Neurotoxicity of Exogenous Cannabinoids

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Contents

Abstract

The endogenous cannabinoid system regulates diverse aspects of physiological functions *via* specific cannabinoid receptors (CB) expressed in the brain and periphery. CB_1 receptors mediate various neurological processes, whereas CB_2

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receptors mainly regulate immune responses and are involved in development of drug addiction and neuroinflammation. The cannabinoids are a heterogeneous group of endo-, phyto-, and synthetic cannabinoids. Cannabis and its products have been used for millennia, and these remain the most frequently used substances around the world. Δ^9-Tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of cannabis, produces psychotic-like symptoms. Acute and chronic cannabis use may impair learning and memory, attention, and psychomotor functions; however, studies on the life-lasting effects of cannabis on brain structure are ambiguous. During the last decade, a worrying trend has been observed regarding the increasing popularity of more potent, addictive, and harmful synthetic cannabinoids (SCs). Unlike Δ^9-THC, SCs use may lead to severe adverse effects including seizures, agitation, aggression, violence, anxiety, and panic attacks. Acute intoxication may be life-threatening or lead to persistent impairments in emotional and cognitive processing as a result of irreversible brain damage. This chapter describes the current state of knowledge regarding various aspects of the neurotoxicity of exogenous cannabinoids, including the harmful effects of their use during pregnancy.

Keywords

Endocannabinoid system · Cannabis · Δ^9-Tetrahydrocannabinol · Synthetic cannabinoids · Neurotoxicity

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	*N*-arachidonoylethanolamine
CB_1	Type 1 cannabinoid receptor
CB_2	Type 2 cannabinoid receptor
CBC	Cannabichromene
CBD	Cannabidiol
CBG	Cannabigerol
CBN	Cannabinol
CPP	Conditioned place preference
CUD	Cannabis use disorder
DAG	Diacylglycerol
DAGLα	Diacylglycerol lipase-α
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
FAAH	Fatty acid amide hydrolase
IVSA	Intravenous self-administration
ICSS	Intracranial self-stimulation
MAGL	Monoacylglycerol lipase
NPS	New psychoactive substances
SCs	Synthetic cannabinoids
UNODC	United Nations Office on Drugs and Crime
Δ^9-THC	Δ^9-Tetrahydrocannabinol

1 Introduction

Marijuana has a very long history of use for both recreational and medical purposes. In the adolescent population, cannabis use poses a significant risk of cannabis use disorder (CUD) and may result in altered neurodevelopment (Jacobus and Tapert 2014). Yet, the therapeutic use of synthetic cannabinoids remains an open issue (De Luca and Fattore 2018). Although often used interchangeably, *cannabis* and *marijuana* have different meanings: *cannabis* refers to all products derived from the plant *Cannabis sativa*, while *marijuana* refers to its parts (or products) that contain substantial amounts of Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive ingredient of the plant and the main responsible for the effects of marijuana. The term *cannabinoids* originally indicated a group of natural bi- and tricyclic products isolated from *Cannabis sativa* showing a structure related to Δ^9-THC. This term has been subsequently enlarged to comprise a broader group of psychoactive substances of different origin; these substances are able to influence a variety of cellular and physiological processes, from neuromodulation to complex metabolic and immune responses by activating endogenous receptors. Cannabinoids exert their effects by interacting with the specific endogenous cannabinoid receptors abundantly expressed in both the brain and periphery. They may be classified into one of the following three main categories, according to their nature/source of production:

1. Endogenous (endocannabinoids) – produced intracellularly
2. Phytocannabinoids – produced in plants, mainly *Cannabis sativa*
3. Synthetic cannabinoids – a large group of compounds with various chemical structures designed to activate cannabinoid receptors

Given the worldwide growth in cannabinoid consumption, this chapter focuses mostly on the neurotoxic effects of Δ^9-THC, the most abundant and psychoactive phytocannabinoid, and of the synthetic cannabinoids currently used as recreational drugs.

2 The Endocannabinoid System

2.1 2-Arachidonoylglycerol and *N*-Arachidonoylethanolamine: Two Major Endocannabinoids

The endocannabinoid system is composed of endocannabinoids, as well as the enzymes responsible for their biosynthesis and degradation, cannabinoid receptors (CB_1 and CB_2) and transporters (Fig. 1). Two major endocannabinoids are 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA; also known as anandamide); both are produced "on demand." Other lesser-known endocannabinoids or non-classical eicosanoids include *N*-acyl dopamine (NADA) and 2-arachidonyl glyceryl ether (noladin ether), both of which bind strongly to CB_1 receptors. 2-AG is synthesized from diacylglycerol (DAG) by diacylglycerol lipase-α (DAGLα) and AEA from

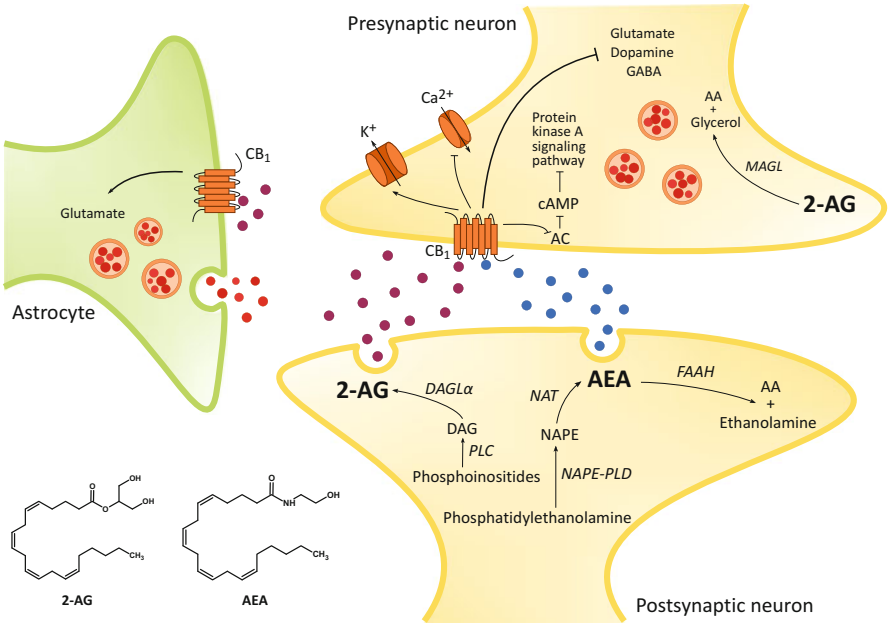


Fig. 1 Overview of the biochemical pathways for synthesis, degradation, and cellular actions of endocannabinoids. Chemical structure of 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA; anandamide)
Abbreviations: *AA* arachidonic acid; *AC* adenylyl cyclase; *cAMP* cyclic adenosine monophosphate; *CB_1* type 1 cannabinoid receptor; *DAG diacylglycerol*; *DAGLα* diacylglycerol lipase-α; *FAAH* fatty acid amide hydrolase; *GABA* γ-aminobutyric acid; *MAGL* monoacylglycerol lipase; *NAPE N*-acyl-phosphatidylethanolamine; *NAPE-PLD* NAPE-specific phospholipase D; *NAT N*-acyltransferase; *PLC* phospholipase C

N-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D (NAPE-PLD). The rate-limiting and Ca^{2+}-sensitive step in 2-AG and AEA production is the formation of DAG and NAPE, which are converted from phosphoinositide by phospholipase C and phosphatidylethanolamine *N*-acyltransferase, respectively.

As lipids, endocannabinoids (mainly 2-AG) readily cross the cell membrane and travel in a retrograde fashion to activate CB_1 receptors located in the presynaptic terminals. Stimulation of presynaptic CB_1 receptors leads to inhibition of the adenylyl cyclase → cAMP → protein kinase A signaling pathway, the blockade of voltage-gated Ca^{2+} channels and the activation of K^+ channels. This chain of intracellular processes inhibits the release of neurotransmitters, such as glutamate, dopamine, and GABA. 2-AG is also able to activate CB_1 receptors located on astrocytes, leading to the release of glutamate. After rapid uptake into cells, both 2-AG and AEA are metabolized by specific enzymes. 2-AG is primarily degraded into arachidonic acid and glycerol by monoacylglycerol lipase (MAGL), located at the presynaptic terminal or in astrocytes, while AEA is hydrolyzed to free arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH), present at the postsynaptic terminals.

AEA and 2-AG significantly differ in terms of their selectivity towards cannabinoid receptors. AEA is a high-affinity, partial agonist of CB_1 receptors and almost inactive at CB_2 receptors, whereas 2-AG acts as a full agonist at both CB receptors with moderate to low affinity. Both endocannabinoids have been reported to interact with various other receptors, including transient receptor potential cation channel subfamily V member 1 (TRPV1) activated by AEA. It is suggested that endocannabinoids, particularly 2-AG, act as retrograde messengers and are involved in the regulation of synaptic plasticity (for an excellent review, see Lu and Mackie 2016).

2.2 Cannabinoid CB_1 and CB_2 Receptors

Both CB_1 and CB_2 receptors are class A, lipid-like GPCRs. CB_1 receptors are widely located in the brain (Fig. 2). They are abundantly expressed in brain regions associated with cognition, memory, reward, anxiety, pain sensory perception, food intake, body temperature, and motor coordination. The highest expression of CB_1 receptors is observed in the olfactory bulb, hippocampus, basal ganglia, and cerebellum, while moderate expression can be found in the cerebral cortex, septum, amygdala,

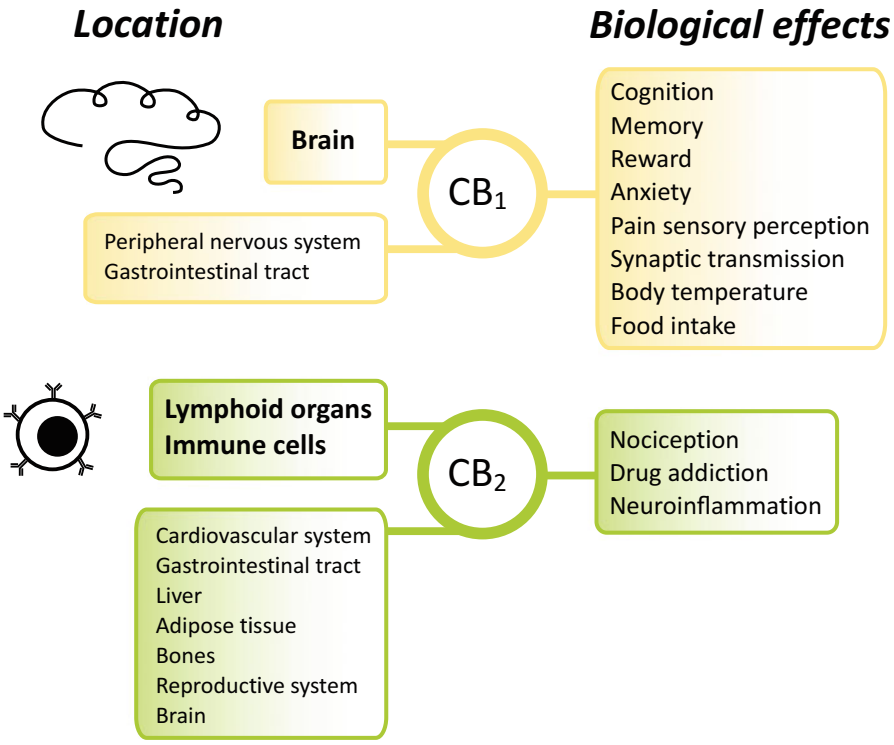


Fig. 2 Tissue distribution and functions of cannabinoid CB_1 and CB_2 receptors

hypothalamus, parts of the brainstem, and dorsal horn of the spinal cord. Finally, low expression is observed in the thalamus and the ventral horn of the spinal cord (Howlett and Abood 2017).

In the brain, CB_1 receptors are expressed in neurons and, albeit to a much lower extent, in astrocytes, oligodendrocytes, and microglia, where they have been shown to modulate synaptic transmission (Howlett and Abood 2017). CB_1 receptors are also abundantly expressed in the peripheral nervous system as well as in the peripheral tissues in a region-specific manner (Fig. 2). In the peripheral nervous system, CB_1 receptors are mostly expressed in sympathetic nerve terminals in the trigeminal ganglion, dorsal root ganglion, and dermic nerve endings of primary sensory neurons, where they regulate nociception from afferent nerve fibers (Howlett and Abood 2017). They are also present in the gastrointestinal tract, both the enteric nervous system and in non-neuronal cells in the intestinal mucosa, including enteroendocrine cells, immune cells, and enterocytes (Pertwee 2001). Interestingly, selected brain regions have displayed sex- and hormone-dependent variation in the density and function of CB_1 receptors (Castelli et al. 2014), which could account for the different responses to exogenous cannabinoids often described in men and women (Antinori and Fattore 2017). Accordingly, sex-specific tonic 2-AG signaling at inhibitory inputs onto dopamine neurons has been reported in rats (Melis et al. 2013). More generally, the actions of the endocannabinoid system and cannabinoids seem to be under the influence of various endogenous and synthetic steroid hormones, including sex hormones (Struik et al. 2018).

CB_2 receptors are predominantly located in the lymphoid organs, such as the spleen, tonsils, thymus, and lymphoid nodes, as well as in the cells of the immune system, including lymphocytes, macrophages, microglia, mast cells, and natural killing cells. These receptors are also found, albeit at moderate levels, in other peripheral tissues, including the cardiovascular system, gastrointestinal tract, liver, adipose tissue, bones, and reproductive system (Howlett and Abood 2017) (Fig. 2). Recent studies have demonstrated that CB_2 receptors are also expressed in the brain, primarily in microglia and vascular elements, albeit at much lower levels than CB_1 receptors. Expression of CB_2 receptors has been also found in neuronal cells in various brain regions, including the cortex, striatum, hippocampus, amygdala, brainstem, and cerebellum (Chen et al. 2017). It has been suggested that CB_2 receptors may be involved in neurological activities, such as nociception, drug addiction, and neuroinflammation. Notably, the expression of CB_2 receptors potently increases after tissue injury or during inflammation, supporting the hypothesis that these receptors may play a role in neuroinflammation (Chen et al. 2017; Howlett and Abood 2017).

3 Cannabis and Phytocannabinoids

Cannabis is defined as the flowering tops or separated resin of the *Cannabis sativa* plant. So far, more than 560 chemical compounds have been isolated from *Cannabis sativa*, including 121 terpenophenolic compounds known as phytocannabinoids or organic cannabis. Of these, the most abundant and prominent phytocannabinoid is Δ^9-THC,

which is responsible for the psychotropic effects associated with cannabis consumption. It is produced mainly in the leaves and flower buds of the plant. Δ^9-THC is a partial agonist of CB_1 and CB_2 receptors, with a preference for CB_1. Some cannabinoids, including cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG), do not induce psychoactive effects (ElSohly et al. 2017) (Fig. 3).

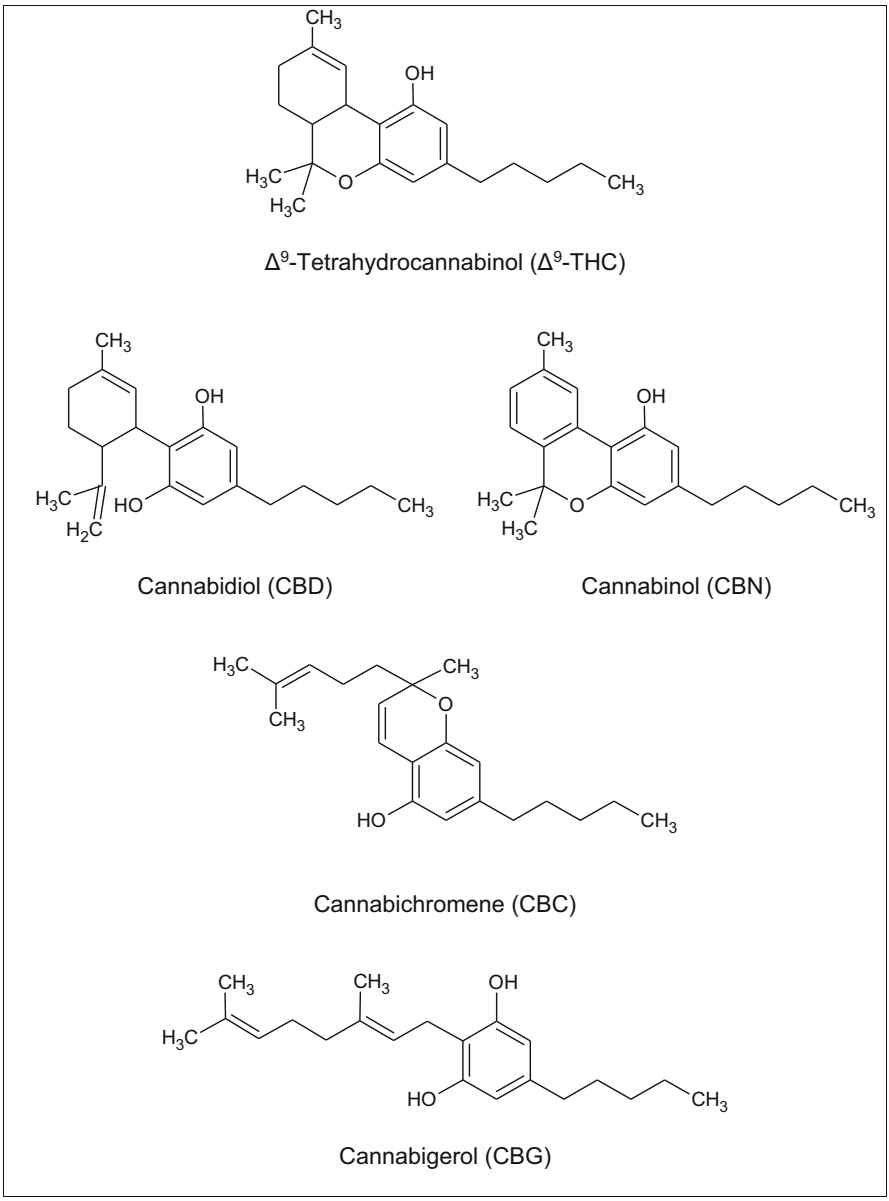


Fig. 3 Chemical structures of major phytocannabinoids

3.1 Cannabis: The Most Used Illicit Drug Worldwide

Cannabis sativa and its phytochemical products (marihuana and hashish) are the most widely produced plant-based illicit drugs. According to the 2020 World Drug Report, cannabis is the most used substance worldwide, with an estimated 192 million users in 2018 (UNODC 2020). There were 12.6 million past-year users of any drug among students aged 15–16 in 2017, with 11.3 million past-year users of cannabis. The annual prevalence of the cannabis use among young adults is highest in Europe (14.4%), North America (13.8%), Oceania (10.9%), and West and Central Africa (10.0%) (EMCDDA 2019a; UNODC 2019). The overall number of annual cannabis users is estimated to have increased by roughly 30% during the period 1998–2017.

3.2 Effects of Δ^9-THC, the Primary Psychoactive Component of Cannabis

3.2.1 Preclinical Studies

In vitro studies on the effects of Δ^9-THC on survival and morphology of neurons have provided mixed results, with both neurotoxic and neuroprotective effects being reported. Treatment of cultured rat hippocampal neurons or hippocampal slices with Δ^9-THC caused shrinkage of neuronal cell bodies and nuclei, and DNA breakage (Chan et al. 1998), and inhibited the formation of new synapses (Kim and Thayer 2001). Exposure of cultured rat cortical neurons to Δ^9-THC induced apoptosis, an effect involving stimulation of CB_1 receptors (Downer et al. 2001). In contrast to neurotoxic activity, Δ^9-THC protected rat hippocampal neurons in culture from excitotoxicity (Gilbert et al. 2007). Similarly, both Δ^9-THC and CDB exerted antioxidant activity and protected rat cultured cortical neurons from glutamate toxicity (Hampson et al. 1998). Additionally, semi-quantitative immunohistochemistry studies have shown that Δ^9-THC prevents methamphetamine-induced brain damage via inhibition of neuronal nitric oxide synthase (nNOS) expression and astrocyte activation (Castelli et al. 2014).

Microdialysis studies in freely moving animals demonstrated that systemic administration of Δ^9-THC stimulated, in the CB_1-dependent manner, release of acetylcholine in the rat hippocampus and prefrontal cortex, of glutamate and dopamine in the rat prefrontal cortex, and of dopamine in the mouse and rat nucleus accumbens. It has been proposed that this effect may be linked to the Δ^9-THC-induced inhibition of GABA release onto acetylcholine-, glutamate-, or dopamine-releasing neurons. A review of this topic is given by Pertwee (2008).

Administration of Δ^9-THC to mice produced a characteristic CB_1 receptor-mediated tetrad of behavioral and physiological effects: suppression of locomotor activity, hypothermia, immobility in the ring test (catalepsy), and antinociception in the tail-flick or hot-plate test. Δ^9-THC also decreased anxiety levels and affected the gait balance and grip strength of mice, as assessed by the latency time to fall from a rod (Schreiber et al. 2019).

The reinforcing effects of drugs and their potential for abuse are evaluated using behavioral tests such as intravenous self-administration (IVSA), intracranial self-stimulation (ICSS), drug discrimination, and conditioned place preference (CPP). Studies on Δ9-THC effects using ICSS, CPP, and drug-discrimination procedures did not return consistent results. Δ^9-THC was self-administered in squirrel monkeys; however, this behavior was not observed in rhesus monkeys or rodents (reviewed by Tanda 2016). A very recent study by Freels et al. (2020), using a self-administered vapor model, demonstrated that volitional exposure to Δ^9-THC-rich cannabis vapor has reinforcing properties. Contrary to Δ^9-THC, the synthetic full CB_1 receptor agonist WIN 55,212-2 was found to be readily self-administered by drug-naïve rats (Fattore et al. 2001). Intriguingly, cannabinoid self-administration behavior tends to differ between male and female animals, which may be associated with the different density and function of CB_1 receptors in the male and female brain (Fattore et al. 2010).

Accumulating experimental evidence indicates that a long-term exposure to Δ^9-THC leads to the development of physical dependence. Abrupt discontinuation of Δ^9-THC treatment or injecting of rimonabant (SR141716A), a CB_1 receptor antagonist/inverse agonist, to animals chronically treated with Δ^9-THC precipitated withdrawal syndrome. In rodents, characteristic withdrawal symptoms include head twitches, paw tremors or scratching, and hyperlocomotion (Cooper and Haney 2009). In rhesus monkeys, abrupt discontinuation of the long-term treatment with Δ^9-THC evoked an immediate (within 24 h) increase in their activity (Wilkerson et al. 2019).

3.2.2 Human Studies

Positive and Adverse Effects of Cannabis Use

Cannabis users typically experience euphoria, easy laughter and talkativeness, distortion of time perception, increased perception of external stimuli, increased appetite, and dry mouth. Common adverse effects after consuming Δ^9-THC containing products include dysphoria, anxiety, panic reactions, paranoia, auditory hallucinations, disorganized thought, and delusions of persecution (Ford et al. 2017). Similarly to findings from animal studies, sex-dependent effects of cannabis and cannabinoids have also been reported in humans (for an elegant review, see Cooper and Craft 2018).

Regular use of cannabis is associated with an increased risk of tolerance and dependence. A cannabis use disorder (CUD) is recognized by the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) and the *International Classification of Diseases, Tenth Revision* (ICD-10). A clinical diagnosis of cannabis withdrawal includes craving, irritability, anger or aggression, nervousness or anxiety, sleep difficulty, strange/wild dreams, decreased appetite or weight loss, restlessness, depressed mood, sweating, shakiness or tremors, headaches, and stomach pains (Bonnet and Preuss 2017). Symptoms of cannabis withdrawal typically occur within 24–48 h of abstinence following a period of regular use, with a peak intensity usually occurring 2–6 days after the last use, and can last between 7 and 14 days (Bonnet and Preuss 2017). A recent meta-analysis by Bahji et al. (2020) found the overall pooled

prevalence of cannabis withdrawal syndrome to be 47% in patients with regular or dependent use of cannabinoids. Cannabis withdrawal, although not life-threating, is clinically significant, as individuals use cannabis to avoid or alleviate withdrawal symptoms. At the molecular level, desensitization and downregulation of CB_1 receptors were observed in the cerebral cortex of regular cannabis users. These changes start to reverse within the first 2 days of abstinence, and the receptors return to normal functioning within 4 weeks of abstinence, which could constitute a neurobiological time frame for the duration of cannabis withdrawal syndrome (Hirvonen et al. 2012).

Effects of Cannabis on Cognitive Function and Brain Morphology

There is strong and consistent evidence that both acute and chronic cannabis use is associated with impaired verbal learning and memory, attention, and psychomotor function. Some, but not all, studies also demonstrated that cannabis use impaired decision-making and certain types of executive function, such as planning, reasoning, and inhibition. The effects are variable and are influenced by various factors, including the dose of Δ^9-THC, first-time vs. repeated use, preexisting vulnerability to mental illness, and personality traits. Impairment in attention, psychomotor function, verbal learning, and memory may persist with abstinence (reviewed by Blest-Hopley et al. 2020; Cohen et al. 2020). Chronic regular (daily or near daily) cannabis use is particularly problematic for young people, whose brains continue to develop into their mid-20s (Mashhoon et al. 2015).

During the last decade, a growing number of studies have been conducted to confirm whether long-term regular cannabis use is associated with structural brain alterations. However, data from structural imaging analyses are inconsistent or even contradictory. The ambiguity of results from neuroimaging studies could be influenced by various factors, including the onset of cannabis use, cumulative cannabis exposure, time of exposure, and Δ^9-THC content in the cannabis-based product (for a critical review see Chye et al. 2021).

3.3 Why Are the Concentration of Δ^9-THC and the Δ^9-THC:CBD Ratio in Cannabis Products so Important for Human Health?

As discussed above, exposure to Δ^9-THC can induce psychotic-like symptoms and anxiety, impairment of memory, and psychomotor control in a dose-dependent manner (Colizzi and Bhattacharyya 2017). In contrast, CBD, a non-psychotomimetic phytocannabinoid derived from *Cannabis sativa*, has been suggested to have beneficial effects over a broad range of neuropsychiatric disorders. Specifically, it seems to exert antipsychotic properties and to counteract the psychotic symptoms and cognitive impairment associated with cannabis use and acute Δ^9-THC administration (Colizzi and Bhattacharyya 2017). Although its mechanism of action is still to be clarified, CBD has been reported to facilitate neurogenesis and attenuate anxiety- and depressive-like states, as well as cause brain damage associated with neurodegenerative and/or ischemic conditions (reviewed in Campos et al. 2016). As a result,

concentrations of Δ^9-THC and CBD in cannabis products and their relative ratio are important factors in determining the level of harm an individual may experience (Colizzi and Bhattacharyya 2017). Cannabis and cannabis resin typically contain 2–8% Δ^9-THC. However, over the last two decades, cannabis potency (Δ^9-THC content) and the Δ^9-THC:CBD ratios have continued to rise in different parts of the world. In the United States, for example, the mean concentration of Δ^9-THC in all analyzed samples increased from 8.9% in 2008 to 17.1% in 2017, and the Δ^9-THC:CBD ratio rose dramatically from 23 in 2008 to 104 in 2017 (Chandra et al. 2019). A similar trend was observed in Europe, where the mean Δ^9-THC content of cannabis resins doubled from about 8% in 2006 to 17% in 2016, and the Δ^9-THC content of cannabis herb increased from 5% to 10% over the same period (Freeman et al. 2019). Therefore, increases in cannabis potency could have important implications for the health effects of cannabis use, especially among adolescents, who may be more vulnerable to cannabis-induced damage. A growing number of observations indicate that higher potency of cannabis preparations is associated with adverse health outcomes, including elevated symptoms of CUD, increased treatment admissions for cannabis problems, higher risk of developing psychosis, and increased risk of relapse to psychosis (EMCDDA 2019c). However, besides the increased concentration of Δ^9-THC in cannabis-based products, another important phenomenon has greatly contributed to increase the health risk of these products, namely, the appearance on the market of synthetic, more potent agonists of cannabinoid receptors (EMCDDA 2019c).

4 Synthetic Cannabinoids

4.1 Synthetic Cannabinoids: The Largest and Most Diverse Group of New Psychoactive Substances

The consumption of new psychoactive substances (NPS), used as alternatives of classical drugs of abuse, has been increasing since the late 2000s worldwide. By the end of 2019, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was monitoring around 790 NPS (EMCDDA 2020). As of January 2020, 120 countries and territories have reported to the United Nations Office on Drugs and Crime (UNODC) a total of 950 NPS (UNODC 2020). Among all NPS reported to the UNODC by the end of 2017, synthetic cannabinoids (251 compounds) constitute the second largest (31%), most structurally diverse and fastest growing group (UNODC 2018). Synthetic cannabinoids also represent the largest group of NPS currently monitored by the European Union Early Warning System – with a total of 189 substances having been notified to the EMCDDA between 2008 and 2018 (Fig. 4) (EMCDDA 2019b). When one compound is, or is about to be, legally controlled, new analogs with increasingly diverse chemical structures appear on the market in order to satisfy demands and avoid criminalization.

Synthetic cannabinoids (SCs) or synthetic cannabinoid receptor agonists (cannabimimetics) represent a heterogeneous group of compounds designed to

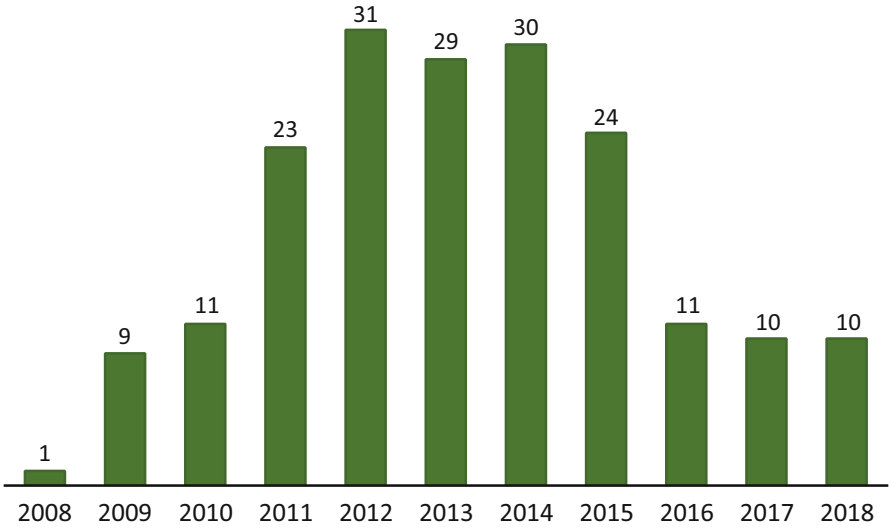


Fig. 4 The number of synthetic cannabinoids notified to the European Union Early Warning System by 2018 (EMCDDA 2019a,b)

mimic the effects of Δ^9-THC by binding to cannabinoid receptors CB_1 and CB_2. SCs emerged in the 1970s and were originally developed as research tools for use in structure-activity relationship studies and/or as part of early phase drugs discovery reports. However, a subset of these compounds was diverted for recreational use beginning in the early 2000s and started diffusing at a global level (Fig. 5). The first SCs detected by forensic toxicologists in Europe were JWH-018 and CP-47,497, found in 2008 in products sold under the brand name "Spice" (EMCDDA 2019c). Canada and Japan reported that SCs had appeared on their markets before 2008, while in the United States, these compounds were reported from 2009 (UNODC 2018).

The major structural groups of SCs include cyclohexylphenols (e.g., CP-47,497 and CP-55,940), classical cannabinoids (such as HU-210), naphthoylindoles (e.g., JWH-018, JWH-073, JWH-073, and AM-2201), napthylmethylindoles (e.g., JWH-184 and JWH-192), naphthoylpyrroles (e.g., JWH-030 and JWH-307), naphthylmethylindenes (e.g., JWH-176 and JWH-220), phenylacetylindoles (e.g., JWH-250 and JWH-251), benzoylindoles (e.g., AM-694 and RCS-4), adamantylindoles (e.g., APICA and AB-001), acylindoles (e.g., AB-005, UR-144, and XLR-11), naphthoylnaphthalenes (e.g., CB-13), indazoles (e.g., AB-PINACA, 5F-MDMB-PINACA, AB-CHMINACA, AB-FUBINACA, and CUMYL-4CN-PINACA), and indole-3-carboxamides and indole-3-carboxylates (e.g., AB-PICA and AB-FUBICA) (reviewed by Banister and Connor 2018; Alves et al. 2020) (Fig. 6).

With the exception of HU-210, SCs are structurally distinct from Δ^9-THC. Their number, chemical diversity, and speed of emergence make this group of compounds

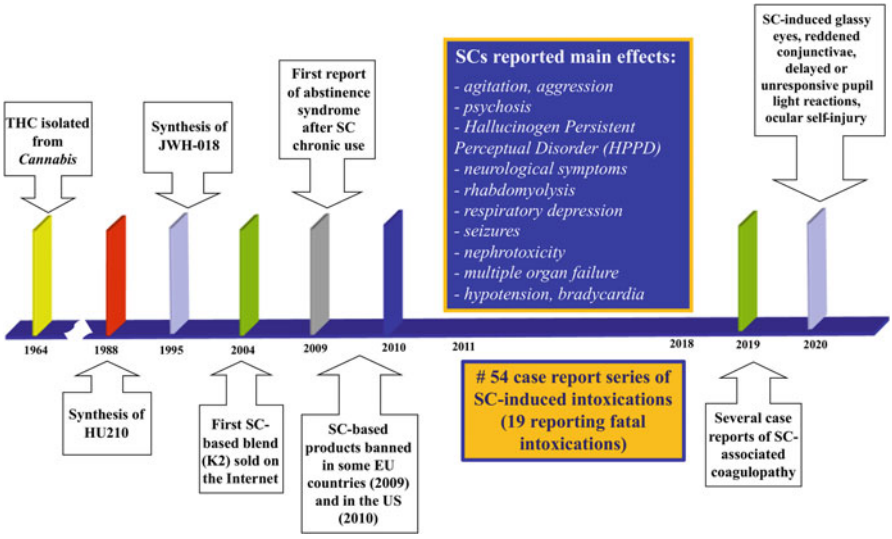
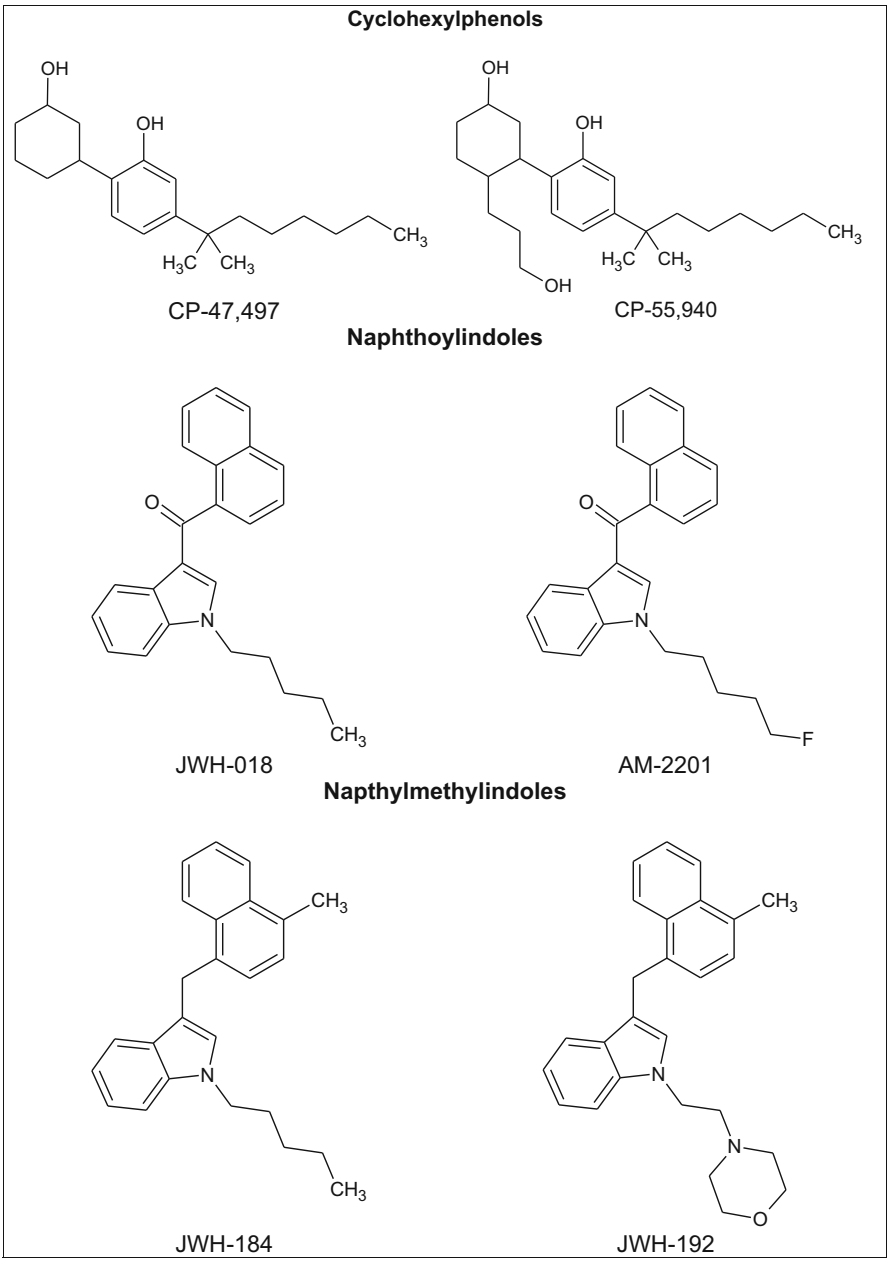


Fig. 5 Timeline of diffusion and effects of synthetic cannabinoids (SCs)

particularly challenging in terms of detection, monitoring, and responding. Together with synthetic cathinones, i.e., psychostimulants related to the alkaloid cathinone found in the khat plant (*Catha edulis*), SCs are the most commonly used NPS that pose a major risk for public health (EMCDDA 2019a).

4.2 Synthetic Cannabinoids and Their Products

In a pure state, SCs are either solid or oils. These compounds typically consist of 20–26 carbon atoms, which explain why they volatilize easily when smoked. Data from seizures and collected samples show that SCs have typically been detected in smoking mixtures, disingenuously marketed as "incense," "potpourri," "air freshener," and "not for human consumption," containing herbal/plant material to which one or more of compounds have been added. For production of smoking mixtures, the substance is dissolved in a volatile solvent (e.g., acetone or alcohol) and applied to plant material, such as damiana (*Turnera diffusa*), marshmallow (*Althaea officinalis*), or *Lamiaceae* herbs like *Melissa, Mentha*, and *Thymus*, either via spraying or soaking. Once the solvent evaporates, the dried plant material is crushed, packed in brightly colored metal-foil sachets, and sold as "spice," "herbal incense," "K2," or with other captivating brand names. The products typically have a pleasant smell and taste, for example, of honey or vanilla (Fattore and Fratta 2011; Zawilska and Wojcieszak 2014). Less commonly, SCs are sold as high-purity bulk powders, liquid formulations for vaporization in electronic cigarettes, as well as liquids or blotters, while others are blended into a dough-like substance as "fake hash" products that look like cannabis resin or as edible products such as candy and



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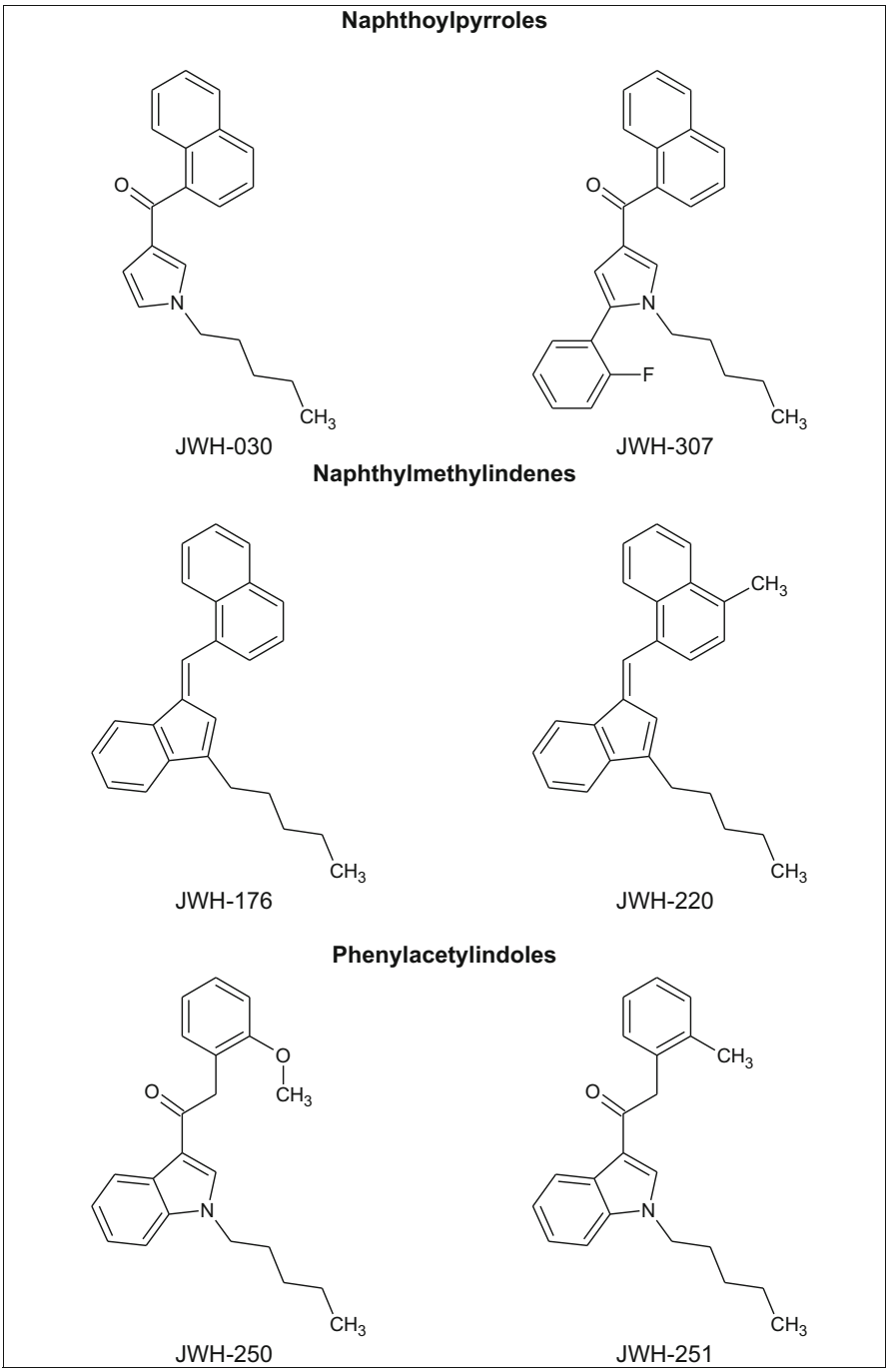

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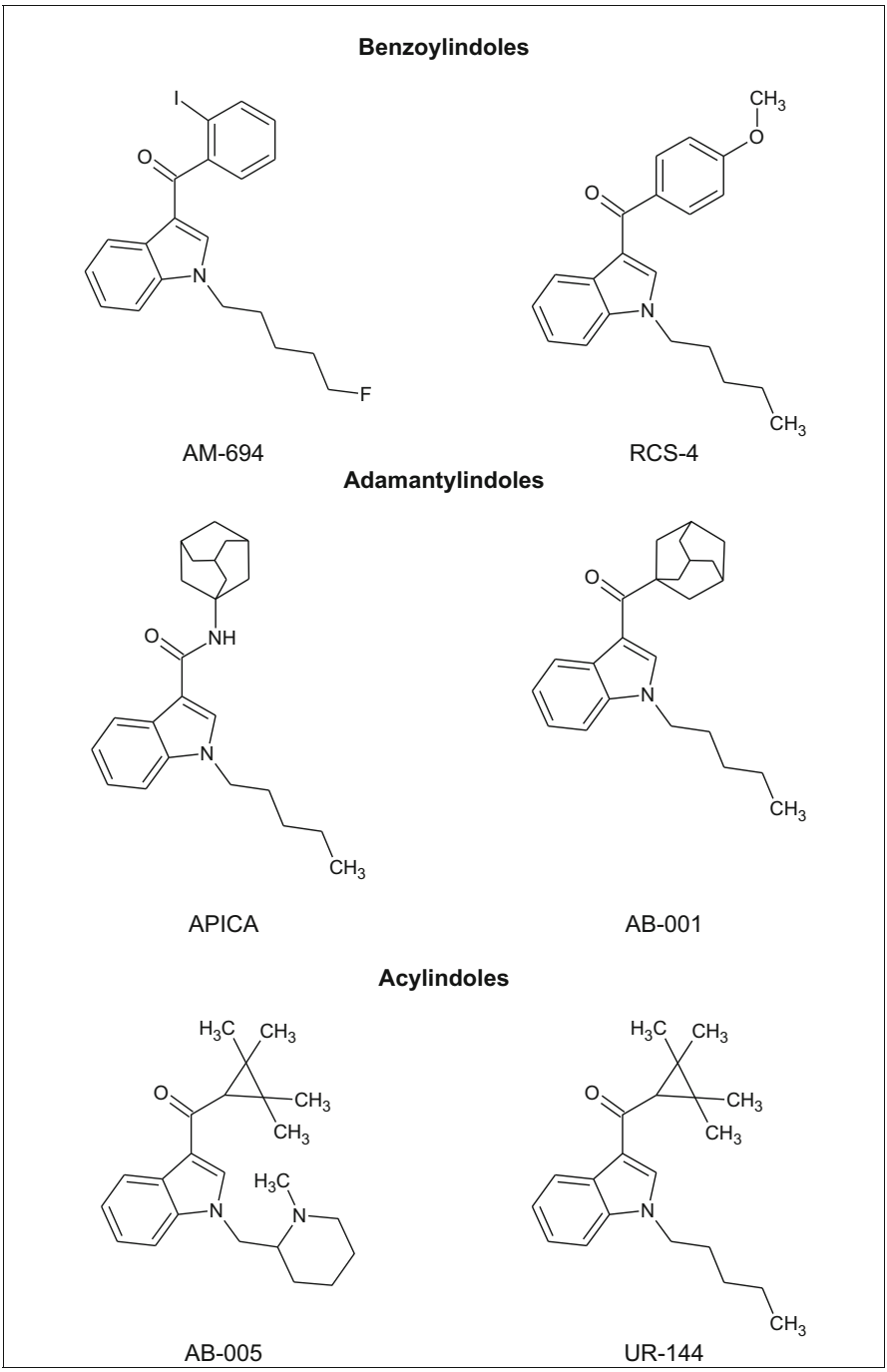

Fig. 6 (continued)

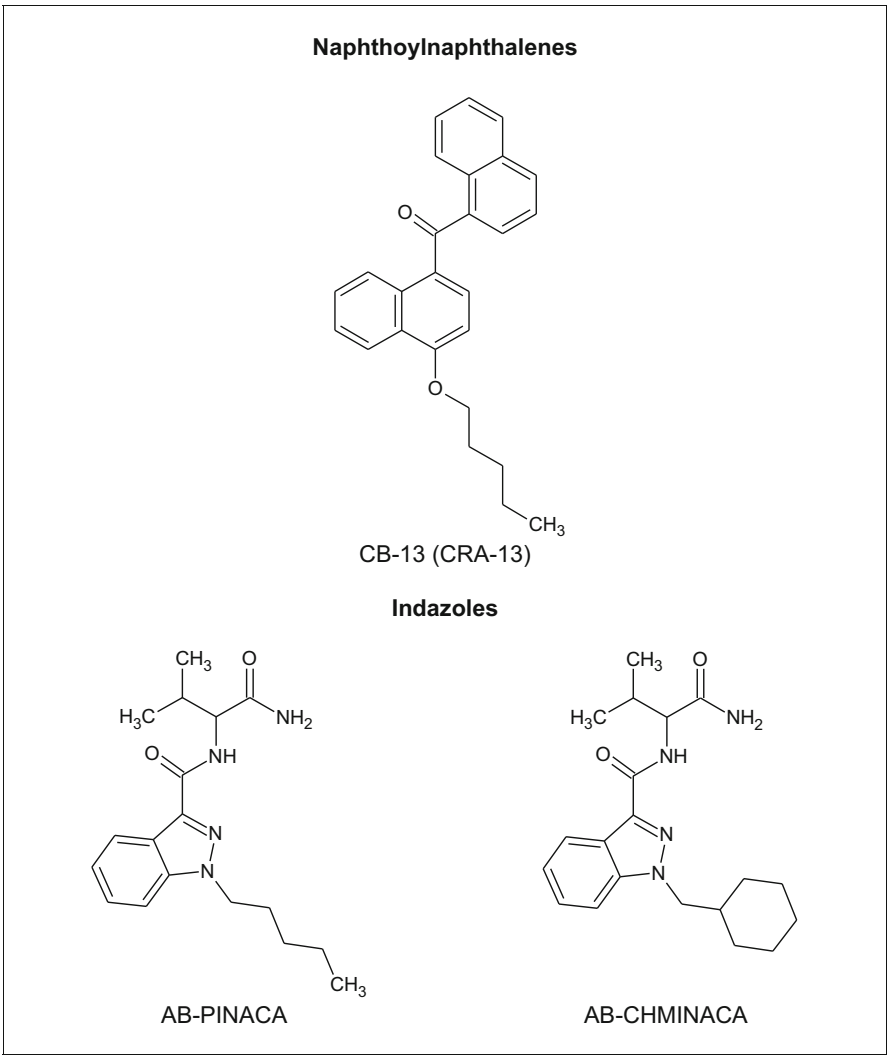

Fig. 6 (continued)

baked goods (EMCDDA 2019c). SCs are typically used by smoking either ready-to-use or homemade "smoking mixtures" as a cigarette ("joint") or by using a vaporizer ("bong" or pipe). Oral consumption as herbal tea or rectal use is uncommon. According to subjective self-reports, smoked SCs typically have a faster onset than natural cannabis, peak more quickly, and have shorter effects (commonly 1–2 h) than marijuana, although much longer lasting effects (10–15 h) have also been reported (Mathews et al. 2019).

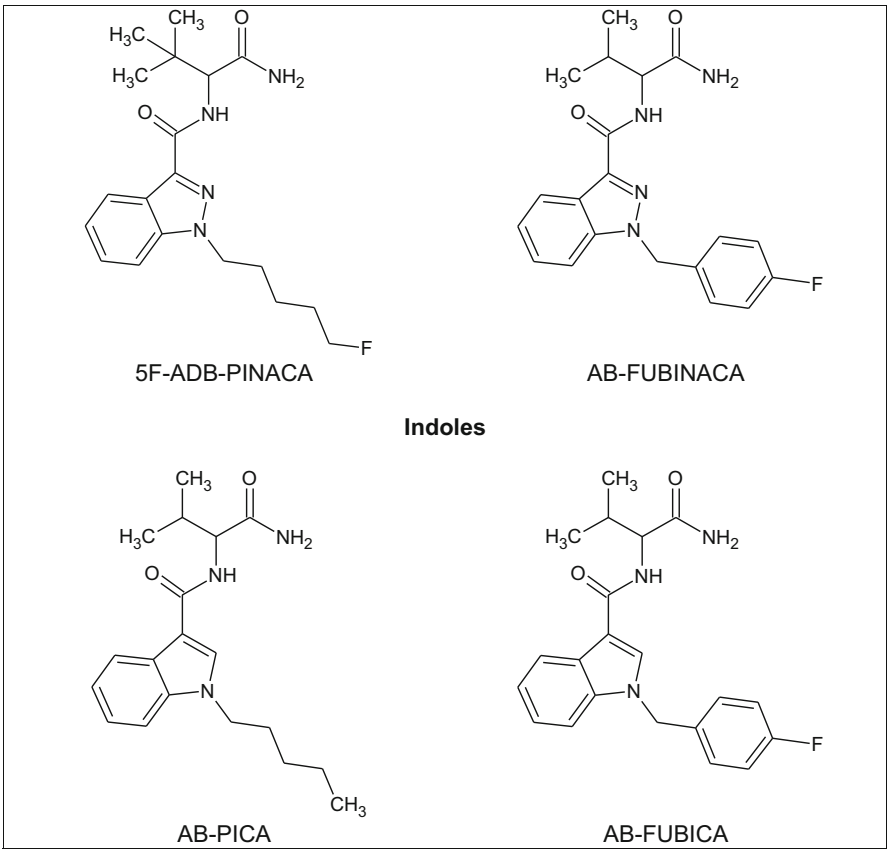

Fig. 6 Exemplar structures of synthetic cannabinoids from different chemical classes

4.3 Who Uses Synthetic Cannabinoids and Why?

The motivation to use SCs is typically associated with curiosity, low cost, easy access, a belief that the products are generally safe, and expectations to achieve marijuana-like effects (euphoria/getting high, relaxation) while avoiding detection in drug tests. Less frequently, SCs are used to reduce or stop cannabis use (Barratt et al. 2013; Fattore and Fratta 2011; Zawilska and Wojcieszak 2014). Users are typically male adolescents and young adults with at least a high school level of education (Barratt et al. 2013; Mathews et al. 2019). Among people who use SCs, there are recreational users, high-risk drug users, groups who experiment with psychoactive substances (such as "psychonauts"), and individuals who are subject to drug testing (e.g., people in drug treatment, prisoners, soldiers, athletes, and drivers) (EMCDDA 2019c; Shapira et al. 2020; Zawilska and Wojcieszak 2014).

Importantly, epidemiological findings support a link between cannabis use and SC use (Gunderson et al. 2014). A recent study conducted on college students in the

United States demonstrated that users of natural cannabis, especially those who reported more frequent use and an earlier age of the first use of natural cannabis, had significantly greater odds of having tried SCs (Mathews et al. 2019). Results of an online survey study of 316 Australian SCs users show that 96% had lifetime use of cannabis, with 61% reporting last-month use and 15% reporting daily use. Other drugs most commonly used in the last month were alcohol (77%) and tobacco (58%) (Barratt et al. 2013).

4.4 Synthetic Cannabinoids Are Potent Agonists of Cannabinoid Receptors

Accumulating experimental data show that despite their structural differences, the vast majority of SCs have higher binding affinity to CB_1 and CB_2 receptors than Δ^9-THC. Similarly, SCs demonstrate higher intrinsic activity (i.e., efficacy) at cannabinoid receptors than Δ^9-THC, which is a partial agonist of these receptors (Table 1). In addition, metabolites of synthetic cannabinoids often retain higher affinity for cannabinoid receptors than Δ^9-THC and thus may produce pharmacological and toxicological effects distinct from those induced by Δ^9-THC (reviewed by Alves et al. 2020). These characteristics, together with their ability to activate dopamine neurotransmission in limbic brain areas, indicate why SCs are more potent (and dangerous) drugs than Δ^9-THC (Fig. 7).

4.5 Effects of Synthetic Cannabinoids

4.5.1 Preclinical Studies

Information on SCs neurotoxicity at the cellular level is scarce. CP-55,940, CP-47,497, CP-47,497-C8, HU-210, JWH-018, JWH-210, AM-2201, and MAM-2201 were found to induce apoptosis of primary neuronal cultures from mouse forebrain through a caspase-3-dependent mechanism (Tomiyama and Funada 2014). These cytotoxic effects were mediated by CB_1 receptors but not CB_2. The potential toxicity of SCs on neuroblastoma cell lines was examined in two studies. Experiments on the murine neuroblastoma neuro-2a cell line demonstrated that exposure of cells to 5F-ADBINACA, AB-FUBINACA, and STS-135 reduced mitochondrial membrane potential, indicating toxic activity (Canazza et al. 2017). In addition, the selective CB_2 receptor agonist JWH-133 induced a decrease in the viability and proliferation rate of SH-SY5Y cells: an experimental in vitro model widely used to study mechanisms of toxicity and protection of nigral dopaminergic neurons (Wojcieszak et al. 2016). The cytotoxic effect of JWH-133 was not mediated by activation of CB_2 receptors or by the caspase pathway, as it was blocked neither by AM-630 (an inverse agonist of CB_2 receptors) nor by Z-VAD-FMK (a pan-caspase inhibitor).

In vivo studies on mice found SCs to produce a tetrad of behavioral and physiological changes characteristic for stimulation of central CB_1 receptors, viz.,

Table 1 Binding affinity (K_i) of selected synthetic cannabinoids from different chemical groups

Compound	Human CB_1	Human CB_2	Cited in
	K_i (nM)	K_i (nM)	
Δ^9-THC	3.87	71.6	Schoeder et al. (2018)
Cyclohexylphenols			
CP-55,940	1.28	1.42	Schoeder et al. (2018)
Naphthoylindoles			
JWH-018	9.0	2.9	Banister and Connor (2018)
JWH-122	0.69	0.69	
JWH-210	0.46	13.8	
AM-2201	1.0	2.6	
Naphtoylpyrroles			
JWH-145	14.0	6.4	Banister and Connor (2018)
JWH-307	7.7	3.3	
Phenacetylindoles			
JWH-203	8.0	7.0	Banister and Connor (2018)
JWH-250	11	33	
Benzoylindoles			
AM-2233	1.8	2.2	Banister and Connor (2018)
AM-694	0.08	1.44	
Acylindoles			
AB-005	5.5	0.48	Banister and Connor (2018)
UR-144	150	1.8	
XLR-12	15	0.09	
Indazoles			
5F-ADB-PINACA	1.43	0.694	Schoeder et al. (2018)
ADB-FUBINACA	0.36	0.339	
MA-CHMINACA	0.339	0.301	
MDMD-CHMINACA	0.135	0.222	
MDMB-FUBINACA	0.0985	0.130	
5F-AB-PINACA	4.96	3.77	
FUB-AMB	0.387	0.536	
CUMYL-PICA	3.27	24.0	
5F-CUMYL-PICA	1.37	29.1	

suppression of locomotor activity, antinociception, hypothermia and catalepsy, and Δ^9-THC-like discriminative stimulus in rodents and nonhuman primates (Canazza et al. 2017; Gamage et al. 2018; Gatch and Forster 2019; Ginsburg et al. 2012; Ossato et al. 2016; Wiley et al. 2015, 2019). In other studies, JWH-018, JWH-250, JWH-073, AKB48, 5F-AKB48, 5F-ADBINACA, AB-FUBINACA, and STS-135 induced hyperreflexia and myoclonia in mice that were not observed after administration of Δ^9-THC (Canazza et al. 2017; Ossato et al. 2016), while 5F-ADBINACA, AB-FUBINACA, and STS-135 impaired sensorimotor responses and promoted aggressiveness (Canazza et al. 2017).

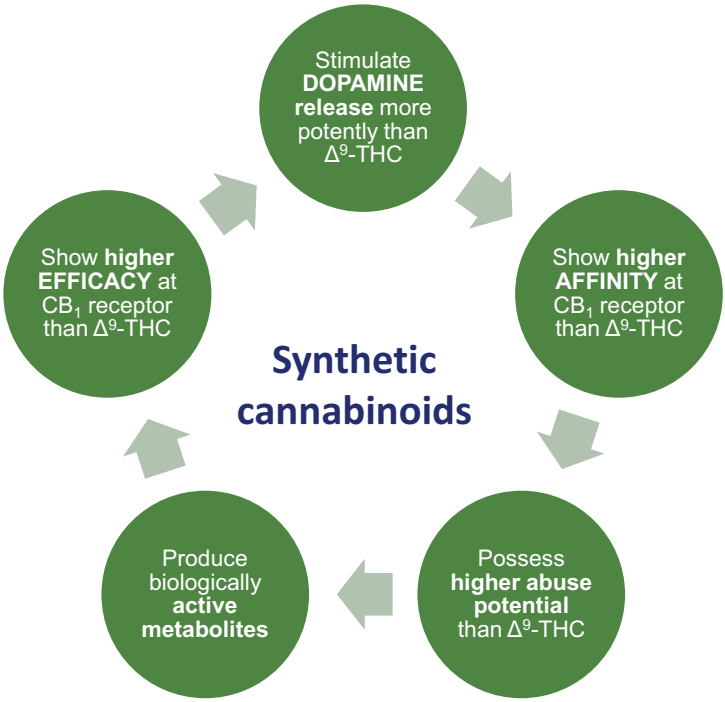

Fig. 7 Factors explaining the different effects of SCs as compared to Δ^9-THC

Malyshevskaya et al. (2017) compared the acute effects of JWH-018 and Δ^9-THC in mice. After administration of JWH-018, they observed behavioral changes including suppression of locomotor activity, impaired walking, ataxia, extensor rigidity in hind limbs, Straub tail, muscular jerks, rearing, and low-intensity behavioral seizures. Animals also showed dyspnea (gasps) and profound catatonia. Similar behavioral changes were also observed after Δ^9-THC administration, but with a lower intensity than those produced by JWH-018. Both drugs evoked electrographic seizures in the form of frequent EEG seizure spikes. The average onset latency of electrographic/behavioral seizures was shorter after JWH-018 (5.4 min) compared to Δ^9-THC administration (11.15 min). Electrographic seizures were apparent for 256 min after Δ^9-THC, but after JWH-018 administration they persisted for a markedly longer time (344 min). Significantly more frequent spikes were observed after JWH-018 (25.1 spikes/min) compared to Δ^9-THC administration (12.3 spikes/min). The epileptogenic effects of JWH-018 were mediated by CB_1 receptors. Proconvulsive activity was also demonstrated for other SCs, including JWH-250 JWH-073, AM-220, 5F-ADBINACA, 5F-PINACA, AB-FUBINACA, and STS-135 (Breivogel et al. 2020; Canazza et al. 2017; Funada and Takebayashi-Ohsawa 2018; Ossato et al. 2016).

Four-day treatment of mice with JWH-073, AM-2201, and Δ^9-THC resulted in the rapid development of tolerance to their antinociceptive and hypothermic effects. Administration to mice of the selective CB_1 receptor antagonist/inverse agonist, SR141716A, precipitated behavioral withdrawal symptoms, including scratching, grooming, and rearing (AM-2201-treated group only) (Breivogel et al. 2020). In addition, JWH-018, JWH-073, JWH-250, BB-22, 5F-PB-22, 5F-AKB-48, and STS-135 were found to facilitate dopamine release in the nucleus accumbens of rats (Bilel et al. 2019; De Luca et al. 2016; Ossato et al. 2016, 2017), suggesting their potential positive role in rewarding mechanisms.

4.5.2 Human Data

The differing toxicological profiles of SCs and Δ^9-THC have been attributed to differences in their chemical structure, metabolism, and pharmacology. It should be emphasized that unlike natural cannabis, products with SCs do not contain CBD, which may protect against psychosis. In addition, several SCs undergo biotransformation to biologically active metabolites with long half-lives (reviewed by Alves et al. 2020). Thus, not surprisingly, the use of SCs-containing products is associated with a higher incidence of severe adverse effects than that of cannabis. SCs are known to have a number of adverse effects: severe cardiovascular toxicity (including myocardial infarction and sudden death), lethargy, confusion, anxiety and fear, distorted perception of time, depersonalization, hallucinations, racing thoughts, paranoia, delirium, impaired motor performance, seizures and convulsions, rapid loss of consciousness/coma, dizziness, ataxia, nystagmus, drowsiness, respiratory depression, pulmonary edema, rhabdomyolysis, nephrotoxicity, and hyperemesis. Psychotic episodes, aggressive and violent behavior, self-harm/suicidal ideation, self-mutilation behaviors, catatonia, and intracranial hemorrhages were also reported (Alipour et al. 2019; Alves et al. 2020; Mathews et al. 2019; Mensen et al. 2019; Tait et al. 2016; Zawilska and Wojcieszak 2014). Notably, as for natural cannabis, the effects of SCs use may considerably vary between males and females, confirming the sex-dependent effects of cannabinoid drugs (Fattore et al. 2020).

Some psychotic/neurologic symptoms of acute intoxication, such as seizures, anxiety, and panic attacks, are unique to SCs and are usually not observed following marijuana use, even after high doses (Mensen et al. 2019). Among psychiatric patients, SC users presented with more severe psychotic symptoms and agitation compared to natural cannabis users (Alipour et al. 2019; Alves et al. 2020). SCs may trigger the occurrence of severe psychosis in psychosis-prone users or the exacerbation of a prodromal psychotic syndrome in healthy individuals (reviewed in Fattore 2016).

There are several reports of fatal intoxications with SCs, taken alone or in combination with other compounds. The list of SCs detected in postmortem samples include EAM-2201, AB-PINACA, 5F-PB-22, 5F-AKB-48, 5F-ADB, AB-CHMINACA, UR-144, XLR-11, JWH-022, MAB-CHMINACA, MDMB-CHMICA, 5F-AMB, mepirapim, JWH-018, AM-2201, JWH-210, JWH-122, JWH-250, JWH-175, ADB-FUBINACA, AB-FUBINACA, 5F-APINACA, MAM-2201, STS135, THJ 2201, AM-1220, AM-2232, PB-22, NNEI, AM-604,

and JWH-073. Some of them, such as 5F-ADB, XLR-11, AM-2201, AB-CHMINACA, and JWH-018, have been identified more frequently than others (for an excellent review, see Giorgetti et al. 2020).

Repeated use of SCs is associated with impairments in emotional and cognitive processing, such as working memory, attention, and executive and visual-spatial functions (Cengel et al. 2018; Cohen et al. 2020; Livny et al. 2018; Umut et al. 2020). Importantly, SCs users demonstrate more severe impairments in cognitive functions than individuals with CUD (Cengel et al. 2018). Brain imaging studies demonstrated reduced total gray matter volume in SCs users compared with control participants and reduced gray matter volume in the thalamus and left cerebellum as well as in several cortical regions, including the middle frontal gyrus, frontal orbital gyrus, inferior frontal gyrus, insula, anterior cingulate cortex, and precuneus (Livny et al. 2018). Long-term use of SCs is also associated with white matter abnormalities in adolescents and young adults (Zorlu et al. 2016). The evidence of neuronal damage associated with the chronic use of SCs is alarming, since it may indicate possible neurotoxic effects. Moreover, SCs users showed diminished brain activations in the precuneus, cuneus, lingual gyrus, hippocampus, and cerebellum while performing an N-back task (Livny et al. 2018).

The use of SCs is associated with a more rapid development of dependence and complex symptoms of withdrawal than those observed after cannabis. Withdrawal often occurs shortly after smoking. A growing number of reports detail adverse effects related to withdrawal from daily use of SCs compared to cannabis. For example, one patient reported that she would wake up every 45 min throughout the night to smoke in order to alleviate withdrawal symptoms. Abrupt discontinuation of daily SCs use can trigger severe symptoms, including reoccurring seizures, as well as cardiovascular and respiratory risks: tachycardia, hypertension, chest pain, palpitations, and dyspnea. Common effects of moderate severity include cravings, headache, impatience, anxiety/nervousness, anger/irritability, mood swings, insomnia, nightmares, tremor, diarrhea, nausea and vomiting, loss of appetite, and diaphoresis due to profuse sweating (Bahji et al. 2020; Livne et al. 2019).

Finally, an innovative study by Matteo Marti's lab has recently revealed for the first time the genotoxic effects of different SCs belonging to the indole and indazole structure families (Lenzi et al. 2020). Authors measured by flow cytometry the mutagenic capacity of four SCs in terms of chromosomal damage induction and showed a significant impact of these compounds on the stability of human genetic material.

5 Cannabinoids Use in Pregnancy

Women are recommended not to smoke during pregnancy. Yet, probably because of its legalization and decriminalization in some countries, marijuana is perceived as a harmless drug, and its use is quite common among pregnant women. Worryingly, women smoke marijuana frequently for its antiemetic properties during the first trimester of pregnancy, thus exposing the fetus to a great risk of teratogenic effects

(Navarrete et al. 2020). Δ^9-THC is lipophilic and can readily cross the placenta and reach the fetus. Marijuana use by breastfeeding mothers is also unsafe, as Δ^9-THC is easily transferred into breast milk, thus prolonging Δ^9-THC exposure to other sensitive periods of development (Navarrete et al. 2020). Notably, Δ^9-THC is detectable in breast milk up to about 6 days after maternal marijuana use (Bertrand et al. 2018).

Both clinical and preclinical studies have shown that use of cannabis during pregnancy and/or lactation can induce significant behavioral alterations (Campolongo et al. 2009; Trezza et al. 2008). Both fetal growth during pregnancy and increased thickness of the brain prefrontal cortex during childhood are associated with prenatal cannabis exposure (El Marroun et al. 2016). Clinical studies suggest that cannabis use during pregnancy may be associated with changes in brain chemistry, including developmental regulation of striatal dopamine D2 receptors in offspring through epigenetic mechanisms (DiNieri et al. 2011). Alterations in dopamine D2 receptor gene expression in other mesocorticolimbic structures of the human brain have also been reported after in utero exposure to cannabis (Wang et al. 2004), a finding strongly corroborated by animal studies (Szutorisz and Hurd 2018). Alterations in the functioning of the mesocorticolimbic system may have great influence on the future psychiatric health of the offspring, which could explain, at least in part, the long debated link between use of marijuana during pregnancy and risk of psychosis in the offspring (Davis et al. 2016; Navarrete et al. 2020). In support to this notion, maternal Δ^9-THC exposure was recently found to dysregulate dopamine cell in vivo activity in prepubertal offspring and to promote both a psychotic-like endophenotype (Frau et al. 2019) and susceptibility to acute stress (Sagheddu et al. 2021).

Whether prenatal and/or perinatal exposure to SCs induces Δ^9-THC-like effects in the fetus/offspring is not known at present. Yet, preclinical studies suggest that this is the case. Studies in rodents have individuated several sites and periods of pregnancy as potential targets of SCs, including preimplantation embryo development, implantation, and placentation (Sun and Dey 2014). Exposure to SCs during gestation and/or lactation may impair hippocampal long-term potentiation (Mereu et al. 2003) and cortical glutamatergic transmission (Antonelli et al. 2006), affect the intrinsic electrophysiological properties of the Purkinje neurons of the cerebellum (Shabani et al. 2011), alter migration of early-born cortical glutamatergic neurons and GABAergic interneurons (Saez et al. 2014), and induce long-lasting alterations in the functional status of the hypothalamic-pituitary-adrenal (HPA) axis (del Arco et al. 2000). The synthetic cannabinoid CP-55,940 has been reported to demonstrate dose-dependent teratogenicity in mice, with fetuses showing significant craniofacial abnormalities and ocular changes (Gilbert et al. 2016).

That marijuana and SCs are able to interfere with neurodevelopment is not surprising, as the endocannabinoid system is present in the animal and human brain since the early stages of development, and during the pre- and postnatal life it plays a crucial role in brain organization. Progenitor cell proliferation and neuronal differentiation, axon growth, and synapse formation in the developing brain are all strongly influenced by the endocannabinoid system (Alpar et al. 2016; Navarrete

et al. 2020). In light of the functional interactions between the endocannabinoid system and hormonal asset (Struik et al. 2018), the use of cannabinoids during pregnancy could impact on fetoplacental development also by altering maternal and placental hormone signaling

6 Conclusion

The endocannabinoid system has gained tremendous interest in recent years. Fruitful studies have been generated during the last decades, unraveling the complexity of the whole endocannabinoid system. Although cannabinoids have therapeutic potential, and the therapeutic action of cannabinoid-based medicines has been acknowledged worldwide, their psychoactive effects have largely limited their use in clinical practice. Yet, scientific interest in this fascinating endogenous system remains strong, with the latest challenges being the effects of SCs of last generations on our brain and behavior and the harmful effects of the use of cannabinoids during pregnancy.

7 Cross-References

► Neurotoxicity: A Complex Multistage Process Involving Different Mechanisms

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