintenance – showed greater lever pressing for access to a heat lamp when exposed to low temperatures because they were no longer able to reflexively regulate their body temperature (Refinetti and Carlisle, 1987; Satinoff and Rutstein, 1970; Schulze et al., 1981). Therefore, the motivation for homeostatic temperature regulation is processed on multiple neural levels, from the hypothalamus as well as from the limbic system, implicated in Pavlovian associative processing and maintaining representations of action-related outcomes (LeDoux, 2000; Morrison and Nakamura, 2011). The eCB system is also involved in this implicit and explicit homeostatic processing. For instance, after short periods of food deprivation, eCB activity within the hypothalamus can regulate action of orexigenic and anorectic mediators that activate appetite through signalling effects of CB₁ receptors (Horvath, 2003). The eCB system may then evoke goal-directed food-seeking behavior through interactions with limbic, motivational, and motor pathways involved in reward and reinforcement (Di Marzo and Matias, 2005).

Such processing is also congruent with the James-Lange and Schachter-Singer theories of emotional regulation, which both hypothesize that the interpretation of interoceptive physiological changes guides the emotional response (James, 1884; Schachter and Singer, 1962). For example, upon seeing a bear, activation of the sympathetic nervous system (i.e., increased heart rate and breathing) is followed by an association of the physiological state with the surroundings, evoking an appropriate, consciously-recognized emotional label. In the Toronto Zoo, the emotional response will likely be labelled as excitement; in the forests of northern Ontario, those same physiological changes will likely be labelled as fear. The insular cortex (IC) plays a crucial role in interpreting these interoceptive states (Namkung et al., 2017; Allen et al., 1991). Initially, interoceptive signals via the thalamus and association cortices connect at the posterior insula where lower-level sensory features are processed (Namkung et al., 2017; Craig, 2009). The anterior insula then integrates this information with emotional and cognitive signals produced in the limbic and prefrontal cortical systems, respectively, leading to labelling motivated behaviors and providing a sense of 'self' (Critchley et al., 2004; Namkung et al., 2017; Craig, 2009; Zaki et al., 2012). The importance of the insula to interoception means that insular dysfunction disrupts motivational, emotional, and cognitive processes, leading to an aberrant interoceptive state, suggesting major implications for the role of the IC in psychiatric disorders and mental illness (Namkung et al., 2017; Goodkind et al., 2015; Naqvi and Bechara, 2010; Paulus and Stein, 2006). Insular hyperactivity has been

associated with affective and anxiety disorders (Stein et al., 2007; Terasawa et al., 2013; Dennis et al., 2011; Duval et al., 2015) driven at least in part by the interconnectivity between the IC and the amygdala (Harris and Gordon, 2015; Baur et al., 2013). Abstinent cannabis users show reduced cortical grey matter and impaired insular function (Tapert et al., 2007), and cannabis dependence or long-term cannabis use is associated with negative affective states and anxiety disorders (Agosti et al., 2002; Stinson et al., 2006; Zvolensky et al., 2008; Moore et al., 2007; Duperrouzel et al., 2018; Mammen et al., 2018; Feingold et al., 2018; Twomey, 2017; Cancilliere et al., 2018). It has been proposed that the reduction of such negative affective states can act as a reinforcer for cannabis dependence. For instance, use of cannabis can be a coping mechanism for the negative feelings related to social anxiety and post-traumatic stress (Buckner et al., 2007; Bonn-Miller et al., 2007). There is extensive research linking the eCB system with the stress-response system (Morena et al., 2016a; Balsevich et al., 2017; Hill and Tasker, 2012; Viveros et al., 2005; Marco and Viveros, 2009), and eCBs are implicated in mood and anxiety disorders as well as motivation, reward, and addictive behaviors, emphasizing the need to target this system when considering comorbidity of anxiety disorders and cannabis use disorder (Mechoulam and Parker, 2013; Solinas et al., 2009; Trigo and Foll, 2017; Witkin et al., 2005; Crippa et al., 2009). Finally, CB receptors have been identified in the IC of both humans and rodents (Moldrich and Wenger, 2000; Westlake et al., 1994; Glass et al., 1997). Therefore, impaired insular function in cannabis use may interact with interoceptive disorders such as anxiety.

4. Anxiety and the endocannabinoid system

The eCB system is of interest for drug development related to anxiety based on the system's physiological and behavioral control over anxiety regulation (Lutz et al., 2015; Metna-Laurent et al., 2012; Patel et al., 2017; Gray et al., 2015). Such regulation shows a dose-dependent biphasic effect. Rats given high doses of the synthetic CB₁ agonist CP 55,940 spent less time socializing immediately following exposure compared to subjects given low- or no-dose treatments. Moreover, the same rats exposed to those higher doses of CP 55,940 also expressed conditioned anxiety when tested the next day (Genn et al., 2004). High doses of CP 55,940 also produced similar anxiogenic responses on the elevated plus maze (EPM), however, lower doses produced an anxiolytic effect compared with controls, with those rats increasing the percent of time spent and entries into the open arms (Marco et al., 2004). The CB₁ inverse agonist SR141716A induced anxiogenic responses in rats as measured by the defensive withdrawal test and EPM test (Navarro et al., 1997). Anxiolytic effects were found in adult mice given synthetic CB₁ agonists AB-FUBINACA, AB-CHMINACA and PB-22 (Schreiber et al., 2018) as well as the CB₁ agonist WIN 55, 212 (Lisboa et al., 2018). Relation between synthetic CB use and affect and anxiety have also been explored in humans (Tait et al., 2016; Every-Palmer, 2011; Castellanos et al., 2011), however, the majority of these studies rely on self-report and individual case-report methods rather than relying on more objective approaches such as those available in animal models. The biphasic anxiogenic versus anxiolytic outcomes of synthetic CB administration in animal models and the reliance on subjective methods used in human studies demonstrate the need to further explore the potential for synthetic CB development for treatment of anxiety-related disorders.

The role of eCBs in anxiety has also been assessed. AEA hydrolysis is catalyzed by fatty acid amide hydrolase (FAAH). Mice lacking the FAAH gene are ultimately unable to metabolize AEA and tend to have enhanced levels of AEA occupying CB₁ receptor sites (Cravatt et al., 2001). Preventing AEA degradation by administration of FAAH inhibitors enhances AEA binding to CB₁ receptors and ultimately promotes anxiolytic-like responses in rodent models (Scherma et al., 2008; Kathuria et al., 2003; Gaetani et al., 2003). Similarly, studies have shown an association between corticotropin releasing hormone (CRH)

and increased anxiety via eCB modulation. Specifically, CRH signalling induces activity of FAAH and ultimately reduces AEA levels in the amygdala (Gray et al., 2015). This decrease of AEA binding to CB₁ receptors is correlated with anxiety-related behavior or anxious phenotypes in both humans and rat models (Gray et al., 2015; Demers et al., 2016). Similarly, 2-AG is metabolized by action of monoacylglycerol lipase (MAGL) and animals with overexpressed MAGL will effectively have lower levels of 2-AG in these areas. At glutamatergic synapses in the hippocampus, overexpression of MAGL in mice has provoked anxiety-like behaviors (Guggenhuber et al., 2016). Conversely, total genetic deletion of MAGL in the basolateral amygdala lead to overexcitation of CB₁ receptors by 2-AG, resulting in glutamate release into the medial prefrontal cortex and subsequent anxiogenic behavioral effects in mice (Imperatore et al., 2015). Based on these results, 2-AG regulatory action of eCB receptors plays a pivotal role in modulating anxiety-related behavior as demonstrated in animal models. Elucidation of the regulatory control of eCB receptor activation by the metabolites of the eCB ligands, specifically within regions of the brain related to anxiogenic responses, shows therapeutic potential for treatment of anxiety disorders. This complexity of the biphasic anxiogenic versus anxiolytic outcomes of CB receptor activation by modulation of eCB metabolites in combination with the importance of baseline levels of arousal on such outcomes (Morena et al., 2016b) and evidence that eCB activity in the amygdala is responsible for coping strategy selection (Metna-Laurent et al., 2012) highlight the need to disambiguate the relations among cannabis consumption, eCB signaling, and the interoception of subjective state.

5. Anxiety and phytocannabinoids

The pharmacological effects associated with cannabis use vary among individuals, and this translates to variations in subjective and objective effects of the drug. Given the increasingly-legal status of cannabis, it is now more important than ever to consider both the potential therapeutic benefits of the drug as well as the possible risks associated with it. In general, individuals diagnosed with anxiety report using cannabis as a means of self-medication (Sarvet et al., 2018b; Menary et al., 2011). At the same time, there is a high prevalence of anxiety disorders among individuals who use cannabis (Crippa et al., 2009; Butler et al., 2019). It is unclear whether cannabis use plays a role in the development of anxiety or whether individuals with anxiety-like symptoms are more likely to use cannabis, and additional research is necessary to tease apart this correlation and try to determine causal relationships. Research regarding the associations between cannabis use and anxiety remain, for the most part, inconclusive (National Academies of Sciences, Engineering, and Medicine, 2017b; Memedovich et al., 2018), making it challenging to identify and prevent risks associated with drug use. Further, the variation in constituent ratios and potency across different strains of cannabis make it even more difficult to study this relationship. In this section, we discuss studies that explore the biphasic anxiogenic versus anxiolytic outcomes of cannabis and its main constituents of current interest, THC and cannabidiol (CBD), in human and animal studies (see Table 1), highlighting some limitations of the experiments, as well as possible future directions.

5.1. Humans

There is conflicting evidence for the potential therapeutic role for cannabis in stress and anxiety disorders (National Academies of Sciences, Engineering, and Medicine, 2017b). Despite the emerging evidence for therapeutic benefits to cannabis use, increased levels of anxiety have also been reported by human subjects exposed to cannabis (Witkin et al., 2005; Crippa et al., 2009; Bhattacharyya et al., 2017a; Kedzior and Laeber, 2014). There are many CBs and other constituents in the *Cannabis sativa* plant that could play a role in the anxiogenic and

Table 1
 Primary literature investigating phytocannabinoids in anxiety and anxiety-related behaviors (Childs et al., 2017; Schreiber et al., 2018; D'Souza et al., 2004; Englund et al., 2013; Englund et al., 2011; Fokos and Panagis, 2010; Onaivi et al., 1990; Stopponi et al., 2014; Harte-Hargrove et al., 2012; Rock et al., 2017; Bitencourt et al., 2008; Resstel et al., 2009; McLendon et al., 1976; Robichaud et al., 2011; Rubino et al., 2008; Fitoussi et al., 2018; Gomes et al., 2011; Mayer et al., 2014; Kamal et al., 2018; Bhattacharyya et al., 2015; Bhattacharyya et al., 2017b; Chait et al., 1988; Freeman et al., 2015; Hunault et al., 2014; Colizzi et al., 2018; Bergamaschi et al., 2011; Bhattacharyya et al., 2010; Fusar-Poli et al., 2009; Martin-Santos et al., 2012b; González et al., 1972b; Keeley et al., 2015; Mohammed et al., 2018; Silva et al., 2016; Urtl et al., 2018; Campos and Guimarães, 2008; ElBatsh et al., 2012; Bedse et al., 2017; Bluet et al., 2017; Brujnzeel et al., 2016; Kastan et al., 2018; Lloyd et al., 2018; Saravia et al., 2019; Myers et al., 2018; Murphy et al., 2017; Todd and Arnold, 2016; Crippa et al., 2004).

Subject	Constituent	Route/brain area	Dose	Relevant task	Results	Study
Anxiety and phytocannabinoids						
Humans	Cannabis/oil Δ9-THC	Varied	Varied	Online survey	Establishing cannabis strain chemotype in treating anxiety	(Kamal et al., 2018)
		Oral	10 mg	Spielberger state-trait anxiety inventory state subscale	Modest dose of THC resulted in acute induction of anxiety	(Bhattacharyya et al., 2015)
		Oral	10 mg	fMRI, PET, Skin conductance, BOLD	Acute effects of cannabis on anxiety in males dedicated to modulation of amygdalar function by Δ9-THC; Extent of cannabis effects on anxiety related to CB1 availability	(Bhattacharyya et al., 2017b)
		Marijuana cigarette	0.0, 0.9, 1.4, 2.7%	POMS, VAS, Addiction Research Centre Inventory	Marijuana cigarettes increased heart rate and elicited changes on several mood scales; increased feelings of anxiety were reported	(Chait et al., 1988)
		Oral capsule	0, 7.5, 12.5 mg	TSST, POMS, ARCIVAS, blood pressure, salivary cortisol, PASA, STAI	Low doses of THC produces subjective stress-relieving effects; higher doses increase stress	(Childs et al., 2017)
		Intravenous	0, 2.5, 5 mg	PANSS, CADSS, VAS, cortisol and prolactin	Δ9-THC produced schizophrenia-like positive and negative symptoms, increased anxiety, altered perception and euphoria, effects on memory, no impairments of orientation and increased plasma cortisol, Δ9-THC plasma levels	(D'Souza et al., 2004)
		Intravenous	1.5 mg in 1 ml pulses every 1 min for 10 min	Real social situation, VAS, immersive virtual reality experiment, and standard self-report and interviewer measures	THC increases paranoia, anomalous experiences, and negative affect. THC decreases scores on working memory. Cognitive awareness may increase paranoia although not significantly and decreases catastrophizing.	(Freeman et al., 2015)
		Marijuana cigarette	29, 49, 69 mg THC	VAS of subjective effects, blood pressure, heart rate, repeated venous blood samples	THC increases self-report anxiety up to 8 h after smoking	(Humault et al., 2014)
		Oral	10 mg	PANSS, STAI, attentional and emotional processing tasks	THC induced significant acute increase in anxiety	(Colizzi et al., 2018)
		Marijuana cigarette	0.0, 2.8% THC	POMS, heart rate	Expectancy instructions and pharmacology play independent roles in effect of THC on anxiety	(Metrik et al., 2011)
Rhesus monkeys	CBD	Oral	600 mg	SPST, VAMS, SSPS-N, physiological measures	CBD decreased anxiety, cognitive impairment, and discomfort	(Bergamaschi et al., 2011)
		Oral	400 mg	VAMS, regional cerebral blood flow at rest	CBD decreased subjective anxiety, increased mental sedation	(Crippa et al., 2004)
		Oral	10 mg, 600 mg	Response inhibition, viewing fearful faces, visual and auditory stimulation task	THC: acute induction of psychotic symptoms and anxiety; CBD: no change in psychotic symptoms and trend for a reduction in subjective anxiety	(Bhattacharyya et al., 2010)
		Intravenous injection, oral	1 ml/min (1.5 mg), 600 mg	State Social Paranoia Scale	Support the idea that high-THC/low-CBD cannabis products are associated with risk for mental health	(Englund et al., 2013)
		Oral	10 mg, 600 mg	fMRI while looking at different anxiety-inducing faces	disruption of prefrontal-subcortical connectivity by CBD, not THC may underlie anxiolytic properties	(Fusar-Poli et al., 2009)
		Oral	10 mg, 600 mg	PANNS, VAMS, ACRI, blood pressure, heart rate	THC increased anxiety, dysphoria, positive psychotic symptoms	(Martin-Santos et al., 2012b)
		Oral, intravenous	10 mg, 1 mg	CAPE, VAS, State Social Paranoia Scale, The University of Wales Mood Adjective Checklist	Demonstration of some protective effects of THCv against THC	(Englund et al., 2016)
		Intravenous	0.2, 0.5, 1.0 mg/kg	Conditioned fear	Physiological expression of conditioned fear	(McLendon et al., 1976)
		Δ9-THC				(continued on next page)

Table 1 (continued)

Anxiety and phytocannabinoids											
Subject	Constituent	Route/brain area	Dose	Relevant task	Results	Study					
Rats	Δ9-THC	Nucleus accumbens shell Intraperitoneal	10–100 mg/0.5 μl 3 ml/kg	Conditioned fear Elevated plus maze	In vivo neuronal electrophysiology Stressful experiences do not alter the effects of THC	(Fitoussi et al., 2018) (Fokos and Panagis, 2010)					
		Intraperitoneal	2.8 mg/kg	Conditioned emotional response paradigm	Chronic administration of THC did not act on conditioned fear acquisition, but blocked conditioned emotional response. Low dose anxiolytic, high dose anxiogenic; females more sensitive to anxiogenic effects	(González et al., 1972b)					
		Intraperitoneal	2.0, 7.5, 15 mg/kg	Locomotor activity		(Harte-Hargrove et al., 2012)					
		Intraperitoneal	5 mg/kg	Elevated plus maze	THC decreased anxiety in females only	(Keeley et al., 2015)					
		Intraperitoneal	1, 5, 10 mg/kg	Predator scent stress, elevated plus maze, acoustic startle response, freezing, corticosterone levels	THC: 5 mg/kg decreases anxiety-like behaviors only if immediately following stress and significantly decreases corticosterone levels response when administered 1 h post stress	(Mayer et al., 2014)					
		Oral gavage	10 mg/kg	Elevated plus maze, social behavior tests, open arm activity	No effects on anxiety	(Mohammed et al., 2018)					
		PFC, hippocampus, and basolateral amygdala	2.5, 5, 10 μg	Elevated plus maze	Anxiolytic responses were produced when low doses of THC were microinjected into the PFC and ventral hippocampus; low doses of THC produced anxiogenic effects when microinjected in basolateral amygdala	(Rubino et al., 2008)					
		Intraperitoneal	3 mg/kg	Elevated plus maze	THC elicited reductions in anxiety in pre-pubertal animals	(Silva et al., 2016)					
		Intraperitoneal, intravenous	2.5, 5, 10 mg/kg	Elevated plus maze, IV heroin self-administration	adolescent chronic THC exposed rats show increased anxiety-like behavior and increased stress-induced reinstatement of heroin seeking	(Stoppioni et al., 2014)					
	CBD	Subcutaneous	3 mg/kg	Open field test	THC elicited anxiolytic-like effect	(Utl et al., 2018)					
		Intracerebroventricular dlPAG	2.0 μg/μl	Elevated plus maze	Attenuation of anxiogenic behavior by CBD	(Bitencourt et al., 2008)					
			0.2 μL infusion	Elevated plus maze, VCT	CBD: anxiolytic effect in EPM prevented by WAY100635 but not AM251	(Campos and Guimarães, 2008)					
		Intraperitoneal	10 mg/kg for 14 days	Locomotion, conditioned emotional response, frontal cortex and hippocampus western blotting	chronic CBD anxiogenic-like effect and decrease in expression of proteins enhanced with antidepressant/anxiolytics	(ElBatsh et al., 2012)					
		BNST	15, 30, 60 nmol infusion	Elevated plus maze, VCT	CBD anxiolytic effect blocked by WAY100635	(Gomes et al., 2011)					
		Intraperitoneal	10 mg/kg	Cardiovascular responses, freezing behavior, elevated plus maze	CBD attenuates anxiety related effects	(Resstel et al., 2006)					
		Intraperitoneal	1, 20, 20 mg/kg	Restraint stress, elevated plus maze	CBD attenuates anxiogenic response from RS and WAY100635 blocked the effects of CBD	(Resstel et al., 2009)					
	Δ9-THC, CBD, CBDA	Intraperitoneal	0.1–10 mg/kg, 5 mg/kg, 0.1–100 μg/kg	Footshock stress exposure, elevated plus maze, light-dark box	CBD and CBDA reduce anxiogenic-like behavior caused by prior stress; THC does not reduce anxiogenic-like behavior caused by prior stress.	(Rock et al., 2017)					
Rats & mice	Δ9-THC	Intraperitoneal	0.3–10 mg/kg, 1–20 mg/kg	Elevated plus maze	THC antagonists functioned to diminish the anxiogenic effects of THC	(Onaivi et al., 1990)					
		Intraperitoneal	2.5, 20, 40 mg/kg	Conditioned fear	THC induced a conditioned fear stimulus evoked response delay	(Robichaud et al., 1973)					

(continued on next page)

Table 1 (continued)

Anxiety and phytocannabinoids							
Subject	Constituent	Route/brain area	Dose	Relevant task	Results	Study	
Mice	Δ9-THC	Intraperitoneal	0.3 mg/kg	Stress exposure, light-dark box test	THC prevents anxiety-like behavior caused by 2-AG depletion	(Bedse et al., 2017)	
		Systemic injection (route unclear)	0.25 mg/kg	Footshock stress exposure, elevated plus maze, light-dark box test, open field test	THC reduced anxiety-like behavior; promotes stress resilience		(Bluett et al., 2017)
			Smoke inhalation	Cigarettes (5.7% THC, 0.02% CBD)	Open field test, elevated plus maze	Cannabis smoke exposure decreased anxiety-like behavior	(Bruijnzeel et al., 2016)
			Intraperitoneal	10 mg/kg	Open field test	THC-locomotor sensitive mice had increased anxiety when given THC	(Kasten et al., 2018)
			Intraperitoneal	10 mg/kg	Elevated plus maze, open field activity	THC resulted in anxiogenic activity	(Kasten et al., 2017)
			Intraperitoneal	3, 10 mg/kg	Open field, social interaction, acoustic startle response, prepulse inhibition	Acute doses of THC lead to increases in anxiety	(Lloyd et al., 2018)
			Subcutaneous	3, 6, 12 mg/kg	Conditioned fear	Simultaneous THC and stress exposure in adolescence lead to increased anxiety in adulthood	(Saravia et al., 2019)
			Intraperitoneal	10 mg/kg	Elevated plus maze, Y-maze, staircase paradigm	THC lead to decreased levels of anxiety	(Schreiber et al., 2018)
		CBD	Intraperitoneal	20 mg/kg	Elevated plus maze	CBD produce anxiolysis	(Myers et al., 2018)
		Δ9-THC, CBD	Intraperitoneal	3 mg/kg, 3 mg/kg	Open field, elevated plus maze	Chronic THC administration led to delayed increase in anxiety, CBD alone had no effect	(Murphy et al., 2017)
		Intraperitoneal	10 mg/kg, 10 mg/kg	Open field test, light-dark box test, social interaction test, prepulse inhibition	CBD attenuated THC effects on anxiety	(Todd and Arnold, 2016)	

anxiolytic effects either individually, or in combination. Studies investigating the effects of cannabis on anxiety in humans have focused on the role of two key exogenous phytocannabinoids: THC and CBD. It appears as though these constituents may work somewhat in opposition of one another, in which higher doses of THC are anxiogenic and CBD can be anxiolytic and counteract the negative effects of THC (Zuardi et al., 2006). With lower doses of THC, participants have been noted to experience positive effects of cannabis including relaxation, decreased subjective anxiety, and decreased catastrophizing and rumination, whereas at higher doses, participants report increases in anxiety using the visual analogue scale (VAS; Zuurman et al., 2009; D'Souza et al., 2004). One study assessed the behavioral and endocrine effects of intravenous THC in healthy individuals and found several psychotomimetic effects including: positive and negative symptoms similar to those found in schizophrenia, increased anxiety, feelings of euphoria, and impaired memory and cognition (D'Souza et al., 2004). Importantly, this study also identified increased levels of plasma cortisol as a result of THC administration (D'Souza et al., 2004). More recently however, oral CBD administration prior to intravenous THC administration inhibited THC-induced paranoia, onset of psychotic-related symptoms, and impaired memory, indicating that CBD can mitigate the adverse effects of THC (Englund et al., n.d.; Englund et al., 2013). In addition to CBD, another phytocannabinoid, Δ -9-tetrahydrocannabinol (THCV), was assessed for its potential protective effects against intravenous THC. Orally-administered THCV before intravenous THC administration prevented the significant increases in psychotic symptoms, paranoia, impaired short-term memory, or heart rate, similarly demonstrating some protective effects of THCV against THC (Englund et al., 2016).

In humans, self-report questionnaires such as the Profile of Mood States (POMS) and the VAS are useful for assessing subjective changes in interoceptive state following cannabis exposure – including those associated with anxiety. For example, one recent study found that greater availability of CB₁ receptors in the amygdala is related to greater levels of THC-induced self-report of anxiety (Bhattacharyya et al., 2017a). This finding indicates that depending on the extent of activation of the eCB system by THC, individuals may experience a range of changes in interoceptive state, including the cognitive interpretation of feelings of anxiety. However, such subjective effects can also be altered by cognitive expectancies. That is, when subjects were told they would receive placebo but were instead given cannabis, there was an 'inherent conflict between the interoceptive cues from significant drug-induced physiological arousal and the instructional set' resulting in increased anxiety (Metrik et al., 2011). Psychosocial stress tests also provide evidence of biphasic self-reported changes in interoceptive awareness related to anxiety following cannabis use. In one study, participants completed several questionnaires that measure anxiety-related subjective effects of THC consumption including: feelings of distress, drug effects, and pre- and post-task appraisals of personal performance (Childs et al., 2017). Participants also completed standardized questionnaires including the State Trait Anxiety Inventory, Perceived Stress Scale, and Perceived Stress Reactivity Scale. Importantly, these self-report measures of anxiety were validated by participants having to complete stress-inducing tasks including a mock job interview and a difficult math activity. While low-dose THC exposure reduced feelings of stress and anxiety, the individuals who received high-dose THC not only reported more stress before and during the completion of the assigned psychosocial tasks, but they also demonstrated poorer performance during the mock interview when compared to the placebo group (Childs et al., 2017).

Another reason why current research regarding the biphasic effects of cannabis on anxiety is quite subjective is because administration of exact quantities of cannabis (or its constituents) is hard to control for. In some cases, depending on the geographical location and sociopolitical era in which the study took place, this subjectivity is attributable to regulatory restrictions of cannabis, meaning administration of cannabis

cannot be included into the experimental protocol. In these studies, investigators do not regulate dose, route of administration, or relative concentrations of cannabis constituents, making cannabis use incredibly varied from participant to participant. Furthermore, variations in chemical makeup across strains of cannabis can make it difficult to disambiguate the link between anxiety-related behaviors and the pharmacological action of the cannabis being administered. Recent legalization of cannabis and its constituents in Canada, however, opens up the opportunity to conduct controlled trials of cannabis, THC, and CBD administration in order to explore and elucidate the relationship between the biphasic, dose-related effects of cannabis on anxiety.

5.2. Animal models

Unlike human participants, non-human animal subjects are incapable of directly communicating subjective changes in interoceptive state after cannabis administration. However, indirect measures of interoceptive state via their measurable behavior in tasks like the elevated plus maze, defensive withdrawal test, light-dark choice, open-field tests, conditioned taste aversion, fear conditioning, and conditioned place preference/aversion tests have demonstrated utility in assaying emotional reactivity in rodent models and provide insight into the aversive or appetitive nature of their environments and experiences. THC has been shown to generally produce conditioned place aversions at higher doses, but can produce conditioned place preferences at lower doses (Murray and Bevins, 2010), further highlighting the variable nature of this drug as a subjective interoceptive stimulus. In the EPM, typically shaped as a plus sign or a "T" having some ends of it open while the others are enclosed (Fokos and Panagis, 2010), animals spending an increased amount of time in enclosed arms and decreased time in the open arms are considered to be demonstrating an anxiety-like response. This assay has been particularly useful in revealing the anxiogenic effects of THC in both rats and mice (Onaivi et al., 1990). Similar to that found in human research, high doses of THC elicit anxiolytic responses whereas lower doses produce anxiogenic responses (Stopponi et al., 2014; Martin-Santos et al., 2012a; Harte-Hargrove et al., 2012). One study even found evidence that females may be more sensitive to the anxiogenic effects of THC (Harte-Hargrove et al., 2012), warranting extensive further research into the sex-dependence of THC effects. Anxiogenic effects of high-dose THC is opposite to that of the anxiolytic GABAergic agent, diazepam, and to CBD (Rock et al., 2017; Bitencourt et al., 2008; Campos et al., 2012a). CBD has also been shown to attenuate behavioral and physiological responses to acute restraint stress via activation of serotonin 5HT_{1A} receptors (Resstel et al., 2009) and it has been suggested that one of the properties of CBD may be attenuating or protecting against the adverse effects of THC (Niesink et al., 2013). Fear conditioning as a purported model of anxiety can also be an effective tool for assessing behavioral, neurological, and physiological responses to fear memory. Typical behaviors associated with re-exposing an animal to a context or cue that was previously paired with an aversive stimulus include freezing, response suppression, increased mean arterial pressure, and increased heart rate. The impact of THC on various measures of conditioned fear in different species has been mixed. In monkeys, THC has been shown to reduce physiological but not behavioral expression of conditioned fear (McLendon et al., 1976), but in rats, THC induced a fear stimulus-evoked response delay (Robichaud et al., 1973). Like diazepam, CBD attenuates anxiety-related effects (i.e., conditioned freezing and cardiovascular responses) evoked by an aversive context in a fear conditioning task (Resstel et al., 2006), a finding dependent on the prelimbic cortex (Lemos et al., 2010). Cannabis extract itself, which purportedly would include both THC and CBD, also attenuates conditioned fear expression in rats (González et al., 1972a).

While these tools are particularly useful for observing anxiety/avoidance-related behavioral effects of phytocannabinoids, the identification of specific neuroanatomical sites underlying these effects are

still being elucidated. One study demonstrated that modulation of eCB receptor activity by THC in areas of the brain dealing with emotion (i.e., the prefrontal cortex, basolateral amygdala, and ventral hippocampus) influenced anxiety-related outcomes on behavior (Rubino et al., 2008). According to this study, low doses of THC microinjected into the prefrontal cortex and ventral hippocampus elicited an anxiolytic response in rats during the EPM, with the opposite effects at high doses. In contrast, low-dose THC was anxiogenic when it was microinjected into the basolateral amygdala (Rubino et al., 2008). Further, in a fear conditioning task, microinfusions of THC into the nucleus accumbens shell dose-dependently potentiated freezing evoked by a shock-associated odor stimulus (Fitoussi et al., 2018). The role of specific brain structures and the underlying neural mechanisms involved in mediating the effects of CBD on anxiety are also being examined (Campos et al., 2012b). For example, the bed nucleus of the stria terminalis appears to be a target wherein the anxiolytic effects of CBD may produce its effects, with 5-HT_{1a} receptors being implicated in this process (Gomes et al., 2011).

The efficacy of THC as an early post stress-exposure therapeutic intervention was investigated in an animal model of posttraumatic stress disorder (PTSD) (Mayer et al., 2014). When THC is administered 1 h post predator scent stress (PSS) it is only effective in attenuating short term PTSD-like behavioral stress responses, whereas 24 h to 7 days later there was no persistent attenuation of these stress responses. This effect may be a result of the ability of THC to blunt the HPA-axis response in the first hours following exposure. Conversely, while CB₁ receptor antagonist AM251 is anxiogenic without exposure to PSS, administration 1 h after PSS results in a reversal of these stress responses (Mayer et al., 2014). These results suggest a role of eCBs in glucocorticoid regulation of the memory of traumatic experiences and help reveal potential targets for therapeutic outcomes using CBs. Finally, chronic THC exposure in adolescence results in increased anxiety-like behavior, and although acquisition and baseline levels of self-administration of heroin do not differ, chronic THC in adolescence resulted in an increased vulnerability to stress-induced relapse to heroin seeking (Stopponi et al., 2014). These studies demonstrate the range of limbic and stress circuitry that are impacted by THC administration.

6. Interoception, anxiety, and cannabinoids

A link between the interoceptive insula and affective disorders including depression, bipolar disorder, psychosis, and anxiety has been established but remains poorly understood. Patients with these mental health disorders display reduced grey matter volume (GMV) in the left anterior insula (AI) that is associated with deficits in neurocognitive performance, changes in the right AI GMV are associated with symptom severity, and there is a positive correlation between AI GMV and social anxiety (Hatton et al., 2012). Furthermore, increased right AI activation, as measured by fMRI, is positively correlated with the degree of social anxiety and neuroticism and negatively correlated with agreeableness and extraversion, possibly via mediation of the left thalamus (Terasawa et al., 2013). Increased activation in the IC and other emotion processing regions like the amygdala are observed in anxiety-prone individuals during processing of emotional faces as compared to healthy controls, and higher scores on anxiety measures are correlated with increased bilateral IC activation (Stein et al., 2007). Anxiety-prone individuals that exhibit less perceived control show greater activity in the dorsal AI, and the anticipation of an unpredictable threat was associated with increased skin conductance and self-reported anxiety levels as well as activation in bilateral IC, anterior midcingulate cortex, and bed nucleus of the stria terminalis (BNST) (Alvarez et al., 2015). This suggests that perhaps one of the ways the IC can exert its effects on anxiety is via a decrease in perceived control and increase in interoceptive awareness. In regards to morphine abstinence-related anxiety-like behavior, glutamatergic neurons in the mouse medial IC are significantly activated during withdrawal which is also associated with

increased anxiety-like behaviors, and lesioning the medial IC attenuates these anxiety-like behaviors (Ma et al., 2018). Thus, insular structure and function is associated with anxiety, but it remains unclear if these alterations are a result of anxiety, or, alternatively, if they precede the occurrence of anxiety traits. These results also support the idea that increased interoceptive awareness is associated with an increase in anxiety symptoms and severity.

Interactions between the insula and the effects of CB administration have also been reported. Following administration of THC, increases in blood oxygen level-dependent (BOLD) levels in the IC have been reported and baseline brain activity was shown to be altered, evidenced by increased amplitude of fluctuations in the resting-state fMRI signal in the IC (van Hell et al., 2011). Therefore, the IC may play a role in the subjective effects of the “high” associated with THC administration. Chronic cannabis users display higher anxiety scores, neurocognitive impairments, and an enhancement in functional connectivity in left AI compared to control subjects (Pujol et al., 2014). Additionally, adolescents with alcohol and cannabis substance use disorders (SUDs) appear to be hypersensitive to interoceptive stimuli that are aversive in nature (Berk et al., 2015), which may be one mechanism whereby aberrant interoceptive processes evident in individuals with SUDs may impact the anxiety-like behavioral outcome. Increases in self-reported ratings of anxiety and positive psychotic symptoms have also been associated with THC administration compared to placebo, with a trend in the opposite direction for CBD, consistent with the divergent effects of THC and CBD discussed previously (Borgwardt et al., 2008). Indeed, unlike THC, CBD administration exerts an inhibitory effect on left IC activity (Borgwardt et al., 2008). If the CB₁ receptor agonist THC can have these effects on functional connectivity in relation to anxiety and interoception, and CBD can have opposing effects, perhaps pharmacological manipulation within interoceptive regions, including the IC, could attenuate or reverse these effects. The CB receptors, their ligands, and enzymes that break down these neurotransmitters within brain regions associated with interoception are therefore all potential therapeutic targets to modulate resulting anxiety.

The neural mechanisms mediating the effects of the IC on anxiety and the role that the eCB system plays in these processes remain unclear, however some circuits have been identified. Connectivity between the IC and stress circuitry appear to be a critical locus where eCB signalling may facilitate anxiogenic and anxiolytic responses. Projections from the IC to the dorsal bed nucleus of the stria terminalis (dBNST) appear to regulate negative affective behavior in a CB₁ receptor mediated fashion. Decreasing neuronal activation specifically in IC→dBNST projections prevent hyperreactivity in the dBNST elicited by ethanol abstinence while increases in IC→dBNST projections resulted in negative affect and enhanced dBNST c-fos expression (Centanni et al., 2019). Systemic administration of the MAGL inhibitor, JZL184, 15 days into ethanol abstinence mitigated the increases in dBNST neuronal activity, similar to when insula→dBNST projections were silenced (Centanni et al., 2019), suggesting a role for 2-AG and CB₁ receptors in mediating this effect. The BNST has been shown to play a critical role in the integration of negative valence, stress reactivity, and anxiety, and these results suggest that insular eCB signalling projecting to the BNST can modulate neuronal activity in the BNST and the resulting affective state (Lebow and Chen, 2016). Bilateral intracranial administration of another MAGL inhibitor, MJN110, in the IC interfered with naloxone-precipitated morphine withdrawal conditioned place aversion and co-administration of the CB₁ receptor inverse agonist, AM251, was able to reverse this effect (Wills et al., 2016). The processing of fear memories may also be one way that the IC exerts its effects on anxiety-like behavior and has also been shown to be mediated by the eCB system. Reactivation of a fear memory followed by activation of CB receptors by the CB₁/CB₂ receptor agonist, WIN55,212-2, in the IC resulted in enhanced fear extinction and attenuated reconsolidation of the fear memory (Zubedat and Akirav, 2017). Perhaps the role of CB₁/CB₂ receptors within the IC during the processing of fearful memories or

stimuli are mechanisms whereby the IC exerts its effects on anxiety and may be a potential target for novel pharmacological therapies.

7. Concluding remarks

The current paper reviews some of the research investigating the role of the eCB system, endogenous cannabinoids, and exogenous cannabinoids on interoception and anxiety-like behavior in human and animal models. Despite its various medicinal and therapeutic effects, cannabis has also been known to have potential negative effects on mental health including high correlation with the incidence psychotic disorders such as schizophrenia, as well as affective disorders including depression and anxiety (National Academies of Sciences, Engineering, and Medicine, 2017b; Agosti et al., 2002; Stinson et al., 2006; Zvolensky et al., 2008; Moore et al., 2007; Duperrouzel et al., 2018). Further, the subjective interoception of CB receptor activation elicited by THC and related agonists appears to be dose dependent with higher doses increasing perception and expression of aversive qualities. The neural substrates of such effects are beginning to be elucidated and have focused thus far on fear memory (e.g., amygdala), extinction (e.g., prefrontal cortex), and reward (e.g., nucleus accumbens) circuitry. However, less published work thus far appears to target the IC, a brain region expressing CB receptors that is necessary for various interoception mechanisms. Notably, activity in this region is related to the subjective self-report of feeling a ‘high’ elicited by THC consumption (van Hell et al., 2011), warranting further investigation of interoception and CB receptor activity. Alterations in the structure and function of the IC have been associated with anxiety-like behavior and are prevalent in anxiety-prone individuals (Stein et al., 2007; Terasawa et al., 2013; Hatton et al., 2012; Alvarez et al., 2015), suggesting a link between increased interoceptive awareness and increased anxiety symptoms and severity. Cannabis use also appears to impact the IC and anxiety activity, such that cannabis users exhibit increased anxiety scores, enhanced functional connectivity in left AI (Pujol et al., 2014), and hypersensitivity to aversive interoceptive stimuli (Berk et al., 2015). THC and CBD appear to have opposing effects on IC activity such that CBD inhibits left IC activity, where THC does not (Borgwardt et al., 2008). Connectivity between the IC and stress circuitry, particularly the BNST, appears to be a critical circuit where eCB signalling may facilitate anxiogenic and anxiolytic responses (Centanni et al., 2019). Although these insights are promising, overall, the research investigating CB effects in relation to anxiety and interoception is still somewhat limited. Much of this limitation has been discussed previously within the context of strict regulatory policies for cannabis (National Academies of Sciences, Engineering, and Medicine, 2017a; Covey et al., 2015; Ware, 2018; Nutt et al., 2013; National Academies of Sciences, Engineering, and Medicine, 2017b). Hopefully, many of these barriers to research will fall as medicinal and recreational cannabis slowly, but steadily, loses its illicit status. With the recent legalization of recreational cannabis consumption across Canada and the steady state-by-state legalization in the United States, the need to understand how cannabis and its constituents interact with the brain and impact behavior has become far more urgent. Such knowledge will guide policy decisions about best practice regarding safety advisements and legal considerations regarding the use of cannabis.

Abbreviations

2-AG	2-arachnidonoyl-glycerol
2-AGE	2-arachidonyl glyceryl ether; 2-AGE
5-HT	5-hydroxytryptamine; serotonin
AEA	anandamide, <i>N</i> -arachidonylethanolamine
AI	anterior insula
ARCI	addiction research center inventory
BOLD	blood oxygen level-dependent
BNST	bed nucleus of the stria terminalis

CADSS	clinician administered dissociative symptoms scale
cAMP	cyclic adenosine monophosphate
CAPE	community assessment of psychic experience
CB	cannabinoid
CB ₁	cannabinoid receptor 1
CB ₂	cannabinoid receptor 2
CBD	cannabidiol
CBG	cannabigerol
CNS	central nervous system
CRH	corticotropin releasing hormone
dBNST	dorsal bed nucleus of the stria terminalis
eCB	endocannabinoid
EPM	elevated plus maze
FAAH	fatty acid amide hydrolase
GABA	gamma amino butyric acid
GMV	grey matter volume
GPCR	G-protein coupled receptor
GPR119	G protein-coupled receptor 119
GPR55	G protein-coupled receptor 55
HPA	hypothalamic-pituitary-adrenal
IC	insular cortex
MAGL	monoacylglycerol lipase
NADA	<i>N</i> -arachidonoyldopamine
OEA	oleoylethanolamide
OAE	virodhamine, <i>O</i> -arachidonoyl ethanolamine
PANSS	positive and negative symptom scale
PASA	primary appraisal secondary appraisal
PFC	prefrontal cortex
POMS	profile of mood states
PTSD	posttraumatic stress disorder
RS	restraint stress
SPST	simulation public speaking test
SSPS-N	negative self-statement scale
STAI	state trait anxiety inventory
SUD	substance use disorder
THC	Δ -9-tetrahydrocannabinol
THCV	Δ -9-tetrahydrocannabivarin
TSST	trier social stress test
VAMS	visual analogue mood scale
VAS	visual analogue scale
VCT	Vogel conflict test

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