Novel therapeutic and drug development strategies for tobacco use disorder: Endocannabinoid modulation

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Abstract

Introduction: Tobacco use disorder (TUD) is a chronic relapsing condition. Existing pharmacotherapy can assist smokers to initiate smoking cessation, but relapse rates remain high. Novel therapeutics are required to help people quit and also to prevent relapse. The endocannabinoid system has been increasingly implicated in reward and addiction processes and the cannabinoid CB1 receptor inverse agonist rimonabant has been shown to be effective at promoting smoking cessation but has been associated with adverse psychiatric side effects.

Areas covered: Multiple converging factors likely contribute to the maintenance of smoking and cause relapse including nicotine reinforcement, propensity to reinstate drug seeking (induced by nicotine priming, nicotine-associated cues, and stress), the severity of withdrawal signs and executive function status. Studies assessing the impact of endocannabinoid (CB1 receptor, CB2 receptor, anandamide, and 2-arachidonoylglycerol) modulation on these addiction-related factors are reviewed.

Expert opinion: Endocannabinoid research in TUD is at a relatively early stage. Based on current evidence, CB1 receptor neutral antagonists and fatty acid amide hydrolase inhibitors demonstrate positive effects in studies assessing several addiction-related factors. This suggests they offer the greatest promise as novel cessation and anti-relapse agents. Future research avenues are discussed, notably to translate findings into humans.

Keywords: Anandamide, Cannabinoid receptor, Endocannabinoid, Executive function, FAAH inhibition, Nicotine Reinforcement, Reinstatement, Tobacco use disorder, Withdrawal, 2-Arachidonoylglycerol.

Article highlights

- Multiple factors likely contribute to continued smoking and relapse including nicotine reinforcement, propensity to reinstate drug seeking (induced by nicotine priming, nicotine-associated cues, and stress), the severity of withdrawal signs and executive function status. We review the impact of endocannabinoid modulation on these factors.
- Inverse agonism and neutral antagonism at CB1 receptors reduces nicotine self-administration, reinstatement of nicotine seeking, as well as some withdrawal signs and may improve executive dysfunction.
- Inhibitors of fatty acid amide hydrolase (FAAH) attenuate reinstatement of nicotine seeking, reduce nicotine self-administration in some animals, and may reduce some withdrawal symptoms. There is mixed evidence for effects on executive function.
- CB1 receptor inverse antagonism is associated with adverse psychiatric effects. Neutral antagonism at this receptor may have an improved psychiatric side effect profile. FAAH inhibitors have anxiolytic and antidepressant effects.
- Research examining the impact of endocannabinoid modulation on addiction-relevant factors is at a relatively early stage. There is currently limited or mixed evidence for effects of alternative endocannabinoid modulating mechanisms on these addiction-relevant factors.
- Preclinical evidence suggests that CB1 receptor neutral antagonists and FAAH inhibitors hold promise as novel smoking cessation and anti-relapse agents. These findings need to be validated in human smokers.

1.0 Introduction

With over 1 billion smokers worldwide [1] and the prevalence of daily smoking estimated at 15% in 2015 [2], tobacco smoking is a global health problem. Specifically, it has been estimated that tobacco use is associated with over 8 million deaths [1] and thousands of billions of dollars in health care costs and productivity losses across the globe annually [3,4]. Further, in 2017 there was estimated to be more than 1.5 million youths (aged 12 to 17 years) using cigarettes over the preceding 12 months in the U.S. alone [5], suggesting tobacco-related problems will likely continue to some degree in to the future. Quitting smoking can significantly improve health outcomes and decrease the risk of dying from smoking-related disease [6]. Unfortunately, tobacco use disorder (TUD) is a chronic condition characterised by multiple cycles of quitting and relapse [7]. Indeed, nearly 70% of smokers report wanting to quit smoking [8] while as few as 3-5% of unaided quit attempts may be successful [9].

Pharmacotherapy can increase cessation success and there are currently three FDA-approved first-line medications for smoking cessation: nicotine replacement therapy (NRT), varenicline, and bupropion. These evidence-based medications show cessation efficacy but there is diminishing benefit of cessation medication over the first 12 months [10,11]. Further, modelling of data from over 40 smoking cessation trials suggests that 12 month abstinence rates are just 23% or less with use of these medications [12]. Therefore, relapse remains the most likely outcome of any cessation attempt even using approved medication. While there is clearly much need for improved cessation pharmacotherapy, there have been no new medications approved for smoking cessation by the FDA since varenicline in 2006 [13].

The lack of efficacious, newly approved smoking cessation pharmacotherapy is not due to an absence of potential candidates. Indeed, several recent reviews on the theme of existing and emerging drug treatments for smoking cessation [14-16] indicate there has been no shortage of pharmacological candidates, and that these have had a wide variety of pharmacological mechanisms of action. For example, Beard et al., [14] compares over 20 potential smoking cessation drugs on a number of criteria including efficacy, cost, ability to serve new patient groups and ease of use. Gómez-Coronado et al., [15] review over 40 conventional and novel pharmacotherapies and our own review of innovative smoking cessation interventions [16] highlights that a range of pharmacological agents have been evaluated in clinical trials in the last decade alone. An adverse side effect profile (such as that observed with rimonabant, discussed briefly below), difficulty translating findings between the preclinical and clinical worlds (perhaps as a consequence of relying on overly-reductionist assays and models to explain a complex disorder), and the small number of high quality studies (i.e. with large sample sizes and adequate abstinence follow-up durations) with any one promising candidate likely contributes to the lack of new approved medications despite an active field of contenders emerging from preclinical studies.

Arguably one of the most promising candidates for smoking cessation in recent years was rimonabant, an anti-obesity drug and inverse agonist at the cannabinoid (CB) receptor 1. Indeed, abstinence at the end of 10 weeks of treatment with rimonabant (20mg/day) and at 48 weeks follow-up was higher than placebo in a pooled analysis of three randomized double-blind controlled trials [17]. However, the high rate of psychiatric side effects, notably the induction of anxiety and depression and risk of emergence of suicidal ideation [18], led to the voluntary withdrawal of rimonabant from the European market in 2008 [19]. Nevertheless, there is an increasing understanding of the role of the endocannabinoid system in reward processing and addiction [20,21] suggesting that there may still be potential tobacco smoking cessation candidates found that work via endocannabinoid modulation. In this review, we provide a brief description of the endocannabinoid system before consolidating existing findings regarding the impact of endocannabinoid modulation strategies on outcomes relevant to TUD and smoking cessation. We focus on presenting studies that assess drug effects on factors thought to drive the maintenance or relapse of

tobacco use including: the rewarding properties of nicotine (reinforcement and motivation), propensity to reinstate use/relapse (induced by nicotine priming, nicotine-associated cues or stress), nicotine withdrawal signs and executive function status. In this way, we draw upon research findings from multiple experimental designs, tasks and assays in order to avoid the pitfalls of a reductionist approach, that could occur from presenting research from one of these research areas alone. At the beginning of each section, we briefly introduce each of these research areas by defining them and describing how they are linked to tobacco use disorder and/or relapse. Then we review the available evidence for effects of CB1 and CB2 receptor modulation, as well as for modulation of the levels of the two main endocannabinoid neurotransmitters, N-arachidonolylethanolamine (anandamide) and 2-Arachidonoylglycerol (2-AG). Ultimately, it is hoped that this review will help to identify which endocannabinoid modulating therapeutic strategies look most promising for TUD given existing findings. In addition, this review may help stimulate further research where this is warranted, either because research is limited in certain areas or because of initial positive findings. Thus, this review will also provide a basis for endocannabinoid modulation drug development strategy. Together, we are hopeful that this will result in a more strategic discovery process that leads to more efficacious pharmacotherapies for smoking cessation.

2.0 Overview of the endocannabinoid system

Within the brain, the endocannabinoid system is a lipid-based retrograde synaptic transmission system [22]. This form of communication fine-tunes information flow within all major neurotransmitter pathways and contributes to synaptic plasticity in several key brain regions involved in neuropsychiatric disorders, including addictions [23]. This system consists of the endocannabinoid neurotransmitters, with anandamide [24] and 2-AG [25,26] being the most well studied. These two neurotransmitters have been shown to have both complementary and mutually inhibitive functions [27-30]. There is some evidence that these endocannabinoids are stored in intracellular adiposomes or are bound to fatty acid binding proteins [31]. However, the most widely supported belief is that unlike conventional neurotransmitters, endocannabinoids are not stored in vesicles. Instead they are thought to undergo de novo synthesis on an as needed basis by receptor-stimulated cleavage of lipid precursors [32]. Such a tightly controlled signalling system may imply that ligands acting directly on the receptors have greater side-effects than those modulating endocannabinoid tone. Anandamide can be synthesized from N-arachidonoyl phosphatidylethanolamine (NAPE) via several pathways including the biosynthetic enzyme N-acylphosphatidylethaloamine-hydrolysing phospholipase D (NAPE-PLD) [33]. Cellular reuptake of anandamide is thought to occur via the hypothetical anandamide reuptake transporter [34-36] and it is predominantly metabolized by fatty acid amide hydrolase (FAAH) in to arachidonic acid (AA) and ethanolamine ($EtNH_2$) [32,37,38]. The major synthetic pathway for 2-AG is from diacylglycerol (DAG), by the action of the biosynthetic enzyme diacylglycerol lipase (DAGL) [39,40], and it is predominantly metabolized by monoacylglycerol lipase (MAGL) in to AA and glycerol [41].

The endocannabinoid system also consists of the brain and peripheral receptors. The CB1 receptor is the most abundant G-protein coupled receptor (GPCR) in the brain. It is localized pre-synaptically on both GABAergic and glutamatergic neurons, in line with its neuromodulatory role [42-44]. The CB2 receptor is mainly found in the immune system [45] but is also found centrally where, like their CB1 counterparts, they can modulate midbrain dopamine neuron activity [46]. Other non-CB1 and non-CB2 cannabinoid-related receptors have been proposed, including GPCR18 and GPCR55, but these remain to be fully validated pharmacologically [47]. The principle components of the endocannabinoid system within the central nervous system are shown in Figure 1. For more details relating to the molecular pathways involved in the biosynthesis, uptake and degradation of the endocannabinoid neurotransmitters see [48].

[INSERT FIGURE 1 NEAR HERE]

3.0 Nicotine reinforcement and motivation

An important driver of tobacco use are the rewarding/reinforcing properties of nicotine. The rewarding and reinforcing effects of nicotine have mainly been assessed using conditioned place preference (CPP) and self-administration procedures. Nicotine self-administration has been observed under both fixed and progressive ratio schedules. Under fixed ratio schedules a fixed number of responses must be achieved before a nicotine infusion is given. Increased nicotine self-administration, often indexed as a higher rate of responding, indicates that nicotine is more rewarding. Under progressive ratios the response requirement for nicotine infusion increases after each nicotine infusion. Progressive ratio schedules are used to assess motivation for nicotine as they provide an index of 'how hard' individuals are willing to work for nicotine infusions. For example, initially naïve non-human primates provided access to intravenous nicotine infusion have been shown to make up to 600 operant responses for a single nicotine injection [49], indicating that they are highly motivated to respond for nicotine and that it is an effective reinforcer. CPP is also used to investigate rewarding effects of drugs of abuse. Typically, CPP studies with nicotine will measure the amount of time animals spend in an area that has previously been associated with nicotine. Animals that find nicotine most rewarding will spend more time in areas associated with nicotine. These animals are described as exhibiting CPP [50]. While the expression of CPP reflects the influence of environmental stimuli previously associated with a drug, the development of drug-primed, or drug-induced, CPP after extinction is thought to reflect the reinforcing effects because approach behavior and time spent in a drug-paired environment can be considered an index of drug reward seeking behavior. The following sections summarize the existing findings regarding the impact of endocannabinoid modulation on nicotine self-administration and nicotine-induced CPP.

3.1 CB1 receptor modulation

Studies have indicated that CB1 receptor antagonism or inverse agonism attenuates self-administration of nicotine [51-53]. For example, Schindler et al., [51] show that high rates of nicotine taking in squirrel monkeys are reduced by both the CB1 receptor antagonist AM4113 and the inverse agonist rimonabant. Studies investigating the effects of central injection into specific anatomical brain regions implicate cortico-limbic CB1 receptors in the control of nicotine reinforcement and subsequent nicotine seeking behavior. For instance, injection of the CB1 receptor antagonist AM251 into rat ventral tegmental area or nucleus accumbens resulted in attenuation of self-administration for ventral tegmental area injection only [52]. In contrast, bilateral injection of rimonabant into the rat nucleus accumbens shell, the basolateral amygdala or the prelimbic cortex leads to reduced nicotine seeking maintained by cues previously associated with nicotine [54]. This suggests there may be subtle regional differences underlying the effects of CB1 receptor blockade on nicotine self-administration and nicotine seeking following extinction. Studies investigating nicotine reinforcement using progressive ratio schedules have also implicated CB1 receptors in nicotine motivation. The non-selective CB1/CB2 receptor agonist WIN 55,212-2 increased self-administration under a progressive ratio schedule, an effect that was reversed by administration of the CB1 receptor inverse agonist rimonabant [55]. Similarly, motivation to respond for nicotine is attenuated by administration of rimonabant [56] or the CB1 receptor neutral antagonist AM4113 [57].

CB1 receptors have previously been implicated in nicotine CPP. For example, evidence from genetic studies shows that while nicotine produces CPP in wild-type mice, this effect is absent in CB1 receptor knock-out mice [58]. Several studies indicate that administration of CB1 receptor antagonists or inverse agonists inhibit nicotine-induced CPP [59-61]. In particular, bilateral injection of the selective CB1 receptor antagonist AM251 in to the ventral tegmental area [62] or basolateral amygdala [63] inhibits nicotine-induced CPP, implicating amygdala-striatal CB1 receptors in drug reward seeking. One study found that a single pre-injection of rimonabant inhibited short-term nicotine-induced CPP i.e. 24 hours after the last

conditioning session, but not long-term nicotine-induced CPP that was 3 or 12 weeks after acquisition [64]. However, further work showed that pre-test injection of rimonabant could retain the capacity to inhibit long-term nicotine-induced CPP when accompanied by daily injection of rimonabant post-acquisition [65]. In contrast, the non-selective CB1/CB2 receptor agonist WIN 55,212-2 can induce a significant place preference to an area previously associated with nicotine when administered alone, or with a low ineffective nicotine dose [63,66]. Taken together, studies evaluating modulation of activity at CB1 receptors demonstrates the pivotal role of this receptor in nicotine reinforcement and motivation. Research demonstrates that these important drivers of nicotine use may be reduced by CB1 receptor blockade. In line with this, CB1 receptor blockade inhibits nicotine-induced dopamine release in the nucleus accumbens [67].

3.2 CB2 receptor modulation

The effects of CB2 receptor ligands on nicotine reinforcement assessed via studies of self-administration or nicotine-induced CPP have provided equivocal findings. Initial studies in rats indicated that there were no effects of CB2 receptor stimulation or blockade on nicotine self-administration under fixed or progressive ratio schedules [55,68]. In contrast, CB2 receptor knock-out mice self-administer less nicotine compared to wild-type mice [69] and do not show nicotine-induced CPP [69-71]. These genetic studies also employed pharmacological CB2 receptor modulation demonstrating that the CB2 receptor agonist O-1966 produced a conditioned place preference when administered with a sub-threshold dose of nicotine [70], and that CB2 receptor antagonists block nicotine-induced CPP [69-71]. In addition, CB2 blockade reduced nicotine self-administration under fixed and progressive ratio schedules in mice [69]. Taken together, these studies suggest that CB2 receptors may play a role in nicotine reinforcement and motivation but that there may be species differences in CB2 mediated control of these factors. However, a more recent study has provided findings that conflict with these previous reports [72]. In this study, administration of the dietary terpenoid and CB2 receptor agonist Beta-caryophyllene inhibited, rather than increased, nicotine self-administration and motivation for nicotine seeking in both rats and mice. In addition, the CB2 receptor antagonist AM630 blocked Beta-caryophyllene-induced reduction in nicotine self-administration. However, the reduction in nicotine self-administration was only partially blocked by CB2 receptor knock-out, with a blocked reduction in self-administration evident in knock-out mice administered low but not high dose Beta-caryophyllene. These findings suggest that the effects of Beta-caryophyllene on nicotine reinforcement may be mediated by both CB2 and non-CB2 receptor mechanisms and this may account for the differences compared to prior findings.

3.3 Anandamide modulation

Conditioned reinforcing properties of drugs of abuse including nicotine are mediated by the dopaminergic system [73]. It has been suggested that phasic dopamine release evoked by abused substances, and important for a range of addictive behaviors, requires cannabinoid receptor activation [74]. Endocannabinoid neurotransmitter tone (anandamide and 2-AG levels) may therefore play an important role in mediating nicotine-reinforcement. Studies of pharmacological modulation of anandamide have used both FAAH inhibitors and anandamide reuptake inhibitors to increase synaptic anandamide by blocking the breakdown and reducing neuronal uptake respectively. Studies assessing the impact of FAAH inhibition or anandamide re-uptake inhibition on nicotine self-administration have found either no effect [56,75,76] or reductions in drug taking [77,78] in studies with rats and non-human primates. Specifically, the anandamide reuptake inhibitors VDM11 and AM404 both failed to affect nicotine self-administration under fixed and progressive ratio schedules of reinforcement in rats [75,76]. Similarly, FAAH inhibition with URB597 did not affect nicotine self-administration under a progressive ratio schedule in rats [56]. In contrast to these reports, URB597 prevented the acquisition of nicotine self-administration in rats [77] and shifted the nicotine self-administration dose response curve consistent with reducing nicotine reward

in squirrel monkeys as did another FAAH inhibitor, URB694 [78]. Interestingly, these effects on nicotine self-administration in squirrel monkeys were reversed by the peroxisome proliferator-activated receptor alpha (PPAR-α) antagonist MK886. It is important to note here that while anandamide reuptake inhibitors should selectively increase anandamide levels, FAAH inhibition will also prevent metabolism of other bioactive fatty acid ethanolamides such as oleoylethanolamide and palmitoylethanolamide. These non-cannabinoid fatty acids may be able to regulate endogenous cannabinoid activity and could modulate anandamide responses (a phenomenon similar to the so called 'entourage effect') [79,80]. They are also PPAR-α ligands and have been shown to suppress mesolimbic dopamine neuron activation [81]. Further, PPAR-α agonists have also demonstrated potential to decrease nicotine self-administration in preclinical studies [82].

Studies assessing effects of FAAH and anandamide re-uptake inhibition on nicotine CPP have also presented mixed findings. In mice, both genetic knock-out and pharmacological inhibition of FAAH, with URB597, enhances the expression of nicotine CPP [83]. In contrast, URB597 and the anandamide reuptake inhibitor AM404 prevented the development of nicotine-induced CPP in rats [77,84]. This species difference is also evident in the effects of FAAH and anandamide reuptake inhibitors on striatal dopamine release. In mice, FAAH inhibition has been shown to enhance nicotine-induced dopamine release in the nucleus accumbens [85]. In contrast, FAAH inhibition blocks nicotine-induced excitation of ventral tegmental area dopaminergic neurons and blocks nicotine-induced dopamine release in the shell of the nucleus accumbens, via CB1 receptor and PPAR-α mediated mechanisms in rats [86]. Similarly, anandamide reuptake inhibition reduces nicotine-induced increases in dopamine levels in the nucleus accumbens in rats [84].

Taken together, the studies described in this section indicate that there are important species differences in the effects of pharmacological manipulation of anandamide on nicotine reinforcement and motivation. Perhaps the strongest evidence for positive effects is that FAAH inhibition reduces nicotine reinforcement in non-human primates [78], although these effects may be mediated by a non-cannabinoid mechanism and further studies are required to confirm this. Interestingly cannabidiol has been proposed to inhibit FAAH and elevate anandamide levels [87,88] and it is noteworthy that one week ad hoc administration of cannabidiol in 12 smokers significantly reduced cigarette smoking (self-administration) relative to placebo [89] (it should be noted that this was a pilot study and this has not yet been replicated). However, the pharmacology of cannabidiol is complex affecting multiple targets [90], including modulation within both GABA and glutamate systems [91]. Therefore, it is not entirely clear that this effect is mediated by actions at FAAH.

3.4 2-AG modulation

It has been suggested that 2-AG may be the main endocannabinoid transmitter regulating phasic dopamine activity and long-term plasticity induced by drugs of abuse [92,93]. However, there have been few studies assessing the effects of 2-AG modulation on nicotine reinforcement and motivation. In mice, the MAGL inhibitor JZL184, which reduces metabolism of 2-AG, had no effect on nicotine self-administration under fixed and progressive ratio schedules of reinforcement [94]. However, inhibition of DAGL (2-AG biosynthesis) reduces nicotine self-administration in rats without disrupting responding for a non-drug reinforcer or motor activity [95]. Further studies are required to establish the full impact of 2-AG modulation on the regulation of nicotine reinforcement and motivation.

4.0 Reinstatement of drug seeking

Drug relapse and craving are commonly precipitated by acute exposure to the self-administered drug, drug-associated cues, or stressors. These relapse-inducing factors are modelled preclinically in laboratory

animals with drug reinstatement following acquisition of self-administration and subsequent extinction of drug-reinforced responding [96]. In the following sections the impact of endocannabinoid modulation on nicotine reinstatement models is summarized.

4.1 CB1 receptor modulation

There is good evidence that CB1 receptor inverse agonism or neutral antagonism attenuates nicotine seeking behavior that is nicotine primed or cue-induced [51,53,54,56,57,97,98]. For example, Gueye et al., [57] found that the CB1 receptor antagonist AM4113 reduces nicotine primed and cue-induced reinstatement of nicotine seeking. The same study also found that AM4113 attenuated stress-induced reinstatement of nicotine seeking using yohimbine as a pharmacological stressor. Future work using other stressors is warranted to assess the generalization of these initial findings. Finally, this study also found, in a different group of animals, that AM4113 decreased dopaminergic neuron firing in response to nicotine in the ventral tegmental area suggesting that the reduction in drug seeking may be mediated by an attenuation in dopaminergic output. Taken together, evidence suggests that the CB1 receptor mediates nicotine seeking behavior. In particular, the effects of CB1 receptor neutral antagonists on nicotine and cue-induced reinstatement suggest they may help with the maintenance of abstinence.

4.2 CB2 receptor modulation

Few studies have investigated the effects of CB2 receptor modulation in models of nicotine reinstatement. Stimulation and blockade of CB2 receptors, with the agonist AM1241 and the antagonist AM630 respectively, failed to effect nicotine seeking induced by nicotine priming or by nicotine-associated cues in rats [68]. Further, the CB1/CB2 receptor agonist WIN 55,212-2 enhanced reinstatement effects of nicotine-associated cues in rats. However, whereas the CB1 receptor inverse agonist rimonabant was able to reverse effects on nicotine seeking, the CB2 receptor antagonist AM630 was not [55]. This suggests that CB2 receptors are unlikely to mediate nicotine seeking. Administration of the dual CB1 receptor antagonist and CB2 receptor agonist Δ^8-tetrahydrocannabivarin attenuated cue-induced and nicotine-induced reinstatement of nicotine seeking in rats [99] but given previous findings with selective CB1 and CB2 receptor ligands, it appears likely that these effects are driven by CB1 receptor antagonism rather than CB2 receptor agonism.

4.3 Anandamide modulation

The anandamide reuptake inhibitors VDM11 and AM404 have both been shown to attenuate reinstatement of nicotine seeking induced by nicotine priming or nicotine-associated cues in rodents [75,76]. Similarly, several studies have found that FAAH inhibition also reduces nicotine primed or cue-induced reinstatement of nicotine seeking [56,77,78,100]. The effect of FAAH inhibition on nicotine reinstatement may be mediated by both endocannabinoid and non-endocannabinoid mechanisms. For instance, one study in rats found that the FAAH inhibitor URB597 reduced cue-induced reinstatement of nicotine seeking and that the effect was reversed by rimonabant, but not by the CB2 receptor or PPAR-α antagonists AM630 and MK886 respectively [100]. This suggests a CB1 receptor mediated mechanism. In contrast, a study in squirrel monkeys found that URB597 and another FAAH inhibitor, URB694, attenuated both nicotine primed and cue-induced reinstatement of nicotine seeking, but the effect on nicotine priming induced reinstatement was blocked by MK886 [78]. This suggests a PPAR-α mediated mechanism. Indeed, PPAR-α agonists have previously been shown to reduce reinstatement of nicotine seeking in both rats and squirrel monkeys [82]. Taken together, evidence suggests that pharmacological modulation of anandamide impacts nicotine seeking behavior. FAAH inhibition, via a CB1 receptor or PPAR-α mediated mechanism, appears to reduce nicotine reinstatement and may offer efficacy as an anti-relapse agent in human smokers. Interestingly, cannabidiol, which appears to inhibit FAAH alongside having other targets, attenuates context and stress-induced drug seeking in rats with alcohol and cocaine self-administration

histories [101]. Moreover, after overnight abstinence, an 800mg dose of cannabidiol reduces the pleasantness of cigarette cues and reverses attentional bias towards cigarette cues in smokers, suggesting that it impacts cue salience [102]. Given the evidence for attenuation of cue-induced relapse by more selective FAAH inhibitors (described above), it is intriguing to speculate that this effect may operate via a reduction in cue salience and further research is warranted in this area.

4.4 2-AG modulation

Few studies have investigated the effects of 2-AG modulation in models of nicotine reinstatement. Administration of the MAGL inhibitor JZL184 increased nicotine cue-induced reinstatement of nicotine seeking in mice [94] suggesting that elevation of 2-AG may induce relapse in the presence of nicotine-associated cues. In support of this finding, another MAGL inhibitor, MJN110, has been shown to enhance cue-induced non-drug reward seeking in rats, an effect that was blocked by rimonabant [103]. Together this implicates 2-AG in cue-induced reward seeking for both drug and non-drug rewards and suggests that endocannabinoid tone at CB1 receptors is an important regulator of cue-induced reward seeking.

5.0 Nicotine withdrawal signs

Symptoms of withdrawal may be experienced after reducing or quitting tobacco use including irritability, anxiety, difficulty concentrating, restlessness, increased appetite, depressed mood and sleep problems. These symptoms can appear 4-24 hours following cessation, peak on approximately the third day of abstinence and gradually reduce over the proceeding 3-4 weeks [104]. Bidirectional relationships between withdrawal symptoms and smoking relapse have been reported. However, analyses evaluating temporal relationships more strongly support a negative reinforcement interpretation [105] whereby negative or aversive states motivate the resumption of tobacco smoking. Therefore, addressing the withdrawal syndrome is an important aspect of smoking cessation treatment. The following sections summarize the studies examining the impact of endocannabinoid modulation on withdrawal.

5.1 CB1 receptor modulation

Genetic knock-out of CB1 receptors does not appear to impact nicotine withdrawal symptoms [58,83]. Castañé et al., [58] induced somatic signs of nicotine withdrawal in chronic nicotine-treated mice using mecamylamine-precipitated abstinence. No difference in severity of nicotine withdrawal signs was found between CB1 receptor knock-out and wild-type mice. Merritt et al., [83] also found that CB1 receptor knock-out mice had equivalent severity withdrawal signs compared to wild-type mice after spontaneous withdrawal induced by termination of nicotine delivery. In contrast, this study also found that mice treated with the CB1 receptor inverse agonist rimonabant had reduced somatic signs of withdrawal compared to vehicle-treated mice. Similarly, the CB1 receptor antagonist AM251 was also shown to significantly reduce withdrawal signs in mice after 24 hours of nicotine abstinence [106]. However, the CB1 receptor partial agonist Δ^9-tetrahydrocannabinol has also been shown to decrease somatic withdrawal signs associated with mecamylamine- or naloxone-precipitated abstinence. In addition, it also reverses conditioned place aversion associated with naloxone-precipitated nicotine abstinence suggesting that it may prevent physical and motivational aspects of nicotine withdrawal [107].

CB1 receptors have been implicated in some specific nicotine withdrawal associated phenomena. For instance, genetic variation in the CB1 receptor of human smokers moderates withdrawal-related cognitive disruption [108]. Similarly, in mice selective genetic deletion of CB1 receptors in forebrain GABAergic neurons or administration of rimonabant was able to block nicotine withdrawal associated memory impairment [109]. The CB1 receptor inverse agonists rimonabant and taranabant moderate weight in smokers during cessation treatment with those of a normal weight tending not to lose weight, while those who are overweight or obese tending to lose weight [110]. This is a useful property given that smoking

cessation may increase appetite. However, rimonabant is anxiogenic [18], may exacerbate anxiety during nicotine abstinence [111] and as noted previously has been withdrawn from the market due to adverse psychiatric side effects. In contrast, the CB1 receptor antagonist AM4113 has no effect on anxiety and shows an antidepressant-like effect [57]. Taken together, CB1 receptors appear to have a role in the manifestation of at least some nicotine withdrawal associated signs. Both CB1 receptor inverse agonists and neutral antagonists may reduce some withdrawal signs, however neutral antagonist may have an improved psychiatric side-effect profile.

5.2 CB2 receptor modulation

Few studies have examined the role of the CB2 receptor in nicotine withdrawal and the limited existing findings have been equivocal. Δ^8-tetrahydrocannabivarin a dual CB1 receptor antagonist and CB2 receptor agonist attenuated nicotine withdrawal signs in mice [99], but the non-selectivity of this ligand does not allow conclusions to be drawn regarding the role of CB2 receptor modulation. Another study found no differences in withdrawal signs when comparing CB2 receptor knock-out and wild-type mice on mecamylamine-precipitated abstinence [70]. In contrast, a further study found that somatic signs of mecamylamine-precipitated withdrawal were absent in CB2 receptor knock-out mice compared to wild-type mice and that AM630, a CB2 receptor antagonist, blocked withdrawal signs in wild-type mice [69]. The reason for differences in the findings of these studies is unclear but may result from other genetic strain differences of the mice used. More research is required to establish the role of CB2 receptors in nicotine withdrawal. Further, CB2 receptor agonism in mice may be associated with an anxiolytic and antidepressant profile that is prevented by pre-administration of a CB2 receptor antagonist [112] however studies examining this effect in relation to nicotine and abstinence-induced anxiety and depressed mood are lacking and further work is required in this area.

5.3 Anandamide modulation

Some degree of species difference has been postulated regarding the impact of FAAH inhibition on nicotine withdrawal [113]. In mice, FAAH inhibition with URB597 or genetic deletion of FAAH exacerbates somatic withdrawal signs. Further, FAAH knock-out mice, but not pharmacological inhibition of FAAH, enhances withdrawal-induced conditioned place aversion [83]. In contrast, URB597 has been shown to reduce anxiety associated with spontaneous nicotine withdrawal and have no effect on somatic withdrawal signs in rats [114]. Several studies find that FAAH inhibitors exert anxiolytic and antidepressant effects [115,116]. It is perhaps somewhat surprising then that one study has found that chronic URB597 administration during nicotine abstinence induces development of a depressive phenotype [117]. In contrast, the anandamide reuptake inhibitor AM404 has been demonstrated to exert antidepressant effects in nicotine withdrawn mice. This effect may be mediated by CB1 and 5-HT1A receptor mechanisms since prior administration of antagonists at these receptors blocked the antidepressant effect [106]. A small number of studies have shown that FAAH inhibition with URB597 can have some pro-cognitive effects, improving attention and memory in rodent models [118,119]. However, further work is required to specifically assess the impact of FAAH inhibition on abstinence-induced cognitive impairment. Interestingly, cannabidiol has been shown to abolish nicotine-withdrawal associated memory impairment in mice [120] but it is important to remember that cannabidiol has multiple pharmacological targets besides the proposed inhibition of FAAH. Taken together evidence suggests that pharmacological manipulation of anandamide levels is likely to improve some aspects of nicotine withdrawal.

5.4 2-AG modulation

The strongest evidence that modulation of 2-AG may impact nicotine withdrawal comes from cross-species work using a range of molecular, genetic and pharmacological techniques [121]. This 2015 report contains data from mouse and human studies and finds that in mice: basal MAGL mRNA expression

correlates with nicotine withdrawal signs, genetic knock-out of MAGL attenuates nicotine withdrawal, and inhibition of MAGL with JZL184 reduces somatic and aversive nicotine withdrawal signs. These effects of MAGL inhibition were blocked by rimonabant providing evidence for a CB1 receptor mediated mechanism. Further, human evidence is presented demonstrating an association between genetic variation within the MAGL gene and smoking withdrawal. In another study, MAGL inhibition with JZL184 had no effect on cognitive deficits associated with nicotine abstinence in mice. However, inhibition of the biosynthesis of 2-AG with O7460 prevented such deficits [109]. MAGL inhibitors have anxiolytic properties [115,122] but this has not been examined in relation to nicotine and abstinence-induced anxiety. Together these findings are consistent with the theory that increasing 2-AG levels may reduce withdrawal signs. Therefore, further work examining the impact of 2-AG modulation on nicotine withdrawal is warranted. This work should consider potential adverse effects of ligands used. For instance, it has been suggested that MAGL inhibition may be associated with impaired motor activity and cannabimimetic side effects whereas this is not the case with FAAH inhibition [122,123]. Dual FAAH/MAGL inhibitors such as SA-57, which is 100-fold more potent at inhibiting FAAH than MAGL, have also been developed. SA-57 appears to have efficacy at reducing withdrawal effects in morphine-dependent mice [123] but research in nicotine dependent animals is lacking.

6.0 Executive function

It is widely accepted that there are three core executive functions, working memory, inhibition, and cognitive flexibility [124]. As these executive functions help us to set and obtain goals amidst changing environments/situations, impairment in these executive functions may contribute to the initiation, maintenance and relapse of drug use. Conversely, enhancing executive function may improve outcomes in substance use disorder. However, there is limited and mixed evidence that existing pharmacotherapy improves executive function [125]. In the following sections we highlight research assessing the impact of endocannabinoid modulation on these three core aspects of executive function.

6.1 CB1 receptor modulation

Evidence suggests that the CB1 receptor is implicated in executive function. In humans, variations in the gene encoding the CB1 receptor (*CNR1* gene) are associated with working memory and attentional control performance [126-128] and a positron emission tomography study also suggests CB1 receptor availability is associated with working memory [129]. In rodents, overexpression of the CB1 receptor in rats impairs cognitive flexibility [130] and CB1 receptor knock-out mice display impaired working memory and cognitive flexibility [131,132].

Several studies have examined the impact of pharmacological modulation of the CB1 receptor on executive function. In rodents, administration of the CB1 receptor inverse agonist rimonabant or the CB1 receptor antagonist SLV330 has been shown to improve executive function. Specifically, rimonabant improves working memory and SLV330 improves inhibition [133,134]. However, rimonabant and another CB1 receptor antagonist, AM251, have also been shown to impair inhibition and working memory respectively [135,136]. Consistent with CB1 receptor blockade improving executive dysfunction, studies show that impairments in working memory, inhibition and cognitive flexibility due to pharmacological (scopolamine, amphetamine, nicotine or nicotine withdrawal) or other (e.g. ischemia, chronic stress) challenges are prevented or attenuated by administration of rimonabant, SLV330 or AM251 [109,136-145].

Impairments in working memory, cognitive flexibility and inhibition have been observed in studies in which rodents have been administered the CB1 receptor agonist ACEA or the non-selective CB1/CB2 receptor agonist WIN-55,212-2 [132,146-150]. However, WIN-55,212-2 exposure has been shown to

improve cognitive flexibility [151] but the non-selective pharmacology limits firm conclusions. On the other hand, studies using animal models of conditions associated with impairments in executive function (e.g. ADHD) and studies that have induced impairments in executive functions using stressors have shown improvements or normalisation of executive function following administration of WIN-55,212-2 or exogenous cannabinoids [152-154]. Taken together, there is reasonably strong evidence that modulation of CB1 receptors impacts executive function. However, there are mixed findings with both improvements and impairments in executive function reported after blockade at this receptor. This suggests that other factors may moderate the effects of CB1 receptor ligands on executive function. Conversely both CB1 receptor blockade and stimulation tend to improve executive function when tested in models of executive dysfunction.

6.2 CB2 receptor modulation

There have been few studies examining the impact of CB2 receptor modulation on executive function. Disruption of CB2 receptor expression in mice using CRISPR-Cas9 genome-editing has been shown to enhance working memory [155]. Similarly, CB2 receptor knock-out mice display enhanced working memory. However, CB2 receptor blockade by AM603 had no effect on working memory [156]. In contrast to these findings, the CB2 receptor agonist beta-caryophyllene has been found to reverse age-associated deficits in working memory in rats [157]. These findings suggest that CB2 receptor modulation does impact executive function. However, there appears to be differences with reports of both genetic deletion and pharmacological stimulation of CB2 receptors enhancing working memory. Further studies are required to fully establish the impact of CB2 receptor modulation on executive function.

6.3 Anandamide modulation

In humans, peripheral anandamide levels positively correlate with cognitive flexibility while there is no significant correlation between anandamide and inhibition [158]. This suggests anandamide modulation may impact some aspects of executive function. Indeed, several studies have examined the impact of FAAH inhibition on executive function with results being somewhat mixed. FAAH inhibition has been shown to improve working memory and inhibition in some rodent assays [118,119]. Further, FAAH inhibition has been shown to reverse impairments in working memory that are induced by head or brain injury models in mice [159,160], and to reverse an impairment in inhibition induced by maternal deprivation in rats [161]. In contrast, one study assessing the effects of five structurally different FAAH inhibitors found that one, AM3506, impaired working memory in rats while four others (URB597, URB694, PF-04457845 and ARN14633) showed no effect [162]. Mixed findings have also been reported regarding the impact of FAAH inhibition on cognitive flexibility with studies showing impaired reversal learning/discrimination reversal in rats after administration of URB597 [145] but no effects of anandamide or URB597 administration in squirrel monkeys [163]. Further, it is noteworthy that cannabidiol, which may inhibit FAAH, had no effect on working memory and impaired inhibition during smoking abstinence [164]. However, as discussed previously cannabidiol has multiple pharmacological targets and these effects may not be mediated via FAAH inhibition. Basal levels of endocannabinoids may explain mixed executive function findings with FAAH inhibition. FAAH knock-out mice, which have elevated levels of anandamide, have an increased sensitivity to the impairing effects of anandamide on working memory compared to wild-type mice [137]. Since nicotine abstinence is associated with increased anandamide in the prefrontal cortex [114], a region implicated in executive function, it will be important to establish if basal anandamide levels do indeed impact the cognitive effects of FAAH inhibitors.

6.4 2-AG modulation

There is a negative correlation between peripheral 2-AG levels and cognitive flexibility and no significant correlation between 2-AG and inhibition [158]. This suggests 2-AG modulation may impact some aspects

of executive function. However, few studies have directly assessed the impact of 2-AG modulation on executive function. There was no effect of the MAGL inhibitor JZL184 on working memory in rats [162]. In contrast, elevation of anandamide by inhibition of alpha/beta hydrolase domain 6 (a novel 2-AG hydrolytic enzyme responsible for some 2-AG metabolism) improved working memory in a mouse brain injury model [165]. As with FAAH inhibition (described above), mixed findings may relate to a moderating effect of basal endocannabinoid levels. In support of this, MAGL inhibition with JZL184 impairs working memory in FAAH knock-out mice but in wild-type mice, only high dose JZL184, and not low dose, impairs working memory [166]. The low number of studies in this area limits further discussion and further research is required to clarify the impact of 2-AG modulation on executive function.

7.0 Conclusions

The studies reviewed here support the involvement of the endocannabinoid system in nicotine reinforcement and motivation, reinstatement of drug seeking, severity of withdrawal signs and executive function. The main findings are summarized in Table 1. In particular, CB1 receptor blockade and FAAH inhibition may represent promising novel pharmacological approaches to smoking cessation and relapse prevention and the main findings supporting this conclusion are summarized below.

[INSERT TABLE 1 NEAR HERE]

Several strands of research implicate CB1 receptors in TUD. For example, positron emission tomography indicates that smoking is associated with an abnormal density of CB1 receptors [167]. In addition, in two independent samples of smokers, genetic evidence shows significant single nucleotide polymorphism and haplotype associations with the Fagerstrom Test for Nicotine dependence for variants within *CNR1*, the gene encoding the CB1 receptor [168]. In particular, CB1 receptors mediate reinforcing, motivational and reinstatement effects of nicotine. As reviewed here, studies tend to find that the inverse agonist rimonabant as well as neutral antagonists reduce nicotine self-administration and attenuate reinstatement of nicotine seeking. There is also evidence that blockade at this receptor may result in reduced severity of some withdrawal signs and may improve impaired executive function. Thus targeting the CB1 receptor in this way appears to have effects that should promote cessation and prevent relapse. While rimonabant has adverse psychiatric side-effects, neutral antagonists may have an improved psychiatric side-effect profile [57]. However, this may not be true of every neutral antagonist [169] and future drug candidates should be thoroughly assessed to ensure they do not induce anxiety and depression-like phenotypes.

As reviewed here, FAAH inhibitors also demonstrate anti-addictive properties in several studies. FAAH inhibition attenuates reinstatement of nicotine seeking and has been shown to reduce self-administration of nicotine in some animals. There is also some evidence that FAAH inhibition reduces severity of withdrawal signs and may enhance executive function, although evidence for the latter is limited and mixed. Importantly, FAAH inhibitors exert anxiolytic and antidepressant effects [115,116]. These properties may make FAAH inhibitors particularly useful for individuals vulnerable to anxiety and mood problems during withdrawal, and for those smokers with comorbid anxiety or depression.

Research examining the impact of modulation of the endocannabinoid system on addiction relevant factors is still at a relatively early stage. Evidence for effects of CB2 receptor or 2-AG modulation on the addiction relevant factors included in this review are either limited or tend to have provided more mixed findings than for CB1 receptor and anandamide modulation. Based on existing research, CB1 receptor neutral antagonists and FAAH inhibitors have the strongest support for continued development but it may be too early to rule out alternative endocannabinoid modulating mechanisms.

8.0 Expert Opinion

The global human and economic cost of tobacco smoking coupled with high rates of relapse among smokers, even when using current first line medication [12], highlights the need for novel and improved pharmacotherapy approaches in the management of TUD. The ideal drug candidate requires both smoking cessation efficacy and anti-relapse efficacy so that initial cessation can be maintained over the long-term. Multiple factors likely converge to maintain drug taking behavior and lead to relapse including the rewarding effects of nicotine, the propensity to reinstate drug seeking (in response to nicotine priming, nicotine associated cues or stress), the severity of withdrawal signs and executive function status. For this reason, a non-reductionist approach should be taken during assessment of candidate drugs for TUD. Here we have reviewed the impact of endocannabinoid modulation in a range of studies relevant to the maintenance of nicotine use and relapse.

While we are still at the beginning of our understanding regarding the impact of endocannabinoid modulation on addiction relevant behaviors, initial research has produced promising findings for CB1 receptor antagonists and FAAH inhibitors. Research efforts in the next few years will increase the understanding of this system in TUD, and in substance use disorders more generally. We will likely see further studies examining reinstatement of nicotine seeking. In particular, data relating to stress-induced relapse models would be desirable given that the majority of reinstatement studies have focused on nicotine primed and cue-induced drug seeking. We know that yohimbine-induced stress provokes reinstatement of nicotine seeking and that CB1 receptor antagonism can attenuate this [57]. Future work using non-pharmacological stressors will assess the generalization of these initial findings. Further, it might be expected that FAAH inhibition will have a larger effect on stress-induced relapse relative to CB1 receptor antagonists given their anxiolytic properties.

Regarding self-administration studies, previous research suggests that studies are most likely to show translational concordance between laboratory assessments and clinical outcomes when the former provide repeated administration (chronic treatment) of the candidate medication of interest, and also demonstrate behavioural selectivity [170-172]. Regarding CB1 receptor neutral antagonists, these criteria have been met. For instance, chronic injections (over 10 days) of the CB1 receptor neutral antagonist AM4113 attenuate nicotine self-administration in rats but have no impact on operant responding for food [57]. To date, none of the studies assessing the effects of FAAH inhibition on nicotine self-administration have used a chronic dosing schedule. However, behavioral selectivity has been demonstrated. For instance, acute FAAH inhibitor administration, over 5 consecutive self-administration sessions, reduced nicotine self-administration in non-human primates, with no effects on cocaine or food self-administration [78]. To increase translational predictive validity, future self-administration studies should aim to use a chronic dosing schedule of the candidate medication and include assessment of behavioral selectivity of drug effects.

Increasing our understanding of the impact of anandamide reuptake inhibitors should also be a research focus given that they attenuate reinstatement of nicotine seeking and may also reduce nicotine self-administration, and some withdrawal signs. To date, the majority of research examining the effects of anandamide modulation has come from studies using FAAH inhibitors. Given the scarcity of studies with the reuptake inhibitors, it has been difficult to draw firm conclusions regarding their impact. An important consideration regarding anandamide reuptake inhibition relates to the potential for abuse. Squirrel monkeys self-administer the anandamide reuptake inhibitor AM404 [173] suggesting reuptake inhibitors may have some risk for abuse. In contrast, the FAAH inhibitor URB597 was not self-administered in squirrel monkeys [174] while the newer FAAH inhibitor URB694 was self-administered at a moderate rate [78].

Whether self-administration of AM404 represents a specific property of this compound or a more general drug class effect requires further research. Careful assessment of abuse risk will be required for both FAAH inhibitors and anandamide reuptake inhibitors going forward.

Given that some of the anti-addiction effects of FAAH inhibitors appear to be mediated by PPAR-α, another focus for future research should relate to establishing interactions of the endocannabinoid system with other systems. However, of note here is that gemfibrozil, a partial PPAR-α agonist, failed to effect nicotine reinforcement, cue-reactivity or smoking cessation relative to placebo in a recent study of treatment seeking smokers [175]. Interestingly, there are interactions between the endocannabinoid and nicotinic cholinergic systems [176]. For instance, anandamide inhibits nicotinic acetylcholine receptor function in mouse thalamic synaptosomes [177] and in amphibian oocytes [178]. Whether these effects occur in other species, and whether they impact on the reported effects of FAAH inhibitors reviewed here will be of interest. Finally, there are some compounds which have yet to be extensively tested in a number of addiction relevant assays, but which may be likely to yield positive findings. Given the promising CB1 receptor antagonist findings, we suggest that allosteric modulators of the CB1 receptor [179] should be evaluated. Also, given that TUD is a complex condition with multiple factors converging to maintain drug taking behavior and cause relapse, it is unlikely that a single pharmacological target will be enough to prevent relapse. Therefore, given that dopamine D3 receptors have been proposed as another alternative pharmacological target for relapse prevention [180], we suggest that innovative multi-target ligands such as dual modulators of dopamine D3 receptors and FAAH [181], or dual modulators of dopamine D3 and CB1 receptors [182] be investigated.

The ultimate goal of this research is to see translation of findings in successful clinical trials and the subsequent availability of novel pharmacotherapeutics for those wanting to quit smoking. We reiterate that we believe that for such successful translation to occur, a non-reductionist, multi-dimensional approach to modelling factors relevant to the maintenance of smoking and relapse is required during preclinical research. We suggest that clinical trials in smokers are now required for neutral CB1 receptor antagonists and FAAH inhibitors. Of these two endocannabinoid modulating strategies, neutral CB1 receptor antagonists might be expected to have the greatest chance of smoking cessation success, given previous clinical findings with rimonabant which blocks the same receptor. However, they might also be expected to have increased risk of psychiatric side effects for the same reason. On the other hand, there is little clinical experience to draw upon regarding FAAH inhibition and none in those with TUD. However, the tools for such a study are available, the FAAH inhibitor PF-04457845 has recently shown efficacy and safety in a clinical trial for cannabis use disorder [183]. Further, unlike CB1 receptor modulation, anxiety and depression risk may be low given the mild CB1 receptor stimulating effects of FAAH inhibition. We are seeing the emergence of cannabinoid pharmacotherapy for several brain disorders [184], and there is potential for development of a novel endocannabinoid modulating medication for smoking cessation.

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** This double-blind, placebo controlled trial suggests that FAAH inhibitors can be used safely in humans to reduce cannabis use and withdrawal symptoms.

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* = of interest
** = of considerable interest

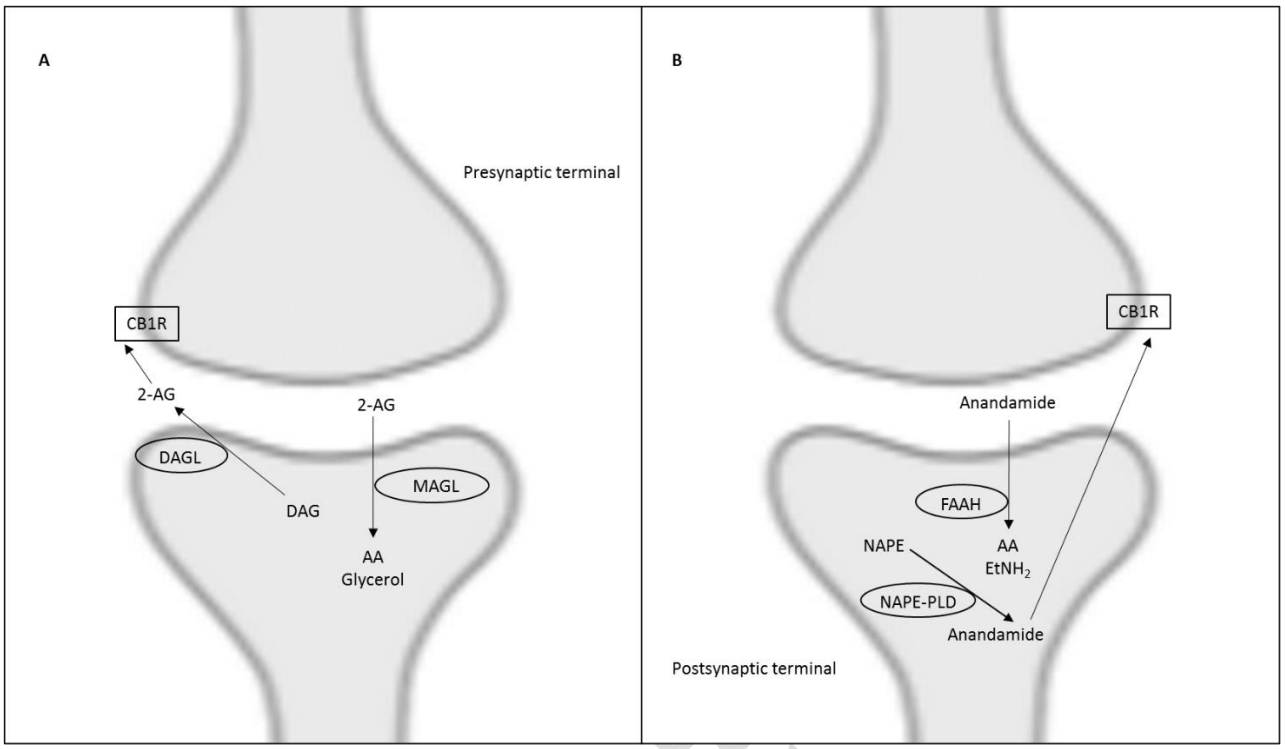

Figure 1: Principle components of the endocanabinoid system in the central nervous system.

The main enzymes involved in biosynthesis and metabolism of **(A)** 2-AG and **(B)** Anandamide. (CB1R = Cannabinoid Receptor 1; 2-AG = 2-Arachidonoylglycerol; DAG = Diacylglycerol; DAGL = Diacylglycerol Lipase; MAGL = Monoacylglycerol Lipase; AA = Arachidonic Acid; Anandamide = N-arachidonolylethanolamine; NAPE = N-arachidonoyl phosphatidylethanolamine; NAPE-PLD = N-acylphosphatidylethaloamine-hydrolysing phospholipase D; FAAH = fatty acid amide hydrolase; $EtNH_2$ = Ethanolamine).

Table 1: Main findings for the impact of endocannabinoid modulation on addiction/relapse relevant factors.

	CB1 Receptor Modulation	CB2 Receptor Modulation	Anandamide Modulation	2-AG Modulation
Nicotine Reinforcement and Motivation	Convincing evidence that CB1R neutral antagonists and CB1R inverse agonists ↓	Few studies and mixed findings but some evidence that CB2R antagonists ↓	Mixed findings but some evidence that FAAH inhibitors ↓	Few studies conducted. DAGL inhibition may ↓
Reinstatement of Nicotine Seeking	Convincing evidence that CB1R neutral antagonists and CB1R inverse agonists ↓	Few studies. Some evidence that modulation has no impact.	Some evidence that anandamide reuptake inhibitors ↓, Convincing evidence that FAAH inhibitors ↓	Few studies conducted. MAGL inhibition may ↑
Withdrawal Signs	CB1R neutral antagonists and CB1R inverse agonists may ↓ some withdrawal signs. CB1R inverse agonists ↑ abstinence-induced anxiety but CB1R neutral agonists may have improved psychiatric side-effect profile.	Few studies. Difficult to draw any conclusions at this point.	Mixed findings but some evidence that anandamide reuptake inhibitors and FAAH inhibitors may ↓ some withdrawal signs.	MAGL inhibition may ↓ some withdrawal signs.
Executive Function	Mixed findings but some evidence that CB1R blockade may reverse impairments.	Few studies and mixed findings. Difficult to draw conclusions at this point.	Mixed findings but some evidence that FAAH inhibitors may reverse impairments.	Few studies and mixed findings. Difficult to draw conclusions at this point.

Abbreviations: CB1R = Cannabinoid Receptor 1; CBR2 = Cannabinoid Receptor 2; 2-AG = 2-Arachidonoylglycerol; FAAH = fatty acid amide hydrolase; DAGL = Diacylglycerol Lipase; MAGL = Monoacylglycerol Lipase.