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**Seeing through the smoke: human and animal studies of cannabis use and
endocannabinoid signalling in corticolimbic networks**

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Highlights

- Corticolimbic circuits mediate cognitive and emotional behavioural processes
- Endocannabinoid activity, and thus exogenous cannabinoids, modulate their function
- The prefrontal cortex, amygdala and ventral hippocampus are key neural loci
- Individual variation in cannabis response is an important consideration
- Both human and animal studies are vital components of such research

Abstract

Public opinion surrounding the recreational use and therapeutic potential of cannabis is shifting. This review describes new work examining the behavioural and neural effects of cannabis and the endocannabinoid system, highlighting key regions within corticolimbic brain circuits. First, we consider the role of human genetic factors and cannabis strain chemotypic differences in contributing to interindividual variation in the response to cannabinoids, such as THC, and review studies demonstrating that THC-induced impairments in decision-making processes are mediated by actions at prefrontal CB₁ receptors. We further describe evidence that signalling through prefrontal or ventral hippocampal CB₁ receptors modulates mesolimbic dopamine activity, aberrations of which may contribute to emotional processing deficits in schizophrenia. Lastly, we review studies suggesting that endocannabinoid tone in the amygdala is a critical regulator of anxiety, and report new data showing that FAAH activity is integral to this response. Together, these findings underscore the importance of cannabinoid signalling in the regulation of cognitive and affective behaviours, and encourage further research given their social, political, and therapeutic implications.

Key words/phrases

cannabis; phytocannabinoids; endocannabinoid system; THC; cannabidiol; CB₁ receptor; AEA; FAAH; corticolimbic circuits; prefrontal cortex; amygdala; hippocampus; individual differences; decision-making; social interaction; emotional salience; associative learning; anxiety; elevated plus maze; striatum; nucleus accumbens; ventral tegmental area; dopamine; reward processing; COMT; NRG1; schizophrenia; executive function; Iowa Gambling Task; delay-discounting; risk-discounting

1. Introduction

In recent years, the social and political landscape surrounding cannabis use has been the focus of heightened scrutiny. Cannabis is the most widely used illicit drug with an estimated 180 million adults using the drug annually (SAMSHA, 2014; UNODC, 2015). However, a growing number of countries and jurisdictions have reformed cannabis laws so that personal consumption of the drug is no longer severely punishable (UNODC, 2015), while others have legalized its use for medicinal purposes. Recreationally, the drug is used for the “high” it produces, which includes feelings of relaxation and euphoria. However, these effects are biphasic, and in some individuals this high can manifest as anxiety, impaired cognition, and psychotic-like states such as in schizophrenia. Such adverse effects highlight the risks associated with cannabis exposure, with a number of studies showing that prolonged use may lead to adverse life outcomes and possible dependence in select users (American Psychiatric Association, 2013; Fergusson and Boden, 2008; Horwood et al., 2010). While for others, cannabis use may provide therapeutic benefits for the relief of pain, spasticity, nausea and vomiting.

The psychoactive effects of cannabis are primarily mediated by Δ^9 -tetrahydrocannabinol (THC), which is one of at least 70 phytocannabinoids found in the plant (Elsohly and Slade, 2005). THC binds to the presynaptic CB₁ receptor that, together with CB₂ receptors and the endogenous cannabinoids, 2-arachidonylglycerol (2-AG) and N-arachidonylethanolamine (anandamide; AEA), comprise the endocannabinoid system. CB₁ receptors are located in key regions throughout corticolimbic brain networks, such as the prefrontal cortex (PFC) and amygdala, which functionally interact with subcortical dopamine pathways (Tan et al., 2014). As such, aberrations of the endocannabinoid system are increasingly recognized as etiological factors in several neuropsychiatric syndromes, including schizophrenia, anxiety, and mood disorders (Bossong and Niesink, 2010; D'Souza

et al., 2005; Hillard and Liu, 2014; Lutz et al., 2015; Papini et al., 2015; Passie et al., 2012; Saito et al., 2013; Semple et al., 2005; Smit et al., 2004; Tan et al., 2014). These conditions may involve deficits in executive function, emotional processing and social behaviours, and/or co-morbidities with affective or addiction-related phenomena—in essence, broad deficits in behavioural processes mediated by corticolimbic circuits. Indeed, growing evidence from clinical and preclinical research demonstrates that CB₁ receptor transmission within these networks strongly regulates the expression of cognitive and emotional behaviours (Arnold et al., 2012; Crane et al., 2013; Hajos and Freund, 2002; Hillard and Liu, 2014; Laviolette and Grace, 2006b; Lutz et al., 2015; Papini et al., 2015; Pattij et al., 2008; Tan et al., 2014)

In this review we will highlight key brain loci that are modulated by cannabinoid transmission, which may subserve the cognitive-impairing, pro-psychotic, and anxiety-regulating actions of cannabis. Given that not all individuals experience adverse effects, we will first describe factors that mediate interindividual variation in the behavioural response to cannabinoid drugs. This includes a number of identified gene variations, as well as different phytocannabinoids in the plant itself, which can modulate the neural and behavioural effects of THC. We will also review evidence suggesting that acute and/or regular THC exposure impairs frontal-cortical functioning, as evidenced by deficits in executive abilities following use. This section specifically focuses on the effects of cannabinoids in both clinical and preclinical models of decision-making, given that optimal cost/benefit decision-making is mediated by corticolimbic circuits. Focus will then shift to the endogenous cannabinoid system, and how CB₁ signalling in the PFC and ventral hippocampus (vHIPP) modulates mesolimbic dopamine activity, dysregulation of which may underlie the emotional processing deficits observed in psychotic disorders like schizophrenia. Lastly, we will review data suggesting that variations in amygdalar endocannabinoid signalling could contribute to

vulnerability to anxiety-related disorders and trait anxiety. Each section reflects a symposium presentation at the 2015 International Behavioural Neuroscience Society meeting in Victoria, BC, Canada, and all include background literature and new data; readers are guided to more comprehensive reviews on the subject throughout each section.

2. Individual differences in response to cannabis: contribution of genetic factors and strain differences

It is clear from human research that there is great interindividual variation in response to cannabis and the cannabinoids. For some, cannabis use is pleasurable and enhances creative thinking, while for others it may provoke anxiety, panic, memory loss and, in rare instances, psychotic-like states. In this section we will review research that seeks to explain variation in response to cannabinoid exposure. One explanation for this variation is genetic disposition, with some individuals being genetically prone to the adverse actions of cannabinoids, while others may be resilient. We will examine clinical and preclinical evidence that demonstrates specific genes modulate the neurobehavioural actions of THC. Another explanation for divergent cannabinoid response centres on the type of cannabis people choose to smoke. Cannabis' psychoactive effects are primarily mediated by THC, but growing evidence highlights that other phytocannabinoids in the plant have unique properties that may modulate the actions of THC. Both human and clinical studies suggest cannabidiol (CBD) may protect against some of the adverse actions of THC on the brain. Therefore, cannabis with a balance of CBD and THC may be better tolerated than high THC varieties. We will also briefly review preclinical research that assesses whether non-psychoactive phytocannabinoids like CBD modulate the effects of THC, and the possible molecular mechanisms that explain these interactions.

2.1 Probing the genetic basis for interindividual response to cannabinoids

The isolation of genes that modulate cannabinoid action on the brain and behaviour may help explain why only a small proportion of cannabis users develop drug dependence or psychosis. Characterisation of vulnerability genes to cannabis-induced addiction or mental illness could pave the way for preventative approaches where those at genetically high risk could be forewarned of the potential danger of experimentation with cannabis. In addition, one of the major impediments to the therapeutic development of cannabinoid medicines is their tendency to increase the risk of addiction and psychosis. Therefore, a pharmacogenetic approach would allow those at risk of detrimental effects to be isolated, leaving low risk patients to benefit from cannabinoid-based therapy.

Genome-wide association studies (GWAS) and analysis of specific single nucleotide polymorphisms (SNPs) has yielded numerous candidate genes that may moderate the risk of developing cannabis-induced psychosis or addiction. Interestingly there is an emerging picture that genes implicated in the pathophysiology of schizophrenia also contribute to the risk of cannabis dependence and vice versa. For example, the largest GWAS to date examining risk genes for cannabis dependence isolated the novel antisense transcript *RP11-206M11.7*, the solute carrier family 35 member G1 gene (*SLC35G1*); and the CUB and Sushi multiple domains 1 gene (*CSMD1*) (see Table 1) (Sherva et al., 2016). Notably, *CSMD1* is expressed in developing neurons, may regulate the complement system, and has been associated with increased schizophrenia risk (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Other genes that have been linked to cannabis dependence are endocannabinoid system genes such as the cannabinoid receptor 1 gene (*CNR1*) and the fatty acid amide hydrolase gene (*FAAH*) (Buhler et al., 2015). Increased risk of cannabis-induced psychosis has been linked to the catechol-O-methyltransferase gene (*COMT*), the dopamine D2 receptor gene (*DRD2*), the brain derived neurotrophic factor (*BDNF*) gene and the protein

kinase B (*AKT1*) gene (Caspi et al., 2005; Colizzi et al., 2015; Decoster et al., 2011; Di Forti et al., 2012; van Winkel, 2011).

GWAS are limited because they cannot infer causation and they seldom probe the molecular, cellular and brain anatomical basis for gene-environment (G×E) interactions in cannabis use disorders. Animal studies have assisted in this regard, enhancing the biological plausibility of G×E interactions by allowing causative links to be drawn and increasing our understanding of the biological mechanisms involved. Here we will review examples of forward and back translation between human and animal research findings that have greatly improved our understanding of the biological bases of gene-cannabinoid interactions.

The identification of susceptibility genes for schizophrenia, such as neuregulin 1 (*NRG1*) and *COMT*, led to the development of animal models with targeted deletions of these genes. In a seminal study, mice with heterozygous deletion of *Nrg1* (*Nrg1* HET mice) displayed increased sensitivity to the behavioural actions of THC (Boucher et al., 2007a). Notably, THC selectively improved sensorimotor gating function in *Nrg1* HET mice but not wild type mice, as measured in the prepulse inhibition of startle paradigm (PPI). The results with THC were also confirmed with a synthetic analogue of THC, CP 55,940, which acutely promoted PPI deficits in wild-type mice, but facilitated PPI in *Nrg1* HET mice (Boucher et al., 2011). The atypical effect of THC on *Nrg1* HET mice appeared to be dependent on stress and correlated with increased activation of stress circuitry in the brain, with pronounced effects in the lateral septum (Boucher et al., 2007b). Interestingly, more recent studies reported that stress decreased expression of *Nrg1* in the brain (Brydges et al., 2014; Makinodan et al., 2012), and partial genetic deletion of *Nrg1* altered behavioural and neurobiological responses to stress (Chohan et al., 2014a; Chohan et al., 2014b; Desbonnet et al., 2012; Taylor et al., 2011).

Nrg1 also moderates the neuroadaptive responses to repeated cannabinoid exposure. *Nrg1* HET mice more rapidly developed tolerance to cannabinoid-induced hypothermia and locomotor suppression than wild-type mice (Boucher et al., 2011). Conversely, *Nrg1* HET mice were resistant to development of tolerance to cannabinoid-induced anxiety-related behaviour. These effects again correlated with a selective induction of Fos transcription factors in the lateral septum of *Nrg1* HET mice. This brain region interfaces with numerous corticolimbic components such as the prefrontal cortex, hippocampus, amygdala and hypothalamus. Repeated THC exposure during adolescence exacerbated the hyperlocomotor phenotype of *Nrg1* HET mice in adulthood and altered the expression of various neurotransmitter receptors (Long et al., 2013). For example, THC exposure selectively increased NMDA receptor expression in the hippocampus of *Nrg1* HET mice, which was associated with increased expression of proteins that transport and stabilise NMDA receptors at the synaptic membrane (Spencer et al., 2013). Studies also suggest Nrg1 influences the endocannabinoid system. *Nrg1* modulated the effects of adolescent THC exposure on the expression of CB₁ receptors (Long et al., 2013) and NAPE-PLD, an enzyme that synthesizes the endocannabinoid anandamide (Spencer et al., 2013). In addition, Nrg1 exposure increased the expression of MAGL, which decreased levels of the endocannabinoid 2-AG and impaired long-term depression in the hippocampus (Du et al., 2013).

The findings that *Nrg1* modulates the effects of cannabinoids in mice have been translated to humans. For example, *NRG1* SNPs were shown to worsen THC-induced information processing dysfunction (Stadelmann et al., 2010) and increase the risk of developing cannabis dependence (Han et al., 2012). Another example of a gene that influences cannabinoid action is *COMT* which encodes an enzyme that catabolises dopamine. Variation in *COMT* was first discovered to increase the risk of developing positive symptoms of schizophrenia in humans (Caspi et al., 2005; Pelayo-Teran et al., 2012; Radhakrishnan et

al., 2014). In addition, SNP of *COMT* moderated THC-induced impairments in executive function (Verdejo-Garcia et al., 2007) and working memory (Tunbridge et al., 2015). *COMT* did not influence reversal learning deficits induced by inhalation of cannabis vapour (Spronk et al., 2015), highlighting that genetic modulation of THC's effects may vary dependent on the presence of other phytocannabinoids. However, this discrepancy may also be simply explained by the lower bioavailability of smoked cannabis compared to intravenous THC, or the greater variation conferred by intersubject variability in smoking dynamics such as inhalation volume and depth. The work of Waddington and colleagues subsequently back translated the human findings showing *COMT* knockout mice display altered behavioural sensitivity to cannabinoids (for a detailed review see O'Tuathaigh et al., 2014). Moreover their research enhanced our neurobiological understanding of the *COMT*-cannabinoid interaction by showing *COMT* mice were more vulnerable to various schizophrenia-relevant neurobiological changes promoted by cannabinoids, such as reductions in parvalbumin-positive GABAergic interneurons, and decreased dopaminergic cell size in the ventral tegmental area of the mesolimbic system.

It is also possible that variation in genes that influence the pharmacokinetics of cannabinoids might play a role in moderating the risk of developing cannabis dependence or psychosis. ABC transporters are drug efflux pumps expressed by blood brain barrier endothelial cells and function to move substrate drugs from brain tissue into the peripheral blood supply. Polymorphism of *MDR1*, the gene that encodes the ABC transporter P-glycoprotein (P-gp), increased the risk of developing cannabis dependence (Benyamina et al., 2009). Mouse studies then showed that THC is a P-gp substrate, as P-gp knockout mice displayed greatly enhanced brain concentrations and hypothermic effects of THC compared to wild-type mice (Spiro et al., 2012). Thus it is likely that SNP of *MDR1* alters the brain uptake of THC in cannabis users, thereby influencing the risk of developing cannabis dependence.

This provides yet another example of the importance of cross-translational research in improving our understanding of how genes contribute to the moderation of risk for cannabis use disorders.

2.2 Examining **non-psychoactive phytocannabinoid** modulation of the pharmacological effects of THC

Aside from genetic factors, individual differences in response to cannabis use are dictated by different cannabis plant strains with their varying compositions of phytocannabinoids. Concentrations of THC in cannabis have dramatically increased over the last 30 years and CBD levels have significantly decreased (Arnold et al., 2012; UNODC, 2015). Consistent with potency data collected in the US and UK, Australian street cannabis contains on average 15% THC with only 0.1 % CBD content (Swift et al., 2013). This may be of significant public health concern, as a growing body of evidence suggests that CBD may protect against the adverse actions of THC.

CBD content does not appear to modulate the subjective high caused by cannabis ingestion (Hindocha et al., 2015; Morgan et al., 2010a). Although, administration of high CBD:THC strains of cannabis reduced attentional bias and liking of drug related stimuli (Morgan et al., 2010a). Further, naturalistic and controlled human studies show that CBD attenuated THC-induced memory impairment, appetite stimulation, anxiety, and psychotic-like states (Englund et al., 2013; Hindocha et al., 2015; Morgan and Curran, 2008; Niesink and van Laar, 2013; Zuardi et al., 1982). Such protective effects of CBD have prompted the viewpoint that breeding CBD back into the plant may make cannabis “healthy” (Mechoulam and Parker, 2013). This issue also pertains to medicinal cannabis that is now legal in various jurisdictions around the world, as utilizing strains with balanced CBD to THC concentrations may maximize therapeutic endpoints while minimizing side effects.

The strength of preclinical research is that it allows controlled administration of various THC and CBD dose ratios, as well as providing mechanisms for their interactions (for a detailed overview of studies see Arnold et al., 2012). Animal studies have shown that CBD and THC interactions are highly complex and various factors need to be considered: the dose ratio, specific doses used, timing of administration (whether administered as a cocktail or temporally separated) and measured endpoint (Arnold et al., 2012; Zuardi et al., 2012). A number of studies indicate that a cocktail of CBD and THC may have greater therapeutic benefits than each of the compounds alone. For example, CBD has been shown to enhance the analgesic effects of THC (Varvel et al., 2006). In addition, combined subthreshold doses of CBD and THC additively disrupted reconsolidation of fear memory in rats (Stern et al., 2015), which may hold promise for cannabis-based pharmacotherapies in individuals with post-traumatic stress disorder.

Preclinical studies have also investigated whether CBD inhibits the adverse effects of THC. For example, CBD was shown to hinder the aversive effects of THC in rodents as measured in the conditioned place aversion paradigm and in animal models of anxiety (Todd and Arnold, 2015; Vann et al., 2008). However, there is conflicting evidence for whether CBD protects against THC-induced impairments in memory. Administration of CBD potentiated the impairing effects THC on spatial memory in mice in the radial arm maze (Hayakawa et al., 2008), yet reversed the impairing effects of THC on spatial working memory in rats in a delayed-matching-to-place version of the Morris water maze (Fadda et al., 2004). The latter study used cannabis extracts, with a CBD-rich extract promoting less memory impairment than a THC-rich extract, raising the possibility that other phytocannabinoids need to be considered in examining interactions between THC and CBD.

Interactions between CBD and THC on behaviour may involve a multitude of both pharmacodynamic and pharmacokinetic mechanisms. With respect to CBD-induced

augmentation of THC effects, combined THC and CBD exposure has been shown to increase CB₁ receptor expression in the hippocampus and the hypothalamus, in a manner that correlated with greater memory-impairing and hypothermic effects of THC respectively (Hayakawa et al., 2008). A more recent study could not replicate this effect in rats, but showed that CBD treatment increases brain and blood levels of THC (Klein et al., 2011). ABC transporters, mentioned in section 2.1, might play a role in this phenomenon. CBD inhibits P-gp as well as the breast cancer resistance protein (BCRP) transporter, and THC is an ABC transporter substrate (Holland et al., 2008; Holland et al., 2007; Spiro et al., 2012). Therefore, CBD might enhance THC brain levels by inhibiting ABC-mediated transport of THC from the brain to the peripheral blood supply. Given that P-gp polymorphisms moderate risk for cannabis dependence (Benyamina et al., 2009), it is clear from the data reviewed thus far that both variation in the genetics of individual users and between strains of cannabis, particularly with respect to the THC:CBD ratio, will dictate the psychobehavioural effects of cannabis use.

CBD's ability to hinder the effects of THC might be explained via cannabinoid receptor modulation. This could be achieved through indirect competition, as CBD inhibits fatty acid amide hydrolase (FAAH) which increases anandamide concentrations that would then compete with THC for the CB₁ receptor (McPartland et al., 2015; Pertwee, 2008), although this may not be a very potent effect. Alternatively, an important new finding suggests CBD inhibits the effects of THC through negative allosteric modulation of the CB₁ receptor (Laprairie et al., 2015), whereas earlier studies indicate a potential involvement of the serotonin system, particularly the 5-HT_{1A} receptor (Russo et al., 2005; Scopinho et al., 2011). At a network level, clues on the brain circuitry involved in CBD's ability to inhibit the neuropharmacological actions of THC are provided by a recent study. CBD administration robustly inhibited THC-induced c-Fos expression, a marker of neuronal activation, in the

medial preoptic nucleus of the hypothalamus, the dentate gyrus of the hippocampus and periaqueductal gray (Todd and Arnold, 2015). These brain regions are implicated in the hypothermic, memory-impairing and anxiogenic actions of THC, respectively. Future work may further explore the precise corticolimbic network mechanisms involved in the interactions of THC with CBD. The subsequent sections of this review will focus on cannabinoid and endocannabinoid actions in different regions and functional domains of corticolimbic circuits.

Future studies could also expand beyond CBD and THC interactions to investigate the interactive effects of other phytocannabinoids such as cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC) and Δ^9 -tetrahydrocannabivarin (THCV). Few studies have been conducted in this area. An early study demonstrated that CBN and THC promoted synergistic depressant effects on animals (Takahashi and Karniol, 1975). More recently, THCV was shown to antagonise some of the effects of THC in mice, such as analgesia and hypothermia, due to THCV behaving as a cannabinoid receptor antagonist (Pertwee et al., 2007). The co-administration of CBC with THC promoted additive effects on catalepsy, analgesia and inflammation in mice, which might be attributable to CBC enhancing the brain concentrations of THC (DeLong et al., 2010). Clearly more studies are needed in this area, to carefully evaluate the pros and cons of cannabinoid combination psychopharmacology, and assist in the development of medicinal cannabis and cannabinoid therapies.

3. Cannabinoid modulation of prefrontal cortical function: focus on decision-making

As described above, genetic vulnerabilities and strain composition can dictate inter-individual response to cannabinoids. This is especially true for the cognitive-impairing effects of the drug, where some individuals are more susceptible to deficits in executive functioning following acute or regular cannabis use. Indeed, the influence of cannabis on the neurocognitive domains of attention, learning, and inhibitory control have been thoroughly

investigated and reviewed elsewhere (see Crane et al., 2013). In contrast, relatively little research has investigated the effect of cannabis on decision-making processes. In its most basic form, decision-making involves evaluating and forming preferences amongst available options, making a choice, evaluating its outcomes, and subsequently using this information to guide future behavior (Ernst and Paulus, 2005). Outside the routine decisions of day-to-day life, optimal cost/benefit decision-making becomes crucial when choices carry with them the possibility of substantial gain or loss; not unsurprisingly, perturbations in decision-making are observed in almost every mental illness (Goschke, 2014). The effects of cannabis are particularly important to consider given that heavy use is associated with impaired decision-making, as evidenced by persistent use despite negative physical, psychological, social, and legal outcomes (American Psychiatric Association, 2013). Neuroimaging studies have shown that prolonged cannabis use is associated with altered activity in the corticolimbic circuitry (i.e. PFC (Wesley et al., 2011), striatum (van Hell et al., 2010), amygdala (Gruber et al., 2009)) subserving cost/benefit decision-making (Floresco et al., 2008). In this section, we will review data from human and preclinical literature on cannabinoid modulation of decision-making, and will identify important questions the field must address in light of changing attitudes towards cannabis use.

3.1 Human studies on the effects of cannabis on decision-making

Naturalistic studies in regular cannabis users have assessed decision-making in a variety of tasks following periods of short (e.g. 12-18 h) or extended abstinence (e.g. >25 days). One such task is the Iowa Gambling Task (IGT), designed to assess real-life decisions involving uncertainty, reward, and punishment. In this paradigm, participants earn as much fictitious money as possible by choosing cards from four decks, each of which leads to varying amounts of monetary gain or loss as determined by set probabilistic schedules (Bechara et al., 1994; Bechara et al., 1999). Cannabis users displayed impaired decision-

making in the IGT, being less able to bias behaviour towards the advantageous decks throughout a session (Moreno et al., 2012; Wesley et al., 2011; Whitlow et al., 2004). Functional imaging revealed that while users did not differ behaviourally from non-users during the initial strategy stage, they displayed reduced activity in the anterior cingulate and medial prefrontal cortices in response to losses (Wesley et al., 2011). Strikingly, neural responses to early IGT losses predicted future improvements in task performance in controls, but not cannabis users, suggesting that impaired decision-making may arise from an insensitivity to losses, consistent with reports that regular and heavy cannabis users show abnormalities in affective and reward processing (Gruber et al., 2009; Martin-Soelch et al., 2009; Nestor et al., 2010). Indeed, computational modelling of IGT performance in cannabis users reveals that these individuals tend to be under-influenced by loss magnitude, treating each loss as a constant and minor negative outcome regardless of the size of the loss (Fridberg et al., 2010); but see Bishara et al., 2009).

By contrast, a number of studies have shown that both adolescent and adult cannabis users show intact decision-making performance in the IGT, and often perform better than other drug-using groups, including cocaine and MDMA users (Dougherty et al., 2013; Gonzalez et al., 2012; Hermann et al., 2009; Quednow et al., 2007; Vaidya et al., 2012; Verdejo-Garcia et al., 2007). Notably, while Hermann et al. (2009) found no difference in IGT performance between cannabis users and controls, high levels of THC metabolites in hair were associated with poor performance in the final block of the task. In another study where cannabis users did not differ from controls in IGT performance, worse performance was associated with increased symptoms of cannabis dependence (Gonzalez et al., 2012). These studies suggest that while cannabis users as a group may not differ from control subjects in IGT performance, a “threshold effect” may exist, whereby decision-making impairments are prominent in heavy, but not casual, users (Bolla et al., 2005). Support for this notion comes

from data showing that IGT deficits in heavy cannabis users persist following a 25-day abstinence period, and correspond to altered patterns of neural activity in the cerebellum, lateral orbitofrontal cortex, and dorsolateral PFC, relative to controls (Bolla et al., 2005).

Naturalistic studies have also assessed decision-making in tasks other than the IGT, in which subjects must decide between two options where the more preferred reward is discounted by temporal delay, risk, or physical effort. For example, adolescent and young adult, but not older adult, cannabis users demonstrate impaired decision-making in such a delay-discounting task, opting for smaller-sooner versus larger-later rewards (Dougherty et al., 2013; Johnson et al., 2010; Moreno et al., 2012). Furthermore, cannabis users make riskier selections in a two-choice gambling task, and are less willing to exert physical effort for large monetary rewards (Griffith-Lendering et al., 2012; Lane et al., 2005a; but see Gilman et al., 2015). Collectively, these findings together with those from IGT studies indicate that cannabis use is associated with impaired decision-making, the severity of which is likely related to age and frequency of use.

Although the acute effects of cannabis on decision-making have received less empirical attention, impairments in various tasks are evident under experimental conditions. Generally no impairments in IGT performance have been observed immediately following, or up to 2.5 h post-cannabis (up to 13% THC) ingestion (Ramaekers et al., 2006; Vadhan et al., 2007); however, the sensitivity of the IGT to detect acute drug effects has been called into question, and decision-making impairments following cannabis intake are observed in other tasks. For example, Lane et al., (2005) employed a task in which subjects chose between a non-risky option associated with guaranteed small monetary reward, and a risky option where payouts were larger, but uncertain. Administration of joints containing 3.58% THC increased selection of the risky option, and made subjects persist on this option following a win or loss relative to placebo (Lane et al., 2005b). This profile suggests that THC-induced risky

decision-making may be due to an altered sensitivity to reward and loss outcomes, similar to the deficits thought to underlie the impaired IGT performance in chronic cannabis users described above (Fridberg et al., 2010; Wesley et al., 2011 but see McDonald et al., 2003). In contrast, THC did not affect delay-discounting of monetary rewards, but decreased the selection of larger rewards discounted by physical effort (Cherek et al., 2002; McDonald et al., 2003). Such acute challenge studies allow us to understand the basic cognitive processes that may be impinged upon by regular exposure to cannabis, including affective and reward processing; these emotionally-guided behaviours will be examined in more detail in section 4 with respect to the downstream effects of disrupted PFC cannabinoid signalling on subcortical dopamine activity.

An overview of the human literature reveals that regular or acute cannabis exposure is associated with impaired decision-making in some conditions but not others. These discrepancies likely relate to differing methodologies, such as the inclusion criteria used to select chronic users, the different dosing regimens used to administer THC, or by insufficient sample sizes required to detect moderate effects. In addition, issues of causation are difficult to tease apart in naturalistic human studies, as it is unknown whether repeated cannabis use per se produces decision-making impairments, or if impaired decision-making precedes, and possibly influences, the development of regular use (Bechara, 2005; Wang et al., 2013). Such issues can be better addressed in cross-translational research using preclinical models.

3.2 Animal studies on cannabinoid modulation of decision-making

The use of animal models of decision-making allows unparalleled experimental control relative to their human counterparts. In these cost/benefit decision models, rodents are typically given the choice of two options, where one is associated with small food reward and a nominal response cost, while the other option offers more reward but at a larger expense. As in human decision-making paradigms, the costs associated with a given decision can vary,

and a substantial body of work has established that valuations involving delay, risk, and effort costs are subserved by overlapping, yet distinct, circuitries (Ghods-Sharifi et al., 2009; Hosking et al., 2014; Rudebeck et al., 2006; Winstanley et al., 2003).

Research using such models has shown that administration of THC modifies decision-making involving delay costs, eliciting rats to increase choice of the larger reward option as the delay to its receipt is lengthened (Wiskerke et al., 2011). This effect was subsequently antagonized by pre-treatment with rimonabant, a CB₁ receptor antagonist/inverse agonist, indicating the THC-induced shift in choice was CB₁ receptor-dependent. Notably, administration of WIN 55, 212-2, a synthetic CB₁-preferring agonist, did not affect delay-based choice in the same task (Pattij et al., 2007), demonstrating that phyto- and synthetic cannabinoids with similar actions at the CB₁ receptor can produce divergent behavioural effects. In contrast, direct infusions of arachidonyl-2-chloroethylamide (ACEA), a potent synthetic CB₁ receptor agonist, into the orbitofrontal cortex (OFC) produced a robust shift towards the low reward option on a T-maze equivalent of the operant delay-discounting task (Khani et al., 2015). This effect is similar to that observed following post-training lesions of the OFC on the same task, and might suggest that presynaptic intra-OFC CB₁ receptor activation induces decision-making deficits by dampening glutamate release in this region (Rudebeck et al., 2006).

Cannabinoid modulation of effort-based decision-making has been investigated using a T-maze task where rats must scale a 30 cm barrier to obtain the larger reward. Infusions of ACEA, but not the synthetic CB₁ receptor antagonist AM251, into the anterior cingulate cortex (ACC) caused a robust shift in choice towards the small, unobstructed reward option, suggesting that rats were less willing to exert physical effort for the preferred reward (Khani et al., 2015). Lesions of the ACC, but not OFC, produce similar deficits in effortful decision-making, and when considered with the delay-discounting data above, it appears that prefrontal

CB₁ receptors in distinct prefrontal regions are necessary when choosing between options varying in delay or effort (Rudebeck et al., 2006). Notably physical costs, such as scaling a barrier or pressing a lever, are typically employed to assess effort-based decision-making in rodents, but these tasks do not address costs that are cognitive in nature. This distinction is important, not only because the two forms of decision-making are subserved by somewhat dissociable neurobiological mechanisms, but because cognitive costs are more representative of the effort costs faced by humans in an industrialized society (Hosking et al., 2015). Indeed, strong links between cannabis use and impaired education, economic, and employment outcomes may reflect a fundamental deficit in effortful decision-making, whereby cannabis use decreases the willingness to expend the greater cognitive load associated with gaining lucrative prospects (Fergusson and Boden, 2008; Horwood et al., 2010; Hyggen, 2012).

A recently developed rodent Cognitive Effort Task (rCET) (Cocker et al., 2012), in which effort costs vary by the amount of visuospatial attention required to complete low- or high-reward trials, provided a behavioural model in which these issues could be addressed. THC administration dose-dependently decreased choice of the difficult, high-reward option requiring accurate detection of a brief (0.2 s) light stimulus amid a five-hole array; correspondingly, rats shifted choice to the easier, low-reward option where the light stimulus was presented for a longer duration (1 s) (Silveira et al., *in press*). Importantly, the lack of effect of THC on attentional accuracy suggests that the choice shift was not due to an inability to complete high-reward trials. Moreover, THC-induced choice impairments were correlated with CB₁ receptor density in the medial PFC, indicating that prefrontal CB₁ receptors may contribute to THC-induced cognitive laziness. These findings complement those of Khani et al., (2015), where CB₁ receptor agonism in the anterior cingulate cortex decreased physical effort-based decision-making. Interestingly, unlike the effects of THC, CB₁ agonism by administration of WIN 55, 212-2 did not shift choice on the rCET, similar to findings noted

earlier in a delay-based choice task (Pattij et al., 2007; Wiskerke et al., 2011). These discrepancies are likely related to the distinct pharmacodynamic profiles of these drugs (Felder et al., 1995), and/or differences in the intracellular signalling pathways they recruit, with THC a potent recruiter of the arrestin2 pathway, and WIN 55, 212-2 a recruiter of the classical G-protein $G_{\alpha i/o}$ and $G_{\beta\gamma}$ pathways (Laprairie et al., 2014). These data emphasise that not all forms of cannabinoid receptor activation produce converging effects, and animal studies aiming to model the psychoactive effects of cannabis would do well to administer THC in lieu of its synthetic counterparts. In contrast, modulating endogenous cannabinoid tone, via CB_1 receptor inverse agonism, or by inhibition of endogenous anandamide hydrolysis by FAAH, did not affect choice on the rCET (Silveira et al., *in press*). Taken together with the null effects of CB_1 antagonism reported with the delay and physical effort tasks, it appears that endocannabinoid signalling does not tonically regulate decision-making, at least under non-pathological conditions (Khani et al., 2015; Pattij et al., 2007; Wiskerke et al., 2011).

Overall, the preclinical literature suggests that THC induces decision-making deficits when choices involve delay and effort (both physical and cognitive) costs, and that this effect is mediated by CB_1 receptors in distinct frontal cortical regions. However, to date no animal studies have investigated the role of the cannabinoid system in decisions involving risk costs (Ghods-Sharifi et al., 2009; Orsini et al., 2015b), despite the existence of validated models. Additionally, a rodent analogue of the IGT has been developed, but to our knowledge cannabinoid drugs have yet to be investigated on this task (Zeeb et al., 2009). Given its potential to parse out the specific neural processes mediating poor IGT performance in regular cannabis users, this would be a worthwhile avenue to pursue. Preclinical models could also benefit research assessing how CBD may moderate THC-induced decision-making impairments. As discussed in section 2.2, ratios of THC/CBD in cannabis can vary widely

(Swift et al., 2013; Syed et al., 2014), CBD can attenuate THC-induced memory and attentional impairments (Morgan et al., 2010a; Morgan et al., 2010b), and both agents differentially influence brain activity when performing cognitive or emotional processing tasks (Bhattacharyya et al., 2015; Bhattacharyya et al., 2010; Borgwardt et al., 2008; Fusar-Poli et al., 2009). However, only one human study has attempted to relate decision-making impairments to CBD concentrations in hair (Hermann et al., 2009), and no studies have assessed how it might modulate THC's effects. We addressed this gap in the literature by testing rats on the rCET following CBD administration (Silveira et al., *in press*). While CBD had no effect in isolation, it modestly attenuated THC-induced decision-making impairments in “slacker” rats (i.e. those that preferred the cognitively easy, low-reward option), when administered at a 1:1, but not 1:10, ratio matching that in cannabinoid therapeutics. This finding both highlights the dose by ratio interactions that mediate the psychoactive effects of co-administered phytocannabinoids, and underscores the importance of considering interindividual differences in the diverse response to cannabis observed in the population, as discussed in section 2. *Finally, it is important to note the various cognitive, emotional, and motivational subprocesses that interact in decision-making, many of which are also mediated by cannabinoid transmission (Orsini et al., 2015a). For example, the ability to form action-outcome (A-O) contingencies, which is crucial as subjects associate different choices with their anticipated outcomes, is dependent on dorsomedial striatal CB₁ receptors (Goodman and Packard, 2015; Hart et al., 2014). Indeed, as will be discussed below, cannabinoid transmission in downstream limbic and striatal regions can have a profound impact on executive processes primarily attributed to the prefrontal cortex.*

4. Cannabinoid modulation of mesocorticolimbic dopamine transmission: focus on emotional salience and memory formation

Cannabis-induced deficits in frontally-mediated executive processes, such as decision-making described in section 3, arise not only from local modulation of PFC activity but also its effects on downstream components of corticolimbic networks. Indeed, emerging evidence is pointing to a critical role for dysregulated cannabinoid signalling in both the PFC and vHIPP as potential underlying mechanisms associated with aberrant dopaminergic signalling, a cardinal feature of neuropsychiatric syndromes including schizophrenia. Several post-mortem studies demonstrate that individuals with schizophrenia have highly abnormal CB₁ receptor expression patterns in the PFC (Dalton et al., 2011; Dean et al., 2001; Eggan et al., 2010; Volk et al., 2014). Furthermore, neuroimaging evidence suggests there are widespread alterations in CB₁ receptor levels in patients with schizophrenia (Ceccarini et al., 2013; Ranganathan et al., 2015; Wong et al., 2010), including significantly reduced levels in the hippocampus (Ranganathan et al., 2015). While PFC dysfunction is primarily thought to underlie cognitive deficits, hippocampal dysfunction is associated with both positive and negative (e.g. blunted affect, social withdrawal) symptomology (Hajos and Freund, 2002; Nguyen et al., 2014). Literature concerning the role of endocannabinoid dysfunction in schizophrenia and the potential for cannabis to induce psychosis has been extensively reviewed elsewhere (Arnold et al., 2012; Bossong and Niesink, 2010; Chadwick et al., 2013; Gage et al., 2015; Radhakrishnan et al., 2014; Saito et al., 2013; Semple et al., 2005; Smit et al., 2004; Volk and Lewis, 2015). In this section, we review recent preclinical work showing that CB₁ receptor transmission in the PFC and vHIPP is capable of functionally controlling mesolimbic dopaminergic activity states in the context of regulating emotional processing and memory formation. Given their breadth and complexity, we first provide an overview of the relevant circuits before moving on to the electrophysiological and behavioural data; avenues for further research are also discussed.

4.1 Prefrontal-cortical cannabinoid regulation of subcortical dopamine transmission

The mammalian PFC is a complex neural region that shares reciprocal functional connections with the mesolimbic dopamine pathway, including the ventral tegmental area (VTA) and nucleus accumbens (NAc). For example, VTA dopamine neurons send functional inputs to the PFC and, conversely, pyramidal output neurons from the PFC send glutamatergic projections to both dopaminergic and non-dopaminergic neurons in the VTA (Carr and Sesack, 2000a, b). Functionally, the CB₁ receptor regulates the presynaptic release of both GABA and glutamate within the PFC and can thus regulate the activity states of pyramidal and interneuron populations (Domenici et al., 2006; Szabo and Schlicker, 2005). As such, CB₁ receptor-mediated changes in PFC neuronal activity can profoundly influence the activity states of the mesolimbic dopamine system (Draycott et al., 2014). In addition, GABAergic interneurons in the PFC normally provide inhibitory, feedforward inputs to pyramidal output neurons. Naturally occurring endocannabinoids, such as 2-AG, are synthesized by pyramidal neurons and travel to CB₁ receptor-expressing GABAergic terminals; CB₁ receptor activation then leads to disinhibition of pyramidal neuron activity. In other words, cannabinoid transmission in the PFC serves to regulate an inhibitory, GABAergic ‘braking’ mechanism on excitatory outputs to VTA dopaminergic neurons. Given the importance of dopaminergic disturbances in schizophrenia and other psychiatric disorders, an important implication is that localised dysfunction in PFC cannabinoid signalling may alter the activity states of GABAergic interneurons versus pyramidal neurons and, in turn, lead to downstream dysregulation of mesolimbic dopaminergic activity.

Preclinical research has characterized a bi-directional effect of CB₁ receptor signalling in the PFC during the processing of emotional salience and memory formation. For example, using an olfactory fear conditioning procedure wherein rats receive pairings of either sub-threshold (normally non-salient) or supra-threshold (highly salient) footshock stimuli, with olfactory cues paired with footshock delivery or its absence, activation of PFC CB₁ receptors

robustly potentiated the emotional salience of normally non-salient fear-related associative memories when measured behaviourally (e.g. fear-related freezing) and in terms of PFC neuronal activity (Laviolette and Grace, 2006a; Tan et al., 2010; Tan et al., 2011). More specifically, acute overstimulation of the local cannabinoid system with CB₁ receptor agonists, including WIN 55, 212-2, strongly increased PFC neuronal associative responses to fear-related conditioned cues and shifted neuronal activity from tonic to bursting modes during affective processing (Laviolette and Grace, 2006a; Tan et al., 2010). In contrast, pharmacological blockade of PFC CB₁ receptor activity attenuated the formation of supra-threshold fear memories at both the behavioural and neuronal levels (Laviolette and Grace, 2006a; Tan et al., 2010; Tan et al., 2011). Together, this evidence shows that aberrant cannabinoid activation can potently modulate PFC neuronal network activity and simultaneously distort affective processing, similar to disturbances observed in schizophrenia and mood disorders.

A critical question remains as to how PFC cannabinoid signalling modulates VTA dopaminergic transmission in the context of emotional salience processing. In terms of cannabinoid-dependent regulation of the PFC-VTA pathway, acute THC administration has been reported to both decrease GABA release and increase dopamine release in the PFC (Pistis et al., 2002). In addition, administration of either synthetic CB₁ receptor agonists or THC increased PFC pyramidal neuron activity while simultaneously blocking the inhibitory effects of VTA dopaminergic inputs (Pistis et al., 2001), suggesting that CB₁ receptor activation can ramp up the excitability of PFC neurons through both local and distal circuit interactions. A recent study characterized a biphasic role for PFC CB₁ receptor transmission in the regulation of emotional salience and memory formation via effects on VTA dopaminergic activity (Draycott et al., 2014). Using single unit, *in vivo* extracellular recordings of VTA dopaminergic neurons in concert with intra-PFC infusions of WIN 55,

212-2, high levels of CB₁ receptor stimulation decreased spontaneous and burst firing activity of VTA dopamine neurons, whereas low levels of stimulation potentiated these parameters. Interestingly, these biphasic effects reflected divergent effects on emotional memory formation, with intra-PFC WIN 55, 212-2 infusions at high doses blocking the formation of normally salient (supra-threshold) fear memory, and lower doses potentiating normally non-salient (sub-threshold) fear memory (Draycott et al., 2014). Furthermore, the behavioural effects of high levels of PFC CB₁ receptor stimulation were dependent on a GABA_B receptor substrate directly in the VTA, whereas those of low levels of CB₁ activation were dopamine-dependent (Draycott et al., 2014). Thus, relative CB₁ receptor tone in the PFC appears to induce either emotional blunting, or potentiation of normally non-salient stimuli via direct actions on subcortical dopamine neurons.

These preclinical findings complement clinical evidence demonstrating that acute cannabis exposure can induce psychotic episodes consistent with hyper-dopaminergic activity (D'Souza et al., 2004). By contrast, repeated cannabis exposure is associated with blunted levels of mesolimbic dopaminergic activity, as indexed by a reduced capacity for dopamine synthesis in the ventral striatum of heavy users (Bloomfield et al., 2014). Indeed, the influence of local cannabinoid signalling over subcortical emotional processing regions corresponds to the top-down executive control function of the PFC, emphasising the importance of downstream factors in cannabinoid modulation of complex cognitive processes, like decision-making discussed in section 3. Beyond the PFC, however, recent studies also reveal an important functional role for hippocampal CB₁ receptor transmission in regulating subcortical dopamine activity and emotionally-guided behaviours.

4.2 Identification of ventral hippocampal cannabinoid transmission as a functional regulator of mesolimbic dopamine activity

The vHIPP is a critical neural region implicated not only in the modulation of dopaminergic transmission (Floresco, 2007; Floresco and Grace, 2003) but in neuropsychiatric-related pathology as demonstrated in both preclinical and clinical studies (Grace et al., 2007; Nguyen et al., 2014; Sams-Dodd et al., 1997; Strange et al., 2014). Anatomically, the vHIPP serves as a nexus point with complex functional connections with the NAc, VTA and PFC (Calhoun and O'Donnell, 2013; Grace et al., 2007; Sotres-Bayon et al., 2012). Located mainly on inhibitory, GABAergic presynaptic terminals within the vHIPP (Takacs et al., 2015), CB₁ receptor activation reduces GABAergic tone and thereby increases the output activity of principal neurons to their targets, including the NAc (Hajos and Freund, 2002). Specifically, through excitatory projections to the NAc shell, vHIPP output neurons have been shown to modulate dopamine neuronal activity via both direct and indirect pathways to VTA cell bodies. For example, vHIPP stimulation increases the spontaneous activity of VTA dopamine neurons via excitatory glutamatergic projections (Floresco et al., 2001). The notion that abnormal mesolimbic dopamine transmission might be secondary to hippocampal dysfunction in schizophrenia is relatively well-established (Schmajuk, 2001); however, a potentially critical involvement of vHIPP CB₁ receptor transmission in modulating schizophrenia-like affective and cognitive disturbances associated with dopamine dysregulation has only recently come to light (Loureiro et al., 2015).

Similar to the work in the PFC reviewed above, a combination of *in vivo* electrophysiology and behavioural approaches were used to assess the effects of vHIPP CB₁ receptor stimulation. Intra-vHIPP infusions of WIN 55, 212-2 increased both spontaneous and burst firing activity of isolated VTA dopamine neurons, yet attenuated the spontaneous activity rates of non-dopaminergic (presumably GABAergic) neurons (Loureiro et al., 2015). Thus, cannabinoid activity in the vHIPP appears capable of modulating VTA neuronal network activity through both dopaminergic and non-dopaminergic circuits, similar to the

effects of CB₁ receptor signalling in the PFC. In contrast, the effects of vHIPP CB₁ receptor stimulation were not biphasic and only led to activation of the VTA dopamine system (Loureiro et al., 2015). At the behavioural level, stimulation of CB₁ receptors in the vHIPP caused a range of affective and cognitive abnormalities. For example, vHIPP CB₁ receptor activation potentiated the reward salience of normally non-salient conditioning doses of morphine in a conditioned place preference paradigm, and disrupted social interaction behaviours by diminishing both the motivation for sociability as well as recognition memory (Loureiro et al., 2015). With respect to reward processing, the effects of intra-vHIPP CB₁ receptor activation differed sharply with those of intra-PFC CB₁ receptor activation. Contrasting reports that PFC CB₁ receptor stimulation switches a normally behaviourally-rewarding dose of morphine into an aversive stimulus (Ahmad et al., 2013), CB₁ receptor stimulation in the vHIPP was found to potentiate opiate-reward salience (Loureiro et al., 2015). Notably, the behavioural effects of intra-vHIPP CB₁ receptor stimulation were blocked by dopamine receptor antagonism within the NAc, indicating that the ability of intra-vHIPP CB₁ receptor stimulation to disrupt rewarding and social interaction behaviours is mediated through a dopaminergic signal directly in the mesolimbic pathway (Loureiro et al., 2015). Therefore, CB₁ receptor signalling within both the PFC and vHIPP can strongly, albeit through distinct neural mechanisms, modulate the activity states of mesolimbic dopamine neurons and induce corresponding changes in affective/reward-related processing and social interaction/cognition behaviours.

Nevertheless, many critical questions remain for future study. For example, given that the vHIPP and PFC share reciprocal connections, it will be interesting to examine how the PFC may regulate subcortical transmission between the vHIPP and mesolimbic dopamine system. Recent evidence demonstrates that the PFC can strongly regulate the ability of the vHIPP to control neuronal activity states in the NAc by gating vHIPP→NAc excitatory

projections (Belujon and Grace, 2008). Thus, how might top-down PFC influences on the mesolimbic pathway control the ability of vHIPP CB₁ receptor signalling to regulate neuronal and behavioural effects within the VTA or NAc? In addition, given that the NAc receives and integrates functional inputs from both the PFC and VTA, how might the biphasic effects of PFC CB₁ receptor activity control neuronal activity states directly within the NAc, and how might these differential effects modulate phasic mesolimbic dopamine release? Characterizing and comparing the effects of cannabinoid transmission within the PFC and vHIPP on dopaminergic activity states will yield important new insights into how dysregulation of the endocannabinoid system, induced by either exposure to cannabis or due to intrinsic, genetically-determined endocannabinoid disturbances, may underlie neuropsychiatric symptoms in disorders such as schizophrenia.

Finally, it is important to note a critical role for the amygdala in regulating emotional processing and associative learning behaviours, with its inextricable anatomical and functional connections to the PFC, vHIPP and mesocorticolimbic dopamine networks (for a detailed overview of studies see Laviolette and Grace, 2006b; Tan et al., 2014). These data were not reviewed herein as complementary work regarding amygdalar endocannabinoid signalling in regulating anxiety is reviewed in the next section.

5. Cannabinoid modulation of amygdalar function: focus on anxiety and fatty acid amide hydrolase activity

Accumulating evidence suggests that the endocannabinoid system is involved in the regulation of anxiety (Korem et al., 2015), and it has long been recognized that cannabis use produces significant effects on anxiety in humans (Patel and Hillard, 2009). Anxiety is a negative emotional state that serves an important protective function, but can become pathological when the intensity of cognitive and behavioral responses does not correlate with actual danger. Hyperactivity in the circuits that induce anxiety can result from repetitive

stress, and/or traumatic life events, which can lead to psychopathologies including generalized anxiety, phobias, hypervigilance and other somatic signs of fear (Calhoon and Tye, 2015). Several corticolimbic brain regions are involved in the detection and assessment of environmental stimuli as being potentially harmful, including the amygdala and bed nucleus of the stria terminalis (BNST), as well as the vHIPP and medial PFC discussed in section 4. In the following section, we first review evidence demonstrating a key role for amygdalar cannabinoid signalling in the regulation of anxiety in both humans and animals. We will then describe new data from studies looking specifically at the modulation of endocannabinoid activity in the amygdala in anxiety-like behaviours in mice.

5.1 Modulation of anxiety by endocannabinoid signalling in the basolateral amygdala

The basolateral amygdala (BLA) receives sensory information in the form of excitatory inputs from the thalamus and sensory cortices (McDonald, 1998). Processing of this sensory information in the BLA results in formation of associations between previously neutral stimuli that predict an outcome that has positive or negative valence through Hebbian learning mechanisms (Maren and Quirk, 2004). The emotional valence that is attached to the stimuli determines whether fear or reward pathways are activated by BLA outputs (Namburi et al., 2015). When the situation is predictive of threat, the BLA activates the central amygdala (CeA) and BNST which, through projections to the hypothalamus and brainstem, provide information to autonomic, neuroendocrine and motor systems to coordinate physiological and behavioral responses to cope with the threat (Calhoon and Tye, 2015).

CB₁ receptors are present in the presynaptic terminals of both GABAergic and glutamatergic neurons in the amygdala (Katona et al., 2001; Marsicano and Lutz, 1999). Thus, as in the PFC, endocannabinoid signalling inhibits the release of both glutamate and GABA in the amygdala, which is consistent with biphasic effects of CB₁ receptor agonists on anxiety-like behaviors in preclinical models (Patel and Hillard, 2006). For example, selective

deletion of CB₁ receptors from cortical glutamatergic axons results in loss of the anxiolytic effects of low doses of CB₁ receptor agonists while the converse occurs when CB₁ receptors are deleted from GABAergic terminals (Rey et al., 2012). Furthermore, CB₁ receptor agonist treatment synergizes with restraint stress to increase c-Fos expression in the CeA, which is consistent with a pro-anxiety role for CB₁ receptor on GABA terminals (Patel et al., 2005). In contrast to the biphasic effects of direct CB₁ receptor agonists, inhibition of FAAH activity, which results in increased concentrations of AEA, exerts a monophasic effect on anxiety-like behaviors in mice (Patel and Hillard, 2006) and does not synergize with stress to promote CeA activation (Patel et al., 2005).

Considerable evidence collected from biochemical, behavioral and genetic studies supports the hypothesis that FAAH activity contributes to amygdalar activation by an acute stress exposure (Gunduz-Cinar et al., 2013a). For example, the potent inhibitor of FAAH, URB597, induces anxiolytic effects in mice in a CB₁ receptor-dependent manner (Busquets-Garcia et al., 2011; Kathuria et al., 2003). Similarly FAAH knockout mice exhibit reduced expression of anxiety-related behaviors in stressful environments (Naidu et al., 2007). Acute exposure to stress results in a reduction of AEA concentrations in the amygdala (Rademacher et al., 2008) which results in the activation of the hypothalamic-pituitary-adrenal axis (Hill et al., 2009). Together, these data lead to the hypothesis that AEA concentrations in the amygdala serve as a gate that inhibits stress responses; and that the catabolism of AEA by FAAH results in a permissive state in which stress can elicit an anxiety response (Hill et al., 2009). Recent studies demonstrate that the stress effect on AEA concentrations is the result of corticotrophin releasing factor-mediated increases in FAAH activity (Gray et al., 2015). In relation to the impact of chronic stress, repeated exposure to restraint stress increases amygdalar FAAH V_{max} and is associated with a significant suppression of AEA concentrations (Rademacher et al., 2008). Additionally, repeated immobilization results in a

significant increase in anxiety-like behavioral responding and dendritic arborisation in the amygdala—effects that do not occur in FAAH knockout mice (Hill et al., 2013). These and other studies support a critical role for FAAH in the regulation of acute anxiety and in the anxiogenic consequences of chronic stress exposure (Gunduz-Cinar et al., 2013a).

Human studies are in accord with a critical role for FAAH in the regulation of amygdalar function. Indeed, complementing data reviewed in section 2, genetic variation in FAAH may contribute to individual differences in reactivity to negative stimuli, including stress. Specifically, a polymorphism in the gene for FAAH (rs324420) has been described that results in FAAH protein that is more labile to degradation (Chiang et al., 2004; Sipe et al., 2002). An imaging study examined the contribution of this polymorphism to amygdalar reactivity evoked by fearful faces in humans and found that individuals with the rare allele demonstrate reduced reactivity (Gunduz-Cinar et al., 2013b). Mice genetically engineered to mimic this human polymorphism exhibit reduced FAAH activity, increased amygdalar AEA and reduced anxiety-like behaviors (Dincheva et al., 2015). These studies lead to the hypothesis that individual differences in FAAH activity could contribute to susceptibility to the development of anxiety disorders.

5.2 Elucidating the influence of age and sex on amygdalar FAAH modulation of anxiety

Evidence suggests that FAAH activity exhibits a development trajectory, demonstrating interesting differences during adolescence that contribute to anxiety-like behaviors (Lee et al., 2015). Although less is known, there is a tendency for FAAH protein to increase in aged mice, particularly in mice null for the CB₁ receptor (Maccarrone et al., 2001). Circumstantial evidence also supports the hypothesis that FAAH becomes a more significant regulator of cardiovascular function and peripheral inflammation (Batkai et al., 2007), together with hippocampal signalling and inflammation (Murphy et al., 2012), as animals age. These data are consistent with an age-related increase in FAAH activity. In support of this

notion, AEA concentrations decrease with aging in the spinal cord, thalamus and cortex (Bishay et al., 2013). Moreover, post-mortem studies in human tissues demonstrate that FAAH expression steadily increases with age in the dorsolateral region of the PFC (Long et al., 2012).

We carried out a set of studies to determine the extent to which FAAH contributes to changes in anxiety-like behaviors in old female and male mice. Male and female mice on the ICR (CD-1) background from three different age ranges, 4-6 (adolescent/young adult), 20-29 (mid-adult) and 60-75 (late-adult) weeks, were exposed to the elevated plus maze (EPM) to evaluate anxiety-like behavior as previously described (Patel and Hillard, 2006). Twenty-four hours after the EPM test, brains were harvested and FAAH activity was determined by measuring the conversion of AEA to ethanolamine as described (Patel et al., 2003). **Female mice were gonadally-intact and had never been pregnant. Although not determined, it is likely that the female mice in the oldest group were no longer cycling through estrous stages.**

Behavior of male and female mice in each of the three age ranges was examined in the EPM. The percent of total entries that occurred into the open arms were calculated for each age and sex group and were examined using two-way ANOVA with age and sex as **between-subjects** factors (Fig 1A). Percent entries into the open arm were affected by both age ($F_{2,33} = 5.50$; $p < 0.01$) and sex ($F_{1,33} = 7.20$; $p < 0.05$) with a significant interaction ($F_{2,33} = 3.40$; $p < 0.05$). *Post hoc* tests reveal that female adolescent/young adult mice exhibit a greater percentage of open entries compared with female late adult mice. Two-way ANOVA also revealed an effect of age on total arm entries (Fig 1B; $F_{2,33} = 15.00$; $p < 0.001$), with the youngest group showing more entries than either of the other groups in both male and female mice. Two-way ANOVA also demonstrated a significant interaction between sex and age in the time spent in the closed arms of the EPM (Fig 1C; $F_{2,33} = 5.40$, $p < 0.01$). The female oldest mice spent a markedly longer time in closed arm exploration than the female youngest

and the male oldest mice. In the time spent in the open arms, two-way ANOVA demonstrated a significant interaction between sex and age ($F_{2,33} = 3.60$, $p < 0.05$; data not shown). *Post hoc* tests revealed that the female adolescent/young adult mice spent more time in open arm exploration compared with female mid-adult and late-adult group.

FAAH activity was measured in membranes harvested from the amygdala 24 h after the EPM. Two-way ANOVA also demonstrated a significant interaction between sex and age (Fig 2; $F_{2,33} = 5.40$, $p < 0.01$). FAAH activity was greater in the female mid-adult mice compared to the female adolescent/young adult and the male late-adult mice. In male mice, FAAH activity was not different in any of the age ranges examined.

Correlation analyses were carried out between the parameters determined in the EPM and amygdala FAAH activity. In female mice, significant, negative correlations occurred between amygdalar FAAH activity and the percentage of open entries (Fig 3A; $r^2 = -0.489$, $p < 0.05$) and the total number of entries (Fig 3B; $r^2 = -0.492$, $p < 0.05$) while a positive correlation between amygdalar FAAH activity and closed time (Fig 3C; $r^2 = 0.490$, $p < 0.05$) was demonstrated. No significant correlations were seen between EPM parameters and FAAH activity in male mice (data not shown).

Our findings, that anxiety-like behaviors are increased in late-adult female mice compared to adolescent/young adult female but not male mice, are consistent with earlier studies (Frick et al., 2000; Lamberty and Gower, 1992) although the opposite has also been reported (Chen et al., 2007). A wide range of EPM behaviors, together with FAAH activity in the same mice, allowed us to examine the contribution of FAAH activity in the EPM behaviors. In the female mice, increased anxiety-like behaviors correlated with increased amygdalar FAAH activity, which supports the hypothesized role of FAAH as a critical regulator of anxiety. In fact, approximately 50% of the variation in the number of entries, percent entries into the open arms and time in the closed arms can be explained by FAAH

activity. However, these correlations were confined to female mice. Notably, the menopause and post-menopausal periods in mice are marked with depressed mood and anxiety-like behaviour, which are related to fluctuations in endogenous estrogen and progesterone levels (Guimaraes et al., 2015). We have reported that old, menopausal mice are more anxious than young mice. Indeed, Lamberty and Gower (1992) showed anxiogenic-like behavior in aged female NMRI mice (17-22 months old). Taken together, the present findings suggest that aging increases anxiety, possibly due to a decrease in the levels of ovarian steroid hormones estrogen and progesterone, in addition to decreasing FAAH activity.

Indeed, there is evidence for an interaction between gonadal hormones and FAAH activity. There is an estrogen response element in the FAAH gene that inhibits FAAH transcription upon translocation of the estrogen receptor to the nucleus (Hill et al., 2007; Waleh et al., 2002). It has been suggested that the anxiogenic effects of estrogen are due, at least in part, to an elevation of AEA and, thus, CB₁ receptor signalling. Data to support this hypothesis include findings that a CB₁ antagonist reversed the anxiolytic effect of estradiol in rats (Hill et al., 2007). With regard to the present findings, estrogen levels would be expected to be lower in older females, which would result in disinhibition of FAAH expression and, thus is consistent with the hypothesis that the anxiogenic effects of estrogen are mediated by AEA. However, although there was a trend, FAAH activity was not significantly greater in the aged compared to youngest group of female mice. Given that we did not measure estrogen levels and have made somewhat arbitrary divisions in the age groups, we cannot make any conclusions from the present studies regarding this potential mechanism.

Together, these results are consistent with the hypothesis that endocannabinoid signalling is part of an endogenous anxiolytic neuromodulator system and suggest that variations in the endocannabinoid system, particularly resulting from changes in FAAH,

could contribute to different vulnerability to anxiety-related disorders and trait anxiety in females compared to males.

6. Summary and conclusions

In this review we have shown that cannabinoid signalling across corticolimbic networks can have profound effects on cognitive and affective behaviours. Effects arise following acute administration of cannabis and related cannabinoids, via interactions with CB₁ receptors in cortical and subcortical regions. However, endogenous cannabinoid tone in the amygdala, hippocampus, and PFC also appears to regulate fundamental emotional and reward-related processes. Understanding cannabis' psychoactive effects and the factors which might minimize adverse reactions is crucial given the widespread use of cannabinoid drugs worldwide. By the same token, understanding how endocannabinoid signalling contributes to basic neural functioning, and how it might be disrupted in pathological states such as schizophrenia and anxiety, suggest a viable therapeutic target for these disorders.

As described in section 2, both human genetic influences and strain differences in the blend of the phytocannabinoids THC and CBD in the plant interact at the level of individual users, leading to a range of psychobehavioural responses. From a psychiatric standpoint, understanding genetic vulnerability for psychotic episodes or cannabis dependence may pave the way for preventative approaches in which “high risk” individuals could be warned of the risks associated with ongoing cannabis use. Alternatively, modifying the composition of cannabis itself may offer utility in curbing the potential for adverse side effects, with phytocannabinoids like CBD evidenced to attenuate some of THC's anxiogenic and cognitive-impairing actions. While concentrations of THC in street cannabis continue to rise, accompanied by a concomitant decline in CBD levels (Burgdorf et al., 2011; Swift et al., 2013), cannabinoid therapeutics, such as nabiximols (Sativex), purposefully contain equal quantities of THC and CBD (Syed et al., 2014). Thus the composition of cannabis and

cannabinoid preparations varies widely as a function of intended use, contributing to vastly different effects. Most clinical and preclinical studies to date have focused on THC exclusively, yet in the interest of ecological validity, ongoing research will benefit by manipulating the ratio of THC relative to other phytocannabinoids present in cannabis.

From a public health and policy perspective, regulating the dose and phytocannabinoid composition of cannabis requires special consideration, given that low doses of THC can induce relaxation and euphoria, but high doses may result in anxiety, psychotic episodes, and impaired cognition (Crane et al., 2013). In section 3, we reviewed clinical evidence for impaired decision-making in regular cannabis users; this is most likely mediated by repeated THC exposure which has been shown to acutely impair this cognitive domain in clinical and preclinical models. As suggested, the poor education and economic life outcomes reported amongst regular cannabis users might reflect decision-making deficits, and such diminished outcomes would place undue burden on the welfare state (Fergusson and Boden, 2008; Horwood et al., 2010; Hyggen, 2012). Given the growing popularity of cannabis concentrates and synthetic cannabinoids, together with evidence that cannabis users report greater risk-taking in social, health, and ethical domains, it is clear that THC-induced deficits in cognitive ability may have severe societal consequences (Gilman et al., 2015; Raber et al., 2015). These could be mitigated by either raising public awareness of the negative outcomes associated with regular use, or regulating the composition of cannabis so that side effects are minimized, as described above.

Importantly, cannabis-induced psychoactive effects reflect the “hijacking” of an endogenous cannabinoid system involved in regulating a variety of reward, emotional, and prosocial processes. Current findings, reviewed in section 4, demonstrate important functional distinctions between modulation of mesolimbic dopaminergic activity states by CB₁ receptor stimulation in the PFC versus the vHIPP (Ahmad et al., 2013; Draycott et al., 2014; Loureiro

et al., 2015). Similarly, the evidence reviewed in section 5 indicates that endocannabinoid signalling in the amygdala, particularly via FAAH activity, is a critical regulator of anxiety in a manner that appears both sex- and age-dependent, adding another layer of interindividual variation in terms of endogenous cannabinoid tone. Interestingly, modulation of both inhibitory and excitatory neurons in the PFC and amygdala seems to underlie biphasic behavioural effects, whereas current data suggests primarily monophasic effects of vHIPP CB₁ receptor signalling. Moreover, administration of synthetic cannabinoid ligands, phytocannabinoids and modulation of endocannabinoid tone, such as through FAAH inhibition, are seen to have divergent outcomes across different behavioural modalities. Taken together, a complicated picture of cannabinoid modulation of corticolimbic function emerges, offering the prospect of site-specific cannabinoid therapeutics via exploitation of regional differences and designer drugs. Indeed, cannabinoid therapeutics, such as CBD and FAAH inhibitors, have been investigated for the treatment of schizophrenia and anxiety, respectively (Batista et al., 2014; Iseger and Bossong, 2015).

Although brief, this review has highlighted the importance of continued research on cannabinoid transmission in a variety of domains, including recreational cannabis and related cannabinoids, given changing social and political attitudes towards their use, as well as medicinal cannabis, in light of its potential to ameliorate neuropsychiatric disorders amongst other medical conditions. For these directives to be achieved, it is imperative that clinical and preclinical research into the cannabinoids be accessible, having long been impeded by strict legislation, with the overarching goals of minimizing harm and maximizing therapeutic potential.

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Figure Captions

Figure 1. Elevated plus maze behaviour in male and female mice at different ages. ICR mice were purchased from Harlan, Madison, WI, and were divided in three age groups of each sex (5-7 mice/group) as shown. Prior to use, mice were group housed on a 12 h light/dark cycle with lights on at 6:00 AM. All studies were carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin. The EPM consists of four arms 30 cm long and 5 cm wide, two open without walls and two closed by 30 cm high walls; the apparatus is elevated 40 cm above the floor (San Diego Instruments). Experiments began by placing a single mouse on the central platform facing an open arm: behaviors were recorded for 5 min as they explored the maze. Data were analyzed using video-based EthoVision System data analysis software (EthoVision 3.1; Noldus Information Technology). A: Percent entries into the open arms were calculated as the number of open arm entries/(open + closed arm entries) $\times 100$. B: Total entries into open and closed arms. C: Total time spent in the closed arms. * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.005$ compared to the 4-6 week old group.

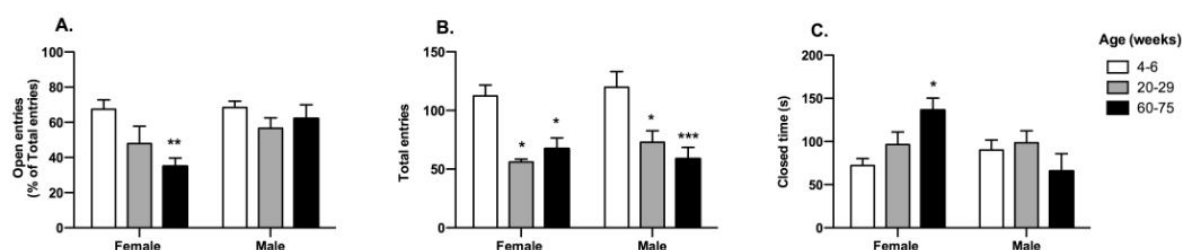


Figure 2. Amygdalar FAAH activity in male and female mice at different ages. Twenty-four hours after EPM, mice were decapitated; brains were removed and frozen on dry-ice. The amygdala was dissected on dry-ice, weighed and membranes were harvested as described previously (Hillard et al., 1995). Membranes were incubated with 0.2 nM [3 H]AEA for 10 min. FAAH activity was calculated as the ratio of dpm (disintegration per minute) in the aqueous phase and the total dpm (aqueous + organic). * $p < 0.05$ compared to 4-6 week old group.

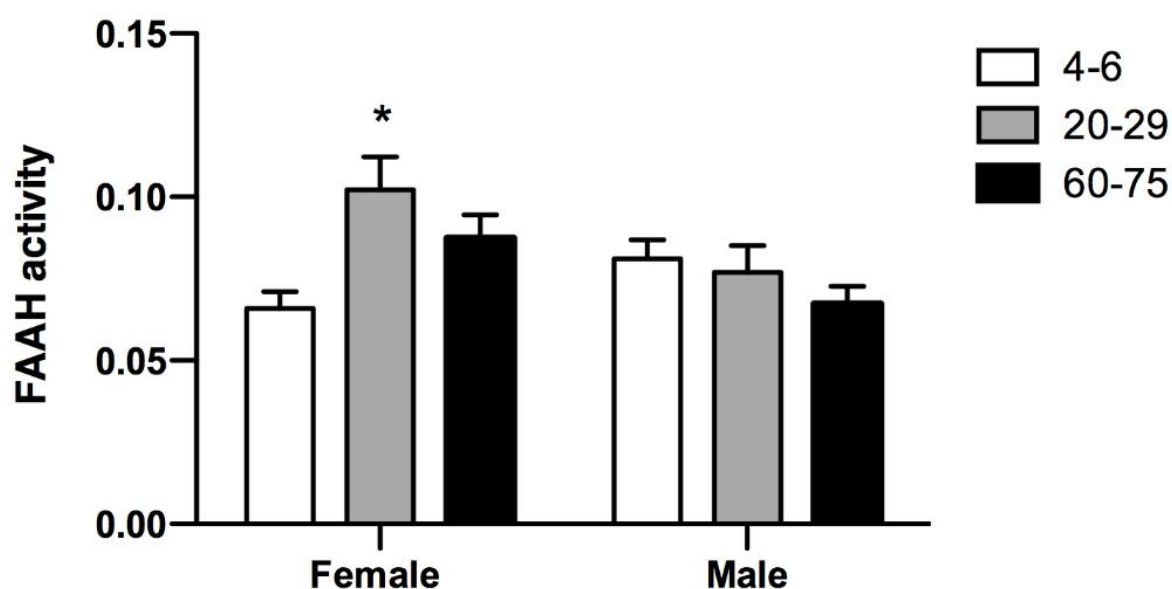


Figure 3. Figure 3. Correlations between elevated plus maze behavior and amygdalar FAAH in female mice and elevated plus maze behavior and age. Correlational analyses were made using Spearman's bivariate test between FAAH activity in the amygdala and the following EPM parameters in female mice of all three ages: (A) percent time spent in the open arms; (B) total entries: and (C) time spent in the closed arms. The correlation between EPM parameters and age in female mice were: (D) time spent in the open arms, (E) percent time spent in the close arms; (F) time spent in the closed arms and (G) percent time spent in the closed arms.

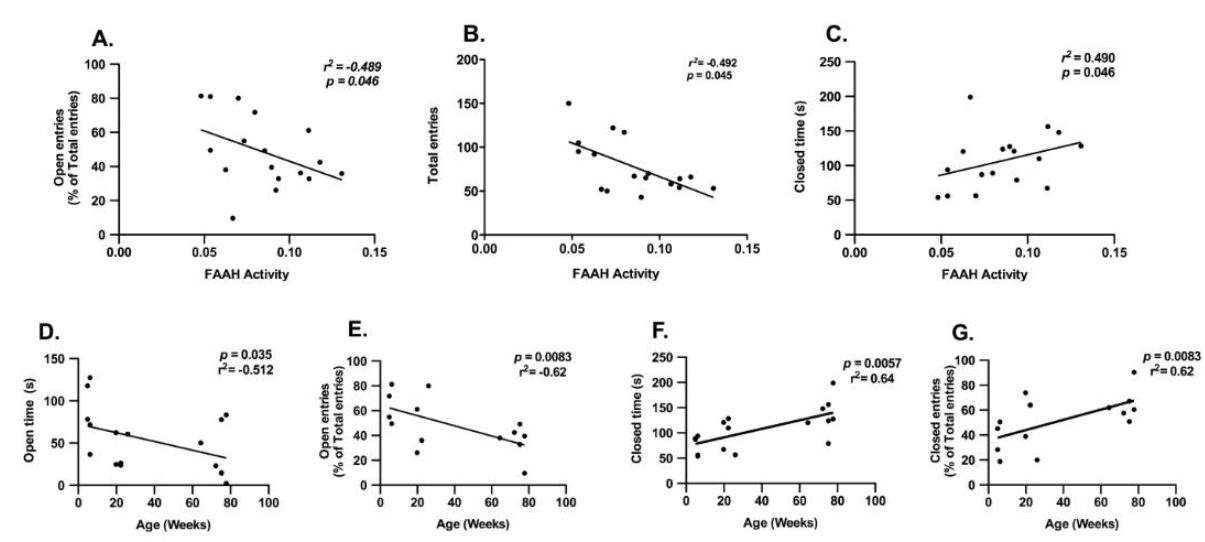


Table 1. Genes associated with increased risk of cannabis dependence and psychosis.

Cannabis dependence genes		
Gene	Function	SNPs
<i>RP11-206M11.7</i>	Unknown	rs143244591
<i>SLC35G1</i>	Nucleotide sugar transport	rs146091982
<i>CSMD1</i>	Complement system regulation	rs77378271
<i>CNR1</i>	Endocannabinoid signaling	rs806380
<i>FAAH</i>	Endocannabinoid metabolism	rs324420
<i>NRG1</i>	Neurotrophic factor	rs17664708
<i>MDR1</i>	Drug efflux pump	rs1045642
Cannabis psychosis genes		
Gene	Function	SNPs
<i>DRD2</i>	Dopamine signaling	rs1076560
<i>BDNF</i>	Neurotrophic factor	rs6265
<i>AKT1</i>	Kinase signaling	rs2494732
<i>COMT</i>	Dopamine metabolism	rs4680