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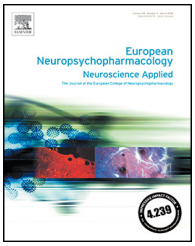

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REVIEW

A systematic review of phytocannabinoid exposure on the endocannabinoid system: Implications for psychosis

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Abstract
Cannabis, the most widely used illicit drug worldwide, produces psychoactive effects through its component cannabinoids, which act on the endocannabinoid system. Research on how cannabinoid exposure affects the endocannabinoid system is limited. Substantial evidence indicates cannabis use as a risk factor for psychosis, and the mechanism(s) by which this is occurring is/are currently unknown. Here, we conduct the first review of the effects of exogenous cannabinoids on the endocannabinoid system in humans with and without psychotic disorders.

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; AMT, anandamide membrane transporter; CB1R, cannabinoid 1 receptor; CB2R, cannabinoid 2 receptor; CBD, cannabidiol; CUD, cannabis use disorder; CYP450, cytochrome P450; DAGL, diacylglycerol lipase; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; GABA, γ-aminobutyric acid; MAGL, monoacylglycerol lipase; mSUV, modified standard uptake value; NAPE-PLD, N-acyl phosphatidylethanolamine-specific phospholipase D; OEA, oleoylethanolamine; PBMC, peripheral blood mononuclear cells; PEA, palmitoylethanolamine; PET, positron emission tomography; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; THC, (-)-trans-Δ9-tetrahydrocannabinol.

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The most well established finding is the down-regulation of cannabinoid CB1 receptors (CB1R) after chronic and recent cannabis exposure, but it remains uncertain whether this effect is present in cannabis users with schizophrenia. We highlight where cannabis exposure affects the endocannabinoid system in a pattern that may mirror what is seen in psychosis, and how further research can push this field forward. In these times of changing cannabis legislation, research highlighting the biological effects of cannabinoids is greatly needed.

1. Introduction

Cannabis is the most widely used illicit substance worldwide (UNDOC, 2018). In light of global changes in cannabis legislation, increasing usage of cannabis among adults in the USA, and an expansion of cannabinoid pharmaceutical trials, it is vital for researchers and policy makers to have an enhanced understanding of the impact of cannabinoids on the body's endogenous cannabinoid (eCB) system (Campbell et al., 2001; Compton et al., 2016; Lynch and Ware, 2015; Zajicek et al., 2003).

Cannabinoids bind to and modify cannabinoid CB1 and CB2 receptors (CB1R and CB2R) with varying intrinsic activities. Cannabinoids consist of several structural homologies, such as the classical tricyclic dibenzopyrans, phytocannabinoid (-)-trans-Δ9-tetrahydrocannabinol (THC) and the synthetic HU-210, and non-classical bicyclic and tricyclic compounds (Pertwee, 2006a). Other structural classes of cannabinoids include the aminoalkylindole *R*-(+)-WIN55212 and the eicosanoids, which consist of the endogenous arachidonylethanolamine (anandamide/AEA) and 2-arachidonylglycerol (2-AG) (Pertwee, 2006a).

Cannabis contains more than 100 phytocannabinoids, with the most abundant and most studied being THC and cannabidiol (CBD) (ElSohly et al., 2016; Pertwee, 2006a). THC is largely responsible for producing the psychoactive effects of cannabis, including cognitive impairments, which may be counteracted by CBD if present in sufficient quantities (Boggs et al., 2017; Curran et al., 2002; Huestis et al., 2001, 2007). In Europe, the concentration of THC in cannabis increased between 2006 and 2013, and has reached an average concentration of 9–13% (EMCDDA, 2018). Between 1995 and 2014 in the USA, the concentration of THC in cannabis increased from ~4 to 12%, with the THC/CBD ratio increasing from 14 to 80 (ElSohly et al., 2016). Despite the increasing potency of cannabis, its use among US adults increased from 10.4 to 13.3%, and the percentage of the population who perceives great risk of harm from using cannabis once or twice per week decreased from 50.4 to 33.3%, between 2002 and 2014 (Compton et al., 2016). This change in perception was accompanied by increasing usage and policy change in the US, specifically the legalization of medical marijuana in 12 states by 2007 (Compton et al., 2016). This discrepancy between increasing potency and decreasing perception of risk is alarming given that cannabis use elevates the risk of adverse mental health outcomes, especially in those who are more vulnerable (Moore et al., 2007). Of particular concern is the association of cannabis use with elevated risk for psychotic disorders, when initiating cannabis use during adolescence (Andreasson et al., 1987; Arseneault et al., 2002; Di Forti et al., 2015; Marconi et al., 2016; Moore et al., 2007). The increasing concentration of THC in cannabis is of concern because THC can cause positive psychotic symptoms, cognitive impairments, and perceptual alterations in a dose-dependent manner in healthy volunteers, and exacerbate symptoms in schizophrenia patients (Curran et al., 2002; D'Souza et al., 2005, 2004).

The task of addressing the public's growing misconceptions of the harmlessness of cannabis is made more difficult given how little is known about how acute or chronic cannabis use alters the eCB system, or how this could be involved in the pathophysiology of psychiatric disorders such as schizophrenia (Radhakrishnan et al., 2014). Although alterations of the eCB system have been observed in schizophrenia (Giuffrida et al., 2004; Ranganathan et al., 2016), the impact that phytocannabinoids have on this system remains unclear, especially within psychosis. A better understanding of the effects of cannabinoid exposure on the eCB system is of pressing importance, given the high rates of cannabis use in the general population and the high prevalence of cannabis use disorder (CUD) among individuals with schizophrenia (Koskinen et al., 2010).

Here, we systematically review the effects of exposure to cannabis and its constituent cannabinoids on the eCB system in healthy individuals including those with CUD, and individuals with schizophrenia. It should be noted that the literature regarding phytocannabinoid exposure in humans typically focuses on cannabis use.

2. Cannabinoids, the endocannabinoid system and psychosis – in brief

The eCB system is one of the most ubiquitously expressed neurotransmitter systems in the brain (Katona and Freund, 2008) and is involved in regulating a wide array of processes including feeding behaviour, memory, anxiety, and stress response (Di Marzo et al., 1998; Matias et al., 2006; Ruehle et al., 2012). The eCB system is composed of the eCB neurotransmitters, their receptors, and metabolizing enzymes (Fig. 1). The eCB system is unique in its retrograde signalling, whereby endocannabinoids are synthesized postsynaptically on-demand to bind cannabinoid receptors on the presynaptic neuron and subsequently attenuate neurotransmitter release (Katona and Freund, 2008).

The levels of endocannabinoids in the central nervous system and periphery are regulated primarily by their metabolizing enzymes (Cravatt et al., 2004). The primary synthetic enzyme of 2-AG is diacylglycerol lipase (DAGL),

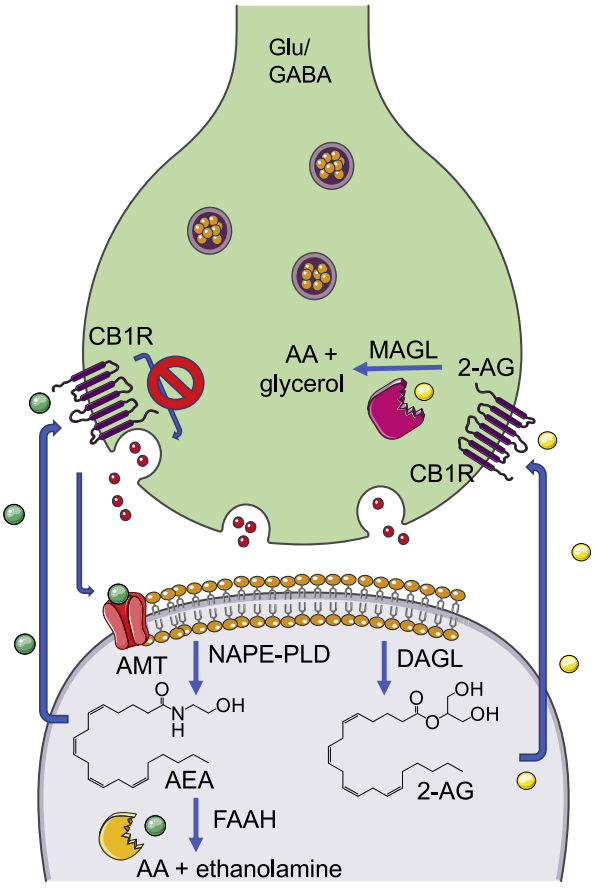

Fig. 1 Endocannabinoid scheme. Both AEA and 2-AG are synthesized from phospholipids by their biosynthetic enzymes, NAPE-PLD and DAGL, respectively. The retrograde signaling of AEA and 2-AG results in an attenuation of neurotransmitter release via CB1R. MAGL degrades 2-AG in the presynaptic neuron. AMT facilitates the re-uptake of AEA into the postsynaptic neuron, where it is degraded by FAAH. AEA is represented by green circles and 2-AG in yellow. This figure was produced using Servier Medical Art, available from https://smart.servier.com/. AEA: anandamide; 2-AG: 2-arachidonoylglycerol; NAPE-PLD: N-acyl phosphatidylethanolamine-specific phospholipase D; DAGL: diacylglycerol lipase; CB1R: cannabinoid receptor type 1; MAGL: monoacylglycerol lipase; AMT: anandamide membrane transporter; FAAH: fatty acid amide hydrolase; AA: arachidonic acid; Glu: glutamate; GABA: γ-aminobutyric acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and its catabolic enzyme is monoacylglycerol lipase (MAGL) (Ueda et al., 2013). The rate-limiting step of AEA synthesis is catalyzed by N-acyl phosphatidylethanolamine (NAPE)-specific phospholipase D (PLD) (Basavarajappa, 2007). Fatty acid amide hydrolase (FAAH) degrades AEA along with oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and to a lesser extent, 2-AG (Fowler et al., 2001). MAGL degrades 2-AG in the presnyaptic neuron, whereas the anadamide membrane transporter facilitates reuptake of AEA into the postynaptic neuron where it is degraded by FAAH (Beltramo and Piomelli, 2000; Gulyas et al., 2004). Notably, alternate metabolizing enzymes exist alongside the canonical FAAH and MAGL degradation pathways (Ueda et al., 2013).

Endogenous and exogenous cannabinoids signal through the canonical cannabinoid receptors, CB1R and CB2R. Additional receptors such as the vanilloid receptor 1 (TRPV1) and the orphan g-protein-coupled receptors GPR55 and GPR18 have been identified as putative cannabinoid receptors, or as part of an extended eCB system (Console-Bram et al., 2014; Panlilio et al., 2013; Pertwee, 2006b, 2008). CB1Rs are highly expressed throughout the brain, particularly in the basal ganglia, cortex, hippocampus, amygdala, and cerebellum (Herkenham et al., 1990; Mackie, 2005). They are predominantly localized to presynaptic axon terminals of GABAergic and glutamatergic neurons, and can also be found on peripheral tissues and immune cells (Galiegue et al., 1995; Katona and Freund, 2008). CB2R expression was initially detected in peripheral immune cells, but the expression of CB2R on human neurons has since been established albeit at an order of magnitude lower than CB1R (Galiegue et al., 1995; Onaivi et al., 2006; Van Sickle et al., 2005).

The two major endocannabinoids, AEA and 2-AG, are agonists for CB1R and CB2R. Both AEA and 2-AG display higher agonist efficacy at CB1R than CB2R, and 2-AG is more potent than AEA (Howlett et al., 2002; Pertwee, 2008). The *N*-acylethanolamines, OEA and PEA, share structural homology and biosynthetic and catabolic enzymes with AEA, but lack affinity for CB1R and CB2R (Brown, 2007). THC is a partial agonist at CB1R and CB2R, and displays lower efficacy than that of AEA and 2-AG (Pertwee, 2008). In contrast, CBD appears to antagonize CB1R agonists CP-55940 and *R*-(+)-WIN55212 (Pertwee et al., 2002; Petitet et al., 1998; Thomas et al., 2007). Numerous non-CB1R targets have been proposed for CBD as well, including the serotonin 1A receptor (5-HT_{1A}), sigma (σ) and mu (μ) opioid receptors, TRPV1, dopamine D2 receptors, and FAAH (Lee et al., 2017; Pertwee, 2008; Seeman, 2016; Watanabe et al., 1996). Synthetic cannabinoids, often called "spice", are most commonly full agonists of CB1Rs (Ford et al., 2017).

THC metabolism involves hydroxylation by cytochrome P450 (CYP450) enzymes, and subsequent glucuronidation before excretion (Pertwee, 2006a). CYP450 enzymes involved in the oxidation of THC include CYP2C9, CYP2C19, and CYP3A4 (Matsunaga et al., 1995). CBD is mainly metabolized by CYP3A4 and CYP2C19 (Jiang et al., 2011), and "spice" compounds JWH-018 and AM-2201 are metabolized by CYP2C9 and CYP1A2 (Stout and Cimino, 2014; Tai and Fantegrossi, 2017). All of THC, CBD, and "spice" undergo glucuronidation (Stout and Cimino, 2014).

A comprehensive account of the eCB system in schizophrenia (with no recent or chronic cannabis exposure) is beyond the scope of this review, and has been reviewed elsewhere (Fakhoury, 2017; Muller-Vahl and Emrich, 2008). We summarize this information here to give context for the interpretation of the effects of cannabinoids on the eCB system relevant to schizophrenia. Succinctly, conflicting reports have identified both elevated (Ceccarini et al., 2013; Dean et al., 2001; Wong et al., 2010; Zavitsanou et al., 2004) and reduced (Eggan et al., 2008, 2010; Ranganathan et al., 2016; Borgan et al., 2018; Hietala, 2018) levels of CB1R via post-mortem autoradiography and in vivo positron emission tomography (PET)

studies. Interestingly, there might be an inverse association between CB1R binding (with CB1R agonist [^{3}H]OMAR) and CB1R mRNA/protein levels in schizophrenia (Volk et al., 2014). While counterintuitive, Volk et al. (2014) presented multiple potential explanations for the discrepancy, such as an alteration of receptor trafficking whereby there are less total levels of CB1R but more CB1R accessible for binding, or a CB1R with higher binding affinity resulting in less CB1R transcription/translation (Volk et al., 2014). Other possibilities include an increased affinity of CB1R as a compensatory mechanism in response to diminished development of CB1R axon terminals in schizophrenia, or that differences in binding of ligands/antibodies with the different methodologies lead to conflicting results (Volk et al., 2014). AEA levels in the cerebrospinal fluid (CSF) are elevated in schizophrenia compared to healthy controls (Giuffrida et al., 2004; Leweke et al., 2007), but are unaltered in the periphery (Giuffrida et al., 2004; Leweke et al., 2007). Peripheral levels of CB2R, NAPE and DAGL enzymes were lower in the peripheral blood mononuclear cells (PBMCs) of first-episode psychosis patients, whereas FAAH and MAGL levels were unaltered (Bioque et al., 2013).

The eCB system is also involved in a number of neuronal developmental processes (Gaffuri et al., 2012), as well as neuronal migration (Berghuis et al., 2005), neuronal connectivity (Berghuis et al., 2007), and neuron specification and connectivity patterns (Mulder et al., 2008). The role of the eCB system in development begins prenatally but remain relevant throughout adolescence (for a review see Bossong and Niesink, 2010; Fernandez-Ruiz et al., 200l; Schneider, 2008). In fact, there is high activity of the eCB system at the onset of puberty, and this had led to the hypothesis of a "window of vulnerability" or a "critical period" when the brain might be most vulnerable towards the development of psychosis from cannabis (Radhakrishnan et al., 2014; Schneider, 2008).

3. Search strategy and selection criteria

We searched PubMed for articles published until May 29, 2017. To our knowledge, no review on this specific topic has been completed before, and therefore we implemented no publication date cut-off for the earliest studies. References of the identified papers were also reviewed to identify additional relevant studies.

The following search query was used in PubMed:

(Cannabis OR marijuana OR synthetic cannabinoid OR phytocannabinoid OR "cannabis use" OR THC OR tetrahydrocannabinol OR "cannabinoid ingestion" OR spice OR K2 OR CBN OR cannabinol OR CBG OR cannabigerol OR thcv OR tetrahydrocannabivarin OR rimonabant OR sativex OR dronabinol OR nabilone OR JWH-018 OR AM-2201) AND ((("CB1 receptor" OR "CB2 receptor" OR "cannabinoid receptor" OR faah OR "fatty acid amide hydrolase" OR dagl OR magl OR nape-pld OR endocannabinoid OR cannabinoid OR cannabinoids) AND (mrna OR expression OR availability OR density OR autoradiography OR "positron emission tomography" OR PET)) OR ((anandamide OR endocannabinoid OR "2-AG") AND (serum OR CSF OR "cerebrospinal fluid" OR plasma))) NOT rat NOT murine NOT mouse NOT rodent.

This query was developed to identify studies that measured alterations in components of the eCB system in response to exogenous cannabinoid exposure, excluding studies completed in murine/rodent models. While rodent models do provide valuable information, our review focuses on the direct impact of cannabinoids in humans.

Inclusion criteria are studies that measure components of the eCB system (CB1R/CB2R, eCB metabolizing enzymes, and eCB levels) in human participants. The study populations included are either healthy volunteers with current or past cannabis exposure including CUD or were challenged by cannabinoids, and patients with schizophrenia/first-episode psychosis/prodromal psychosis who used cannabis (recently or chronically). The inclusion criterion for the reviewed studies in schizophrenia is the disclosure of whether each study participant had either recent or chronic exogenous cannabinoid exposure (see Supplement 1 for cannabis exposure reported in each study). Studies measuring the eCB system in alternate clinical populations were excluded, such as an obese population, as well as studies in healthy/schizophrenia cannabis users with different outcome measures (e.g. behavioural changes after acute THC exposure but no results in the eCB system), and studies with insufficient data on cannabis exposure demographics.

4. Results

The search yielded 24 papers that met inclusion criteria (Fig. 2). This includes 13 studies in healthy volunteers exposed to phytocannabinoids, including individuals with CUD, and 11 studies in schizophrenia that reported cannabinoid exposure. Two studies investigated the effects of acute THC administration in healthy volunteers, one study examined the effects of CBD on the eCB system in schizophrenia, and the remaining studies measured the eCB system after chronic cannabis exposure. The synthetic cannabinoid search terms yielded no results, and to our knowledge no studies examining the effects of these cannabinoids on the eCB system in humans have been published to date.

5. Studies in the central nervous system

5.1. Post-mortem findings: effects of cannabis on cannabinoid receptors

A number of post-mortem studies have measured CB1R density in relation to cannabis exposure. Daily cannabis users exhibited lower CB1R protein and mRNA levels throughout the basal ganglia, dopaminergic midbrain nuclei, and hippocampus versus controls (Table 1; Villares, 2007). In contrast, Dean et al. (2001) found higher CB1R protein levels in combined schizophrenia patients and controls with THC metabolites in blood versus those without (Table 1). Other post-mortem reports of CB1R in individuals with schizophrenia and a history of cannabis use detected no effect of cannabis exposure on CB1R binding in the cortical regions (Table 1; Eggan et al., 2008, 2010; Volk et al., 2014; Zavitsanou et al., 2004). However, the studies in

Table 1 Post-mortem findings: effects of cannabis on cannabinoid receptors and metabolizing enzymes.

Reference	Groups	M/F	Method	Target	Regions of interest		
					Cortical	Limbic	Subcortical
Villares (2007)	Heathy, non-users	6/0	Autoradiography [^{3}H]-SR141716A (CB1R inverse agonist)	↓CB1R	Central gray area*	HPC proper CA1*, 2*, 3*, 4*, Subiculum, Dentate gyrus	VTA*, SNpc, SNpl*, SNpr*, GPe*, GPi*, CN*, Put*, NAc* Mesencephalon*
	Healthy, cannabis users	6/0	In situ hybridization mRNA (Md grain density)	↓CB1R		Hippocampal CA1*, 2*, 3*, 4* Subiculum	CN*, Put*, NAc*
Dean et al. (2001)	Healthy Non-users	8/2	Autoradiography [^{3}H]CP-55940 (CB1R/CB2R agonist)	↑CB1R		HPC	Striatum*,[1]
	Healthy, cannabis users	4/0					
	Schizophrenia, non-users	7/2					
	Schizophrenia, cannabis users	4/1					
Zavitsanou et al. (2004)	Healthy, non-users	8/1	Autoradioagraphy [^{3}H]-SR141716A	CB1R n.s.		ACC n.s.	
	Schizophrenia, non-users	4/1					
	Schizophrenia cannabis users	5/0					
Eggan et al. (2008)	Healthy, non-users	17/5	CB1 in situ hybridization & immunoreactivity mRNA & protein	CB1R n.s.	Cortex n.s.		
	Schizophrenia, Non-user	11/5					
	Schizophrenia, cannabis users	6 /1					
Eggan et al. (2010)	Healthy, non-users	17/9	Immunoreactivity	CB1R n.s.	Cortex BA46		
	Schizophrenia, non-users	8 /7	protein		n.s.		
	Schizophrenia, cannabis users	5 /1					
Volk et al. (2014)	Healthy, non-users	16/5	Autoradiography [^{3}H]-OMAR (CB1R antagonist)	CB1R n.s.	PFC n.s.		
	Schizophrenia, non-users	10/4					
	Schizophrenia, cannabis user	6/1					

(*continued on next page*)

Table 1 (*continued*)

Reference	Groups	M/F	Method	Target	Regions of interest		
					Cortical	Limbic	Subcortical
Volk et al. (2013)	Healthy, non-users	31/11	ABHD6 mRNA qPCR	ABHD6 n.s.	PFC n.s.		
	Schizophrenia, non-users	19 /9					
	Schizophrenia, cannabis users	12 /2					

↑: increased; ↓: decreased.
n.s.: Non-significant effect of cannabis exposure on CB1R or ABHD6 within schizophrenia patients.
ABHD6: α-β-hydrolyzing domain 6; ACC: anterior cingulate cortex; CB1R: cannabinoid CB1 receptor; CN: caudate nucleus; GPe: globus pallidus external; GPi: globus pallidus internal; HPC: hippocampus; NAc: nucleus accumbens; PFC: prefrontal cortex; Put: Putamen; qPCR: quantitative polymerase chain reaction; SNpc: substantia nigra pars compacta; SNpl: substantia nigra pars lateralis; SNpr: substantia nigra pars reticulata; VTA: ventral tegemental area.
* Significant reduction of CB1R in healthy cannabis users versus non-users.
[1] Higher CB1R binding in all individuals - healthy and schizophrenia - that were positive for THC.

schizophrenia reported mixed results with respect of CB1R levels in schizophrenia. There were reports of both elevated CB1R binding (Dean et al., 2001; Volk et al., 2014; Zavitsanou et al., 2004) and lowered CB1R mRNA or immunoreactivity in patients versus controls (Eggan et al., 2008, 2010).

Several factors could account for the apparently disparate results between studies reporting elevated and reduced CB1R in cannabis users. Villares (2007) observed reduced CB1R binding in a sample with current daily use until time of death. In contrast, Dean et al. (2001) reported elevations in a mixed group including non-CUD users along-

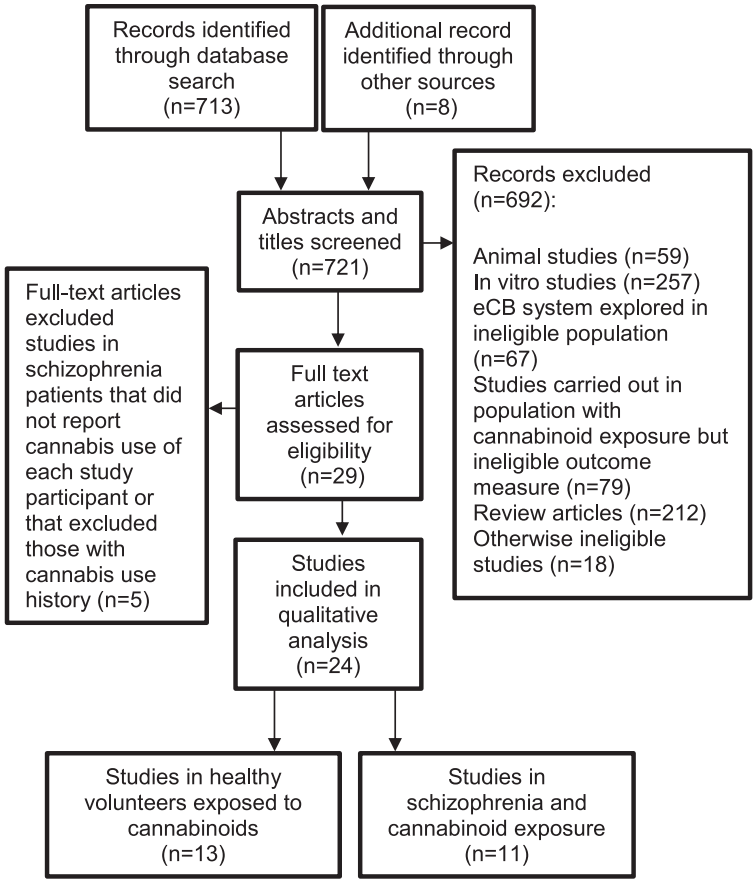

Fig. 2 Systematic review study selection.

side current and past CUD. The detected levels of cannabinoids in urine and blood confirm higher cannabis exposure at time of death in the Villares (2007) sample (Musshoff and Madea, 2006; Schwilke et al., 2011). Alternative confounding factors include cause of death, post-mortem interval, and medication effects. All participants in Villares (2007) died by gunshot, were not taking antipsychotics and the post-mortem interval was 7–11 h, whereas half of the schizophrenia patients in Dean et al. (2001) died by suicide, patients had antipsychotic exposure, and the post-mortem interval ranged from 15–69 h. It is also possible that similar explanations for inconsistent results in post-mortem CB1R studies, as reported by Volk et al. (2014) and discussed above, apply here as well.

5.2. Positron emission tomography findings: effects of cannabis on cannabinoid receptors

PET studies on CB1Rs support the post-mortem findings of lower CB1Rs in current cannabis users. First, Hirvonen et al. (2012) observed a ∼20% reduction in CB1R binding across various cortical and limbic regions, but this finding did not reach significance in the striatum, in daily users versus controls (Table 2). Ceccarini et al. (2015) detected ∼12% lower CB1R availability in cortical regions and the nucleus accumbens in a cohort of daily or near-daily cannabis users. D'Souza et al. (2016) reported ∼15% lower CB1R availability across cortical, limbic and striatal regions in diagnosed CUD compared to controls (Table 2).

This reduction of CB1Rs in daily users can be reversed with sustained abstinence. Hirvonen et al. (2012) no longer detected significant reductions in CB1Rs after two-to-four weeks of cannabis abstinence. D'Souza et al. (2016) no longer detected a statistically significant reduction in CB1R availability in cannabis users after two or 28 days of abstinence. However, the average time of abstinence in the Ceccarini et al. (2015) cohort was four days when the effect of cannabis on CB1R levels was significant. Thus, the reduction in CB1R availability in cannabis users appears to begin to normalize within a period of two days to two weeks of abstinence.

Apparent variation in the duration of CB1R reduction in Ceccarini et al. (2015) and in D'Souza et al. (2016) could reflect differences in population samples or in PET tracers used. The three PET studies used different PET tracers (Table 2), each associated with unique pharmacology and quantification methods. Notably, [^{18}F]MK-9470, used by Ceccarini et al. (2015), displays slow in vivo brain kinetics making typical kinetic models for quantification difficult to use (Hirvonen et al., 2010). To overcome this problem, binding was reported as an mSUV (modified standard uptake value; Sanabria-Bohorquez et al., 2010).

Schizophrenia patients with a history of cannabis use did not display altered CB1R levels versus non-using patients (Ceccarini et al., 2013). An exploratory analysis found that patients who had used cannabis multiple times daily (at least six months prior) tended to have lower, albeit non-significant, CB1R receptor availability compared to patients who used cannabis multiples times per week or month ($p \geq 0.16$) (Ceccarini et al., 2013). Overall, the study reported elevated CB1R availability in schizophrenia versus controls (Ceccarini et al., 2013).

Despite discrepancies in regional binding and complexities in receptor quantification, the evidence supports an overall consensus that near-daily (or more frequent) cannabis exposure results in a reversible down-regulation of CB1R, primarily in cortical regions. According to the current post-mortem and in vivo literature, exploratory analyses have failed to observe cannabis-related changes in CB1R levels in schizophrenia with recent or chronic cannabis exposure. To confirm this finding, research will need to evaluate CB1R in cannabis-using schizophrenia patients as a primary outcome measure. Further, future PET studies may investigate CB1R response to varying THC/CBD ratios, or CBD alone, in these populations.

5.3. Post-mortem findings: effects of cannabis on eCB metabolizing enzymes

To our knowledge, there have been no post-mortem studies completed on eCB metabolizing enzymes in heathy cannabis users. The only study measuring eCB metabolizing enzymes in the brain in psychosis with concurrent cannabis exposure measured ABHD6 (α-β-hydrolase domain six), a 2-AG catabolic enzyme, in postmortem tissue. Cannabis exposure did not significantly affect ABHD6 transcript levels, and further, ABHD6 mRNA levels did not differ between schizophrenia subjects and controls (Table 1; Volk et al., 2013).

5.4. Positron emission tomography findings: effects of cannabis on endocannabinoid-metabolizing enzymes

FAAH is the only eCB metabolizing enzyme to have been investigated in the brains of cannabis users with PET. Using the highly selective FAAH radioligand [^{11}C]CURB, Boileau et al. (2016) reported that current cannabis users exhibited 14–20% lower FAAH binding across cortical, striatal, and limbic regions versus non-users (Table 2; Boileau et al., 2016). This reduction was observed across more brain regions than were reported for CB1R PET studies of cannabis users with comparable cannabis use (Ceccarini et al., 2015; D'Souza et al., 2016; Hirvonen et al., 2012). The findings may reflect a possible compensation for CB1R reduction with cannabis use, since reduced FAAH levels might result in increased cannabinoid signaling via increased AEA levels (Boileau et al., 2016). Whereas CB1R downregulation is reversible with sustained abstinence, it is unknown whether reductions in FAAH are reversible with abstinence.

Another factor to consider in the association between FAAH and cannabis is the FAAH C385A single nucleotide polymorphism, which results in lower FAAH expression and steady-state activity and, consequently, elevated AEA plasma levels (Dincheva et al., 2015). Interestingly, the AA genotype of this FAAH mutation is associated with lower rates of cannabis dependence than the AC and CC genotypes (Tyndale et al., 2007). However, heavy cannabis users display lowered levels of FAAH, thus highlighting the complexities underlying FAAH in the context of cannabis use.

Table 2 Positron emission tomography findings: effects of cannabis on cannabinoid receptors and metabolizing enzymes.

Reference	Groups	M/F	Radioligand, Pharmacology (outcome measure)	Abstinence period			Regions of interest		
				~8-24 h	2-4 days	2-4 wk +	Cortical	Limbic	Subcortical
Hirvonen et al. (2012)	Non-users	28/0	[^{18}F]FMPEP-d_2, CB1R inverse agonist (V_T)	↓CB1R		CB1R n.s. (2 wk)	PFC*, PC*, TC* Occ*,	Ins*,	CN, Put, VST, midbrain, Cb, pons, white matter
	Cannabis users	30/0							
Ceccarini et al. (2015)	Non-users	7/3	[^{18}F]MK-9470 ,CB1R inverse agonist (mSUV)		↓CB1R		FC, PC, TC*, mTC, Occ, central area	ACC*, PCC*, Tha, Ins,	CN, Put, GP, NAc*, pons, Cb
	Cannabis users	8/2							
D'Souza et al. (2016)	Non-users	19/0	[^{11}C]OMAR ,CB1R antagonist (V_T)	↓CB1R n.s.	CB1R	CB1R n.s. (4 wk)	FC*, PC*, TC*, Occ*	Tha, HTH, Ins*	Put*, CN*, Cb, GP
	Cannabis users	11/0							
Boileau et al. (2016)	Non-users	11/11	FAAH inhibitor (λk_3)	↓FAAH			PFC*, mPFC*, PC*, TC*, Occ*,	ACC*, HPC*, Amy*, Tha*, Ins*	VST*, DST*
	Cannabis users	7/3							
Ceccarini et al. (2013)	Healthy	8/4	[^{18}F]MK-9470 CB1R inverse agonist (mSUV)			CB1R n.s. (6 + mo)	Global grey matter		
	Schizophrenia	43/24							
:	Non-users	32							
	Cannabis users (past)	35							

n.s.: Non-significant effect of cannabis exposure on CB1R availability; ↑: increased; ↓: decreased.
ACC: anterior cingulate cortex; Amy: amygdala; Cb: cerebellum; CN: caudate nucleus; DST: dorsal striatum; FC: frontal Cortex; GP: globus pallidus; HPC: hippocampus; HTH: hypothalamus; Ins: insula; mo: month; mPFC: medial prefrontal cortex; mSUV: modified standard uptake value; mTC: mesotemporal cortex; Occ: occipital cortex; PC: parietal cortex; PCC: posterior cingulate cortex; PFC: prefrontal cortex; PHG: parahippocampal gyrus; Put: Putamen; NAc: nucleus accumbens; Tha: thalamus; TC: temporal cortex; VST: ventral striatum; VT: total volume distribution; wk: week.
* Significant reduction of CB1R availability/FAAH in cannabis users versus non-users.

Table 3 Cerebrospinal fluid findings: effects of cannabis on endocannabinoid levels.

Reference	Groups	M/F	Cannabis users versus non-users		Schizophrenia versus healthy controls
			Healthy cannabis users versus non-users	SCZ cannabis users versus SCZ	
Morgan et al. (2013)		15/18	↑AEA*		
	Healthy, non-users	13	(>10x/month versus <10x/month)		
	Cannabis users (< 10x/mo)	10	PEA n.s., OEA n.s., 2-AG n.s.		
	Cannabis users (> 10x/mo)	10			
Leweke et al. (2007)	Healthy, non-users	29/26	AEA n.s.	↓AEA *	↑AEA *
	Healthy, cannabis users	16/10	OEA n.s.	OEA n.s.	(SCZ non-users only)
	Schizophrenia, non-users	16/9	PEA n.s.	PEA n.s.	OEA n.s.
	Schizophrenia, cannabis users	16/3			PEA n.s.
Koethe et al. (2009)		45/36	AEA n.s.	AEA n.s.	↑AEA *
	Healthy, non-users	55	OEA n.s.	OEA n.s.	OEA n.s.
	Healthy, cannabis users	26			
		19/8			
	Prodromal schizophrenia, non-users	16			
	Prodromal schizophrenia, cannabis users	11			

n.s.: No significant difference between groups; ↑: increased; ↓: decreased; > greater than; < less than.
2-AG: 2-arachidonoyl glycerol; AEA: anandamide; CSF: cerebrospinal fluid; HV: healthy controls; OEA: N-oleoylethanolamine; PEA: N-palmitoylethanolamine; SCZ: schizophrenia.
* Significant difference between AEA levels in indicated cannabis-using group versus comparison group; or between healthy versus schizophrenia.

5.5. Cerebrospinal fluid findings: effects of cannabis on endocannabinoid levels

Central levels of endocannabinoids can be measured by sampling CSF (Table 3). In cannabis users with varying degrees of use, AEA CSF levels did not differ from controls, after 24 h of abstinence (Morgan et al., 2013). However, the AEA levels were significantly lower in those who used cannabis more than 10 times monthly, versus those who used cannabis less than 10 times monthly (Table 3). No group differences were detected for 2-AG, OEA or PEA levels (Morgan et al., 2013). Leweke et al. (2007) also measured CSF AEA levels in healthy volunteers and detected no difference in AEA, OEA or PEA levels between cannabis users and non-users, but did not measure 2-AG. However, the cannabis users in Leweke et al. (2007) used cannabis a total of 20-50 times throughout their lives. Therefore, frequency of cannabis use in Leweke et al. (2007) was low, which could in part explain why AEA levels were not altered.

In the same study, Leweke et al. (2007) also found no change in OEA or PEA levels between schizophrenia patient cannabis users versus non-using patients (Table 3). Remarkably, AEA levels were lower in the cannabis-using patients versus their non-using counterparts (Leweke et al., 2007). Previous studies have failed to find an effect of cannabis use on CSF AEA levels in both schizophrenia and its putative prodromal state (Giuffrida et al., 2004; Koethe et al., 2009). However, details of cannabis exposure were unclear in the schizophrenia study (Giuffrida et al., 2004). Also, the negative urine screens for THC in the prodromal study indicate the cannabis exposure was neither recent nor necessarily extensive (Koethe et al., 2009). While it is interesting that Leweke et al. (2007) found a reduction of CSF AEA in the cannabis users with schizophrenia but not healthy cannabis users, it must be noted that the cannabis use in the schizophrenia group was more recent and extensive. The healthy cannabis users were six weeks abstinent and had up to 50 lifetime exposures, whereas the schizophrenia cannabis users were three days abstinent and some had more than 500 lifetime cannabis exposures (Leweke et al., 2007). Only the non-cannabis using schizophrenia patients displayed elevated CSF AEA levels compared to healthy controls in Leweke et al. (2007). This elevation of CSF AEA in schizophrenia was previously reported in cohorts of patients including those who used cannabis, albeit the cannabis exposure was less explicitly stated, as discussed above (Giuffrida et al., 2004; Koethe et al., 2009).

6. Studies in the peripheral endocannabinoid system

The peripheral endocannabinoid system is involved in numerous physiological responses. A notable example is inflammation, which is partially reflected by the high ex-

pression of CB2R on PBMCs (Pandey et al., 2009). The expression patterns of these PBMC eCB proteins could be related to a myriad of inflammatory responses occurring in the individuals, independent of their response to cannabinoid exposure (Pandey et al., 2009). Nonetheless, the eCB system markers in PBMCs are relevant to psychosis through the inflammation hypothesis of schizophrenia (Kirkpatrick and Miller, 2013; Pandey et al., 2009; Stefansson et al., 2009). Endocannabinoids are also highly involved in feeding behaviour and metabolic regulation (Matias et al., 2006), which are other potential confounds when interpreting peripheral eCB data. For example, circulating endocannabinoids are affected by blood-glucose levels (Di Marzo et al., 2009). Other factors that affect levels of peripheral endocannabinoids include whether measurements are made from plasma or serum, the time of day the samples are taken, recent exercise, hours of sleep, and acute or chronic stress (Hillard, 2018).

6.1. Peripheral findings: effects of cannabis on cannabinoid receptors

PBMC CB1R expression was elevated in current cannabis users with and without CUD, but not past users, relative to healthy controls without cannabis exposure (Table 4; Muhl et al., 2014; Nong et al., 2001; Rotter et al., 2013). CUD participants also displayed elevated promoter methylation versus controls (Rotter et al., 2013). However, since promoter methylation is known to silence gene expression, and CB1R mRNA expression and mean promoter methylation were inversely related, it is unclear if increased CB1R gene expression is truly representative of higher cannabinoid signaling. In contrast to CB1R, CB2R mRNA measured in the same studies was elevated in the current and past cannabis users, but not CUD (Muhl et al., 2014; Nong et al., 2001; Rotter et al., 2013). Within schizophrenia, lifetime incidence of cannabis abuse or dependence did not affect PBMC CB2R protein levels, however the patients with lifetime cannabis abuse/dependence did have lower CB2R protein levels than healthy controls (Bioque et al., 2013). PBMC CB1R protein levels fell below the limit of detection in the same study (Bioque et al., 2013).

The apparent upregulation of CB1R mRNA expression in the periphery of cannabis users/CUD contrasts with the reduced availability of brain CB1R detected by PET and postmortem studies. However, a direct comparison between findings would be misleading due to the different methodologies used and extent of cannabis exposure. The three peripheral studies included both past and current cannabis users with varying severities of use, while the PET studies only looked at heavy and chronic cannabis users. Another factor to consider is the difference in expression patterns between CB1R and CB2R in the brain versus the periphery, with CB1R being more highly expressed on neurons and CB2R more highly expressed on immune cells (Pandey et al., 2009). This may help to explain the incongruence between findings, as the dissimilar expression patterns may reflect the independent regulation of peripheral and central eCB function (Cravatt et al., 2004). Furthermore, the limitations of experimental design and techniques should also be considered when comparing results among these studies. Only Rotter et al. (2013) detected the increased CB1R expression in CUD using qPCR, a quantitative method for measuring gene expression, as opposed to the semiquantitative RT-PCR (Muhl et al., 2014; Nong et al., 2001; Rotter et al., 2013). Moreover, CB1R protein and mRNA levels do not measure CB1R function, a piece of information that could be helpful in reconciling challenging findings, such as elevated CB1R protein alongside elevated promoter methylation.

6.2. Peripheral findings: effects cannabis on eCB metabolizing enzymes

Decreased FAAH protein levels were detected in PBMCs of daily consumers of bhang, a cannabis-containing beverage (Table 4), which is in agreement with the lowering of brain FAAH detected by PET imaging in cannabis users (El-Gohary and Eid, 2004; Boileau et al., 2016). FAAH was significantly decreased in those with longer (24–36 months) versus shorter lengths of bhang consumption (6–24 months; El-Gohary and Eid, 2004).

Protein levels of the primary eCB metabolizing enzymes (MAGL, DAGL, NAPE and FAAH) were unaffected by lifetime cannabis abuse /CUD in first-episode psychosis patients (Table 4; Bioque et al., 2013). Notably, the first-episode psychosis cohort combined past and current use. Protein levels of biosynthetic enzymes NAPE and DAGL were lower in patients compared to healthy controls, with no change in catabolic enzymes FAAH or MAGL (Bioque et al., 2013).

6.3. Peripheral studies: acute and sub-chronic effects of THC and CBD on endocannabinoid levels

The effects of THC have been explored through the acute administration of THC to healthy volunteers in two studies (Table 5). Intravenous administration of THC (0.1 mg/kg) produced a transient increase of AEA and 2-AG plasma levels within 30 min, a decrease at 300 min after administration (versus baseline and 15 min post-exposure, respectively), and a normalization within 24 h after administration (Thieme et al., 2014). Another group detected decreased values of peripheral eCB concentrations at 2 and 3 h post-administration of a high, oral dose of THC (20 mg) in healthy volunteers (Walter et al., 2013). However, the peripheral eCB levels were in fact elevated when compared to those in the placebo condition (Walter et al., 2013). Therefore, THC administration might lead to acutely elevated eCB levels in the periphery. Previous reports of peripheral 2-AG demonstrate that eCB levels fluctuate throughout the day and begin to decrease in the late afternoon, which could potentially account for the decrease in circulating endocannabinoids detected in both studies (Hanlon et al., 2016).

To our knowledge, the effects on peripheral endocannabinoids from acute or chronic exposure to CBD have not been measured in healthy volunteers. In schizophrenia, peripheral endocannabinoid levels were elevated in a cohort of patients who were treated with CBD in a phase II double-blinded, randomized, parallel-group clinical trial (Leweke et al., 2012). After two weeks of treatment, peripheral levels of AEA, PEA and OEA were increased in the serum of the CBD-treated group compared to baseline, with the effect

Table 4 Peripheral findings: effects of cannabis on eCB system receptors, enzymes, and endocannabinoids.

Reference	Groups	M/F	Sample	Cannabis users versus non-users		Schizophrenia versus healthy controls
				Healthy cannabis users versus non-users	SCZ cannabis users versus SCZ	
Rotter et al. (2013)	Healthy, non-users, non-smokers	15/6	PBMC	↑CB1R* (versus all healthy or versus non-smokers)		
	Healthy, cigarette smokers	14/6	mRNA	↑CB1 methylation* (versus all healthy or versus non-smokers)		
	Cannabis users	28/8	qPCR	CB2R n.s.		
Nong et al. (2001)	Healthy, non-users	10	PBMC	↑ CB1R*		
	Cannabis users	7/3	mRNA RT-PCR	↑ CB2R*		
Muhl et al. (2014)		14/14	PBMC	CB1R n.s.		
	Healthy, non-users	15	mRNA	↑CB2*		
	Past cannabis users (6 months)	13	RT-PCR	↓FAAH *		
El-Gohary and Eid (2004)	Healthy, non-users	30/0	PBMC			
	Cannabis users	60/0	Protein			
Bioque et al. (2013)	Healthy	71	PBMC		FAAH n.s.	↓CB2R n.s.
			protein		MAGL n.s.	↓NAPE n.s.
	First episode psychosis	67/28			CB2R n.s.	↓DAGL n.s.
	Non-user	49			NAPE n.s.	FAAH n.s.
	Cannabis users	46			DAGL n.s.	MAGL n.s.
Morgan et al. (2013)		15/18	Serum	↑2-AG* (> 10x/month versus non-users)		
	Healthy, non-users	13		AEA n.s., PEA n.s., OEA n.s.		
	Cannabis users (< 10x/mo)	10				
	Cannabis users (> 10x/mo)	10				
Muhl et al. (2014)		14/14	Serum	↑AEA*		
	Healthy, non-users	15		↑OEA*		
	Past cannabis users (6 months)	13		↑PEA*		
Desfossés et al. (2012)	Healthy, non-users	16/11	Plasma	↓OEA*	OEA n.s.	
				↓AEA*	AEA n.s.	
	Substance use disorder[1]	25/13		(SUD vs Healthy)		
	CUD	20				
	Schizophrenia, non-users	17/8				

(*continued on next page*)

Table 4 (*continued*)

Reference	Groups	M/F	Sample	Cannabis users versus non-users		Schizophrenia versus healthy controls
				Healthy cannabis users versus non-users	SCZ cannabis users versus SCZ	
Leweke et al. (2007)	Healthy, non-users	29/26	Serum	AEA n.s.	AEA n.s.	AEA n.s.
	Healthy, cannabis users	16/10				
	Schizophrenia, non-users	16/9				
	Schizophrenia, cannabis users	16/3				
Koethe et al. (2009)		45/36	Serum	AEA n.s.	AEA n.s.	AEA n.s.
	Healthy, non-users	55		OEA n.s.	OEA n.s.	OEA n.s.
	Healthy, cannabis users	26				
		19/8				
	Prodromal SCZ, non-users	16				
	Prodromal SCZ, cannabis users	11				

n.s.: No significant difference between indicated groups; ↑: increased; ↓: decreased; > greater than; < less than.
1SUD includes SUD for cannabis, alcohol and stimulants, 20 out of 38 had cannabis use disorder.
2-AG: 2-arachidonoyl glycerol; AEA: anandamide; CB1: cannabinoid CB1 receptor; CB2: cannabinoid CB2 receptor; CSF: cerebrospinal fluid; CUD: cannabis use disorder; DAGL: diacylglycerol lipase; FAAH: fatty acid amide hydrolase; HV: healthy controls; MAGL: monoacylglycerol lipase; NAPE: N-acyl phosphatidylethanolamine phospholipase; OEA: N-oleoylethanolamine; PBMC: peripheral blood mononuclear cells; PEA: N-palmitoylethanolamine; qPCR: quantitative polymerase chain reaction; RT-PCR: reverse transcriptase polymerase chain reaction; SUD: substance use disorder; SCZ: schizophrenia; THC: Δ9-tetrahydrocannabinol.
* Significant difference between indicated cannabis-using group versus comparison group, or between healthy versus schizophrenia.

remaining for AEA and PEA at four weeks (Table 5; Leweke et al., 2012). This effect was not detected in the control (amisulpride) group, suggesting an effect specific to CBD.

6.4. Peripheral findings: chronic effects of cannabis on endocannabinoid levels

Morgan et al. (2013) detected elevated 2-AG levels versus controls in the periphery, remarkably in the same cannabis users who displayed decreased AEA CSF levels (in users who used more than ten times per month versus those who used less than ten times monthly; Table 4). There was no difference in peripheral AEA, OEA or PEA levels between these cannabis users and non-users (Morgan et al., 2013). Leweke et al. (2007) also detected no difference in peripheral AEA levels in healthy cannabis users. Surprisingly, a study in healthy individuals with past (6 month abstinent) cannabis use reported higher AEA, PEA, and OEA levels in serum compared to non-users (Table 4; Muhl et al., 2014). In a study comparing peripheral eCB levels between substance use disorder, schizophrenia, and controls, only the individuals with substance use disorder displayed lower plasma AEA and OEA levels compared to healthy controls, with no change in the schizophrenia group (Table 4; Desfossés et al., 2012). While this substance use disorder group was not solely made up CUD participants, an exploratory subgroup analysis did not reveal a difference in plasma eCB levels between those dependent on alcohol, cannabis, or stimulants (Desfossés et al., 2012).

In schizophrenia patients who used cannabis, serum AEA levels were unchanged relative to non-users, failing to mirror the effect found in the CSF (Leweke et al., 2007). There was also no detected effect of cannabis on peripheral AEA or OEA levels in putative prodromal psychosis (Koethe et al., 2009). Thus, both of these studies failed to detect the effect of illness on peripheral eCB levels that was found in the CSF (Koethe et al., 2009; Leweke et al., 2007).

Overall, evidence points toward a distinction between the effects of exogenous cannabinoids on the eCB system in the periphery and central nervous system. Effects of cannabis on CSF AEA levels were not replicated in the periphery of the same subjects (Koethe et al., 2009; Leweke et al., 2007; Morgan et al., 2013). Likewise, there is a lack of correlation between peripheral and central eCB levels (Giuffrida et al., 2004; Koethe et al., 2009).

Table 5 Peripheral findings: acute and sub-chronic effects of THC and CBD on endocannabinoid levels.

Reference	Groups	M/F	Treatment	Comparison	Methodology	Post-administration
Thieme et al. (2014)	Healthy, non-users (acute challenge, no control condition)	11/14	(0.1 mg/kg THC, intravenous)	Baseline	Plasma	30 min: ↑AEA[1] ↑2-AG[1] 6 h: ↓AEA[2] ↓2-AG[2] 24 & 48 h: n.s. AEA n.s. 2-AG
Walter et al. (2013)	Healthy, non-users (double cross-over design)	15/15	THC condition (20 mg, oral)	Placebo condition	Plasma	2–3 h: ↑AEA*, ↑OEA*, ↑1-, 2-AG* n.s. PEA
Leweke et al. (2012)	Schizophrenia, non-users: cannabidiol amisulpride (Parallel group design)	15/5	Cannabidiol (800 mg/day, oral)	Amisulpride (800 mg/day) control group	Serum	2 weeks** ↑ AEA ↑ OEA ↑ PEA
		17/2				4 weeks** ↑ AEA ↑PEA n.s. OEA

n.s.: No significant difference between groups or versus baseline measure; ↑: increased; ↓: decreased.
2-AG: 2-arachidonoyl glycerol; AEA: anandamide; OEA: N-oleoylethanolamine; PEA: N-palmitoylethanolamine; SUD: substance use disorder; THC: Δ9-tetrahydrocannabinol.
[1] Significantly higher AEA levels versus 2, 5, 300-2880 min post-THC injection, significantly higher 2-AG levels versus 2, 45, 90, 180, 300 min post-THC injection.
[2] Significantly lower AEA levels versus baseline, 1, 2, 5, 10, 15, 45, 90, 2880 min post-THC injection, significantly lower 2-AG levels versus 10, 15 min post-THC injection.
* Significantly higher endocannabinoid levels in THC versus placebo condition.
** Significant increase of endocannabinoid levels in cannabidiol group versus baseline but not versus amisulpride group.

7. Implications in context of psychosis

Alterations in the eCB system in response to cannabis are especially interesting in the context of schizophrenia and related psychotic disorders. CSF AEA levels were found to be inversely correlated with psychotic symptoms in a group of schizophrenia patients with fewer than 50 lifetime exposures to cannabis (Giuffrida et al., 2004). Therefore, an increase of AEA is theorized to be an adaptive mechanism in schizophrenia (Giuffrida et al., 2004). Leweke et al. (2007) detected negative correlations between CSF AEA levels and positive and general psychotic symptoms, only in schizophrenia without cannabis use. Morgan et al. (2013) also reported a negative correlation between CSF AEA levels and persistent psychotic symptoms in their heathy cannabis users when drug-free. Interestingly, CSF AEA was lower when comparing those who use cannabis more than 10 times per months versus those who use less (Morgan et al., 2013). This mirrors the reduction of AEA found in the cannabis-using schizophrenia population versus their non-using counterparts (Leweke et al., 2007; Morgan et al., 2013). Should further research consolidate the hypothesis that AEA is an adaptive mechanism and inversely related to psychotic symptoms, AEA reduction could be a key mechanism whereby cannabis is associated with psychotic symptoms.

In the periphery, first-episode psychosis participants with lower FAAH levels exhibited more severe negative symptoms, despite the low symptom severity exhibited at the time of study (Bioque et al., 2013). While a direct comparison cannot be made between the two methodologies, it is interesting that lower FAAH levels were detected in the brains of cannabis users via PET. Peripheral CB2R levels did not correlate with symptomology (Bioque et al., 2013), but Ceccarini et al. (2013) did report a correlation between negative symptoms and CB1R availability in the brain. While the PET studies have yet to report an effect of cannabis on CB1R within schizophrenia, the CB1R appears to be downregulated with chronic cannabis exposure in healthy controls, and this decrease may parallel a reduction of CB1R availability in schizophrenia.

The relationship between peripheral AEA levels and symptomology remains ambiguous, due to conflicting reports between symptom relief and AEA levels in schizophrenia (Leweke et al., 2012; Potvin et al., 2008). The CBD-treated schizophrenia group displayed elevated AEA levels accompanied by an attenuation of psychotic symptoms as efficacious as amisulpride (Leweke et al., 2012). The authors attribute the antipsychotic effects of CBD to FAAH inhibition, based on the detected association between AEA levels and symptom relief, and the increase in levels of all FAAH substrates (AEA, PEA, OEA) in the CBD-treated group (Leweke et al., 2012). In order to prove this mechanism, the authors also report to have tested CBD inhibition of FAAH in rat brain membranes with effective concentration $8.6 \pm 0.2\,\mu M$ ($n = 12$; results not shown) although it is unclear whether the administered dose would lead to an effective concentration in the brain. In an unrelated study, healthy volunteers were given an oral 800 mg dose of CBD, and plasma concentrations reached a maximum of $221.1\,\mu g/L$ ($\sim 0.7\,\mu M$) (Manini et al., 2015). Furthermore, recent evidence shows that, unlike in mouse

brain microsomes (Bisogno et al., 2001; Watanabe et al., 1996), CBD does not inhibit human FAAH (Elmes et al., 2015). Further research on the relationship between FAAH, CBD, and psychotic symptoms will provide important information for potential alternative therapies for psychotic symptoms, as well as for legislation regarding the potency of legalized cannabis with respect to THC/CBD ratios.

8. Limitations

One major limitation when comparing multiple studies is the inconsistent criteria applied by different studies to qualify cannabis users. Another factor contributing to the variable results between studies is the unknown ratio of THC/CBD and potency of cannabis used by participants. Further limitations with regard to cannabis exposure among studies is the dependence on participants' self-report, and inconsistent time of cannabis abstinence at the time of study. Considering that abstinence leads to a reversal of the cannabis effect on CB1R observed in PET studies, this is a factor that must be controlled for in future CSF AEA studies. Moreover, even when drug screens are performed, it is unknown if the THC metabolites detected in the study participants' blood/urine are from recent use or if they are remaining after an extended period of abstinence, since THC metabolites can be detected in chronic users' urine up to 90 days after last use (Musshoff and Madea, 2006). Also, it is unknown whether it is the chronic exposure to cannabis, or these residual levels of THC metabolites that influence the regulation of the eCB system. Due to the limited research conducted with synthetic cannabinoids, this review only reflect effects of phytocannabinoids, mostly cannabis.

The studies reviewed here contained small sample sizes, not necessarily matched for tobacco use or antipsychotic exposure. For studies in psychosis patients, typical versus atypical antipsychotics seem to have different effects on the eCB system (Giuffrida et al., 2004). There are CYP450 enzymes common to the metabolism of both cannabinoids and antipsychotics, such as CYP2D6 and CYP3A4 (Jiang et al., 2011; Matsuda et al., 1990; Urichuk et al., 2008). Because of these shared enzymes it is possible that the metabolism of these drugs affect each other (Ogu and Maxa, 2000). There is some evidence to suggest that CBD can both inhibit (Jiang et al., 2013) and induce CYP450 enzymes (Bornheim et al., 1994). However, to our knowledge, there are no reports of significant induction/inhibition of CYP450 enzymes by either cannabinoids or second-generation antipsychotics, with a possible exception of clozapine (English et al., 2012; Kennedy et al., 2013; Prior et al., 1999; Stout and Cimino, 2014). Interestingly, THC might affect binding of antipsychotics to ATP-binding cassette transporter P-glycoprotein, thus increasing the efflux of antipsychotics and decreasing brain concentrations (Brzozowska et al., 2017). Nonetheless, there is little evidence to suggest that any inconsistent findings presented in this review are a result of drug–drug interactions where antipsychotic treatment alters eCB levels via drug metabolism pathways. On the other hand, there is compelling evidence to suggest an interplay between the eCB and nicotinic systems (for a review see Gamaleddin et al., 2015; Scherma et al., 2016). In preclinical models, nicotine exposure can alter AEA and 2-AG levels in brain (Buczynski and Parsons, 2010; Gonzalez et al., 2002). It is likely that these alterations in the eCB system occur not only as a response to pharmacological alteration, but also because of the involvement of the eCB system in addictive behaviours (Parsons and Hurd, 2015). However, among the studies included in this review that did not exclude tobacco use, all concluded that effects of tobacco did not explain the study findings (Boileau et al., 2016; Ceccarini et al., 2015; Eggan et al., 2008; Hirvonen et al., 2012; Leweke et al., 2007; Muhl et al., 2014). Only Rotter et al. (2013) included independent groups of cannabis users and tobacco users. They found the effects of tobacco on CB1R expression to be in the same direction as cannabis (Rotter et al., 2013).

The studies are also not necessarily matched for sex, age, or body mass index. The eCB system is thought to be sensitive to effects age and sex, as demonstrated in PET studies that reported different CB1R binding in men and women (Normandin et al., 2015; Van Laere et al., 2008), and increased binding with age in women only (Van Laere et al., 2008). There may also be a sexual dimorphism with respect to the effects of cannabis, with women reporting higher withdrawal symptoms when in cannabis abstinence, and men reporting more severe subjective effects (Craft et al., 2013). The peripheral eCB system is upregulated in obesity, presenting another possible confound (Engeli et al., 2005). An additional limitation is that with the exception of Boileau et al. (2016), none of the studies included in this review have taken into account the FAAH C385A single nucleotide polymorphism. Furthermore, only Bioque et al. (2013) and Leweke et al. (2007) drew samples at consistent times to limit diurnal effects, and only Bioque et al. (2013) used fasted samples. Finally, the studies in this review cannot address the question of a "window of vulnerability". When reported, the average initial age of cannabis use was similar among the studies in this review, and pubertal age was unknown. In order to effectively test the "critical window" hypothesis, future studies should include participants who began cannabis use at a wide range of ages, and the studies should be longitudinal in order to investigate whether changes to the eCB system are maintained overtime. Overall, this review highlights the challenges in performing studies, both centrally as well as in the periphery, to understand the role of phytocannabinoids on the eCB system.

9. Conclusion

We have reviewed the current literature on the effects of cannabinoids on the central and peripheral eCB system in humans with and without a psychotic disorder. The most supported finding is the down-regulation of CB1R after chronic and recent cannabis exposure (Ceccarini et al., 2015; D'Souza et al., 2016; Hirvonen et al., 2012), but it remains uncertain whether this effect is replicated in schizophrenia patients who recently or chronically used cannabis (Ceccarini et al., 2013). There is evidence indicating that cannabis exposure influences CSF AEA levels in individuals with and without psychosis (Leweke et al., 2007; Morgan et al., 2013). Some data suggests a

potential biphasic effect of cannabis whereby eCB signaling may be initially elevated with cannabis use but downregulated after heavier/chronic use. This pattern is exhibited in Morgan et al. (2013), where CSF AEA levels were lower in the participants with heavier cannabis use versus those who used cannabis less frequently. In postmortem studies, CB1Rs were elevated and decreased with cannabis use in Dean et al. (2001) and Villares (2007), respectively, with higher THC metabolite concentrations reported in Villares (2007). Also, Ceccarini et al. (2013) detected a (non-significant, $p = 0.16$) downregulation of CB1R in the (past) cannabis users who used multiple times *daily* versus multiple times *weekly*. This finding is broadly in line with the report that there is lower FAAH availability in the brain after chronic cannabis use (Boileau et al., 2016). In schizophrenia, cannabis use results in lower CSF AEA levels than in non-using schizophrenia patients (Leweke et al., 2007).

To date, there is minimal research on effects of phytocannabinoids on the eCB system. It is clear that meaningful progress in this field is needed to provide insight towards the effects of cannabis, which will soon be legalized widely in North America and Europe.

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Conflict of interest

Dr. Mizrahi reports and discloses to have received speaker and consultant from Otsuka-Lundbeck Canada. All other authors declare they have no conflict of interest.

Contributors

RM conceived the study and reviewed the manuscript, MJ prepared the manuscript, and JW, IB and JT provided input towards the manuscript.

Supplementary material

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